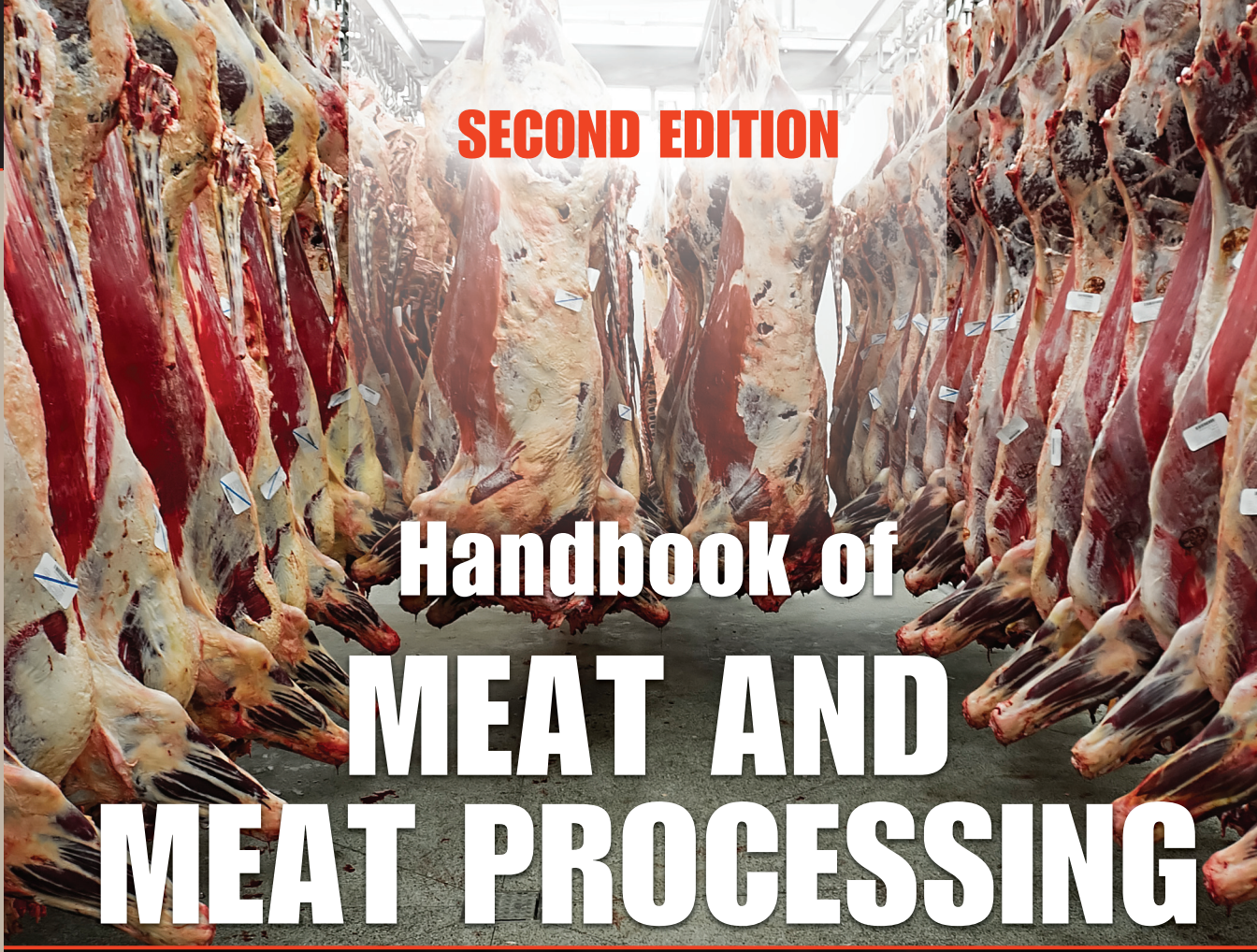

SECOND EDITION

Handbook of MEAT AND MEAT PROCESSING

Edited by Y. H. Hui, PhD

Associate Editors

J. L. Aalhus, PhD

L. Cocolin, PhD

I. Guerrero-Legarreta, PhD

L. M. Nollet, PhD

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CRC Press
Taylor & Francis Group
Boca Raton London New York

CRC Press is an imprint of the
Taylor & Francis Group, an **informa** business

CRC Press
Taylor & Francis Group
6000 Broken Sound Parkway NW, Suite 300
Boca Raton, FL 33487-2742

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CRC Press is an imprint of Taylor & Francis Group, an Informa business

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Version Date: 20111117

International Standard Book Number-13: 978-1-4398-3684-2 (eBook - PDF)

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Preface

The consumption of red meat and meat products has a long history in most cultures. Meat is a source of nutrients, as well as a sign of wealth in some countries. Various techniques have been developed in different parts of the world over the centuries to preserve meat for extended shelf life and enjoyment. Even nonedible parts of animals are used for various reasons. Thus, meat, meat products, and by-products are important to our daily life.

In the past three decades, many books on the science and technology of meats and meat products have been published. Many of these books are useful reference texts and well received by professionals in the meat industries, academia, and the government. *Meat Science and Applications* is one such example. It was published in 2001 by Marcel Dekker of New York. A decade later, CRC Press is publishing a second edition of the book with a new title, *Handbook of Meat and Meat Processing.* The change in title reflects the expansion of coverage in depth and breadth from the first edition, as illustrated in the table of contents of this book. Apart from updating materials in the first edition, the new edition contains 51 chapters instead of 27.

This second edition is divided into seven parts. Part I covers an overview of the meat-processing industries in the United States. Part II discusses the basic science if meat, including chapters on muscle biology, meat composition, and chemistry. Part III covers meat attributes and characteristics such as color, flavor, and analyses. Part IV describes the primary processing of meat, including antemortem and postmortem slaughter, carcass evaluation, religious status, and so on. Part V discusses the principles and applications in the secondary processing of meat, for example, breading, curing, and fermentation. Part VI focuses on the manufacture of some processed meat products that include sausages, ham, and others. Part VII is devoted entirely to the safety of meat products and meat workers in the United States.

Of the new chapters, half is distributed among Parts I through VI. The remaining is located in Part VII. Apart from new developments in science and technology, the materials in Part VII should serve as excellent frames of reference for countries exporting meat to the United States. This book is unique in several aspects: it is an updated comprehensive source, it contains topics not covered in similar books, and its editors and contributors include experts from government, industry, and academia worldwide.

The editorial team is made up of eight associate editors from six countries. Each has unique contributions in the planning and execution of this enormous project, as illustrated by each person's professional status:

- Dr. Aalhus is a meat scientist with the Lacombe Research Centre, Alberta, Canada. She is an expert on meat tenderness, as documented by her numerous professional publications.
- Dr. Cocolin is the editor-in-chief of *International Journal of Food Microbiology*, with expertise in food fermentation. He is with the University of Turin, Italy.
- Dr. Guerrero, an established scientist in meat and poultry, is also a chemical engineer. She is a distinguished professor at the Metropolitan University Iztapalapa Campus, Mexico City. She is the editor of the two-volume texts *Handbook of Poultry Science and Technology*, Wiley, 2010.
- Dr. Nollet teaches at the Katholieke Universiteit Leuven, Belgium. He is the editor-in-chief of two books on meat quality and analysis. His research interest is reflected by the more than 20 professional reference texts on food and water analyses that he has edited.
- Dr. Purchas of Massey University, New Zealand, is the only academician in the team with extensive research activities in both animal science and meat science, with special interest in the characteristics of meat quality.
- Ms. Stanfield is a consultant to the health industry and has authored five books in allied health and many chapters in professional books in food science, technology, and engineering.

- Dr. Xiong of the University of Kentucky is a meat scientist. He is an elected IFT fellow and an associate editor for several food science/meat science journals.
- Dr. Schilling of Mississippi State University has expertise in meat science, sensory science, and food chemistry, as documented by his extensive professional publications. He is the recipient of the Achievement Award by the American Meat Science Association.

This book is a result of the combined efforts of nearly 100 highly qualified individuals from 16 countries: Belgium, Canada, China, Egypt, France, Germany, Hungary, Italy, Mauritius, Mexico, New Zealand, Singapore, Spain, Taiwan, Turkey, and the United States. Each contributor or editor was responsible for researching and reviewing subjects of immense depth, breadth, and complexity. Care and attention were paramount to ensure technical accuracy for each topic. This volume is unique as stated earlier, and it is our sincere hope and belief that it will serve as an essential reference on meat and meat products.

We wish to thank all the contributors for sharing their expertise throughout our journey. We also thank the reviewers for giving their valuable comments, leading to improvements in the content of each chapter.

The production of a volume of this size could not have been accomplished without the hard work and excellent suggestions of professionals in the production team assigned by CRC Press to manage the project. You are the best judge of the quality of their work.

Y. H. Hui

Editor

Y. H. Hui received his PhD from the University of California at Berkeley. He is currently a senior scientist with the consulting firm of Science Technology System at West Sacramento, California. He has authored, coauthored, edited, and coedited more than 35 books in food science, food technology, food engineering, and food laws, including *Handbook of Food Product Manufacturing* (2 volumes, Wiley, 2007), and *Handbook of Food Science, Technology, and Engineering* (4 volumes, CRC Press, 2006). He is currently a consultant for the food industries.

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Leo M. L. Nollet received his MS (1973) and PhD (1978) in biology from the Katholieke Universiteit Leuven, Belgium. Dr. Nollet is the editor and associate editor of numerous books. He edited for Marcel

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Part I

Meat Industries

1

Meat Industries: Characteristics and Manufacturing Processes[*]

Y. H. Hui

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1.1 Introduction

This chapter will give you an overview of the processes used and the products produced by the industries that we regulate. Each plant is unique, and the production processes used by plants have their own

[*] The information in this chapter has been modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. Used with permission.

details. There are a wide variety of products produced and a number of different activities conducted by these establishments. The industry as a whole is dynamic, in that over time, production of products that are not favored by consumers are decreased or discontinued, and new products are created to meet consumer needs.

In the United States, the Food Safety Inspection Service (FSIS), a unit of the Department of Agriculture (USDA), is responsible for the safety of meat and meat produces. In accordance with the following, please note that, for this chapter:

1. The text and tables have been collated from multiple USDA public documents (legal, regulatory, and training), distributed in USDA'S website, http://www.FSIS.USDA.gov.
2. The figures were originally adapted from a series of USDA continuing educational documents divided into 3 categories:
   a. *USDA HACCP Generic Models on Raw Ground and Not Ground Meat Products.*
   b. *USDA HACCP Generic Models on Shelf-Stable Processed Meat Products.*
   c. *USDA HACCP Generic Models on Not Shelf-Stable Processed Meat Products.*

They are available at USDA's website, www.FSIS.USDA.gov. It must be emphasized that, in this chapter:

1. All information has been designed by the USDA for training purposes only and is not meant to detail the actual manufacturing processes.
2. All abbreviations are standard ones used in law, science, and technology as issued by FSIS.
3. All terms with legal implications apply only in the United States.

We have organized these materials by what the FSIS calls *processing categories.* The nine different processing categories that we will cover include the following:

- Slaughter
- Raw product—not ground
- Raw product—ground
- Heat treated but not fully cooked—not shelf stable
- Fully cooked—not shelf stable
- Product with secondary inhibitors—not shelf stable
- Not heat treated—shelf stable
- Heat treated—shelf stable
- Thermally processed—commercially sterile

There are some processes and technologies that are not specifically addressed by a processing category in the Hazard Analysis and Critical Control Points (HACCP) regulations as listed below:

- Mechanically Separated and Advanced Meat Recovery
- Irradiation
- Egg Products

The Agency developed and distributed generic HACCP models that addressed most of these processing categories. We will be referring to some examples given in these models as we discuss the processing categories.

Every product produced by an establishment (when the hazard analysis reveals any food safety hazard that is likely to occur) must be produced according to a written *HACCP* plan. Many different products

may be grouped within a single processing category, as long as the food safety hazards, critical control points, and critical limits are essentially the same.

In this chapter, we will discuss both quality and safety issues. Both of these issues are important to both the agency and the industry. There are many quality issues, sometimes referred to as *nonfood safety consumer protection*, which would render the product adulterated. Some examples are products with low net weights or with water added above the allowed limits. Safety or *public health protection* issues are given an extremely high priority because of the potential to cause food-borne disease outbreaks. The most common hazard to public health is the presence of harmful bacteria. Throughout this module we will point out processes where quality or safety issues are important.

1.1.1 Definition of Terms

Before we go further, let us define a few terms:

- *Amenable*—accountable or liable to an order or regulation.
- *Product*—any carcass, meat, meat by-product, or meat food product, poultry, or poultry food product capable of use as human food.
- *Process*—is a procedure consisting of any number of separate, distinct, and ordered operations that are directly under the control of the establishment employed in the manufacture of a specific product, or a group of two or more products.
- *Process flow diagram*—process flow diagrams provide a simple description of the steps involved in the process. The flow diagram covers all steps in the process which are directly under the control of an establishment. It may also include steps in the food chain which are before and after the processing occurs.

We will discuss each of the nine categories of meat processing and meat products. However, one or two of the illustrations will be reused in another chapter discussing a specific category of meat product under the section on meat safety. This approach facilitates reading in those chapters, avoiding frequent referral to a specific illustration in this chapter.

1.2 Slaughter

Slaughter is the process whereby healthy, live animals are humanely stunned, bled, dehided, dehaired and/or defeathered, and eviscerated. The resulting carcass may be split and/or fabricated in some fashion. During the process, inedible waste and products (e.g., products not used for human food such as the hides) are produced. Edible by-products (e.g., livers and gizzards) are also produced. The establishment must keep inedible materials separate from edible ones. We will cover by-products when discussing another processing category.

Slaughter includes all amenable red meat species, and all poultry classes. Some examples are beef and pork carcasses, and ready-to-cook whole chickens and turkeys. Some of the products, such as whole poultry, will be distributed for sale following the slaughter process. However, most products go for further processing.

1.2.1 Beef Slaughter Process

Look at the process flow diagram (Figure 1.1) for the beef slaughter process. They are only *examples* of the slaughter process. These processes will vary from plant to plant. The examples we use are more typical of large plants. Large plants (large volume/many employees) are typically highly mechanized and may process thousands of carcasses daily. Smaller plants follow many of the same steps, but with fewer employees and less automatic equipment.

Beef slaughter covers all market classes of cattle. Class is determined based on maturity and sex of the animal at the time of slaughter. The *classes* of beef carcasses are calves, steers, bulls, heifers, and cows.

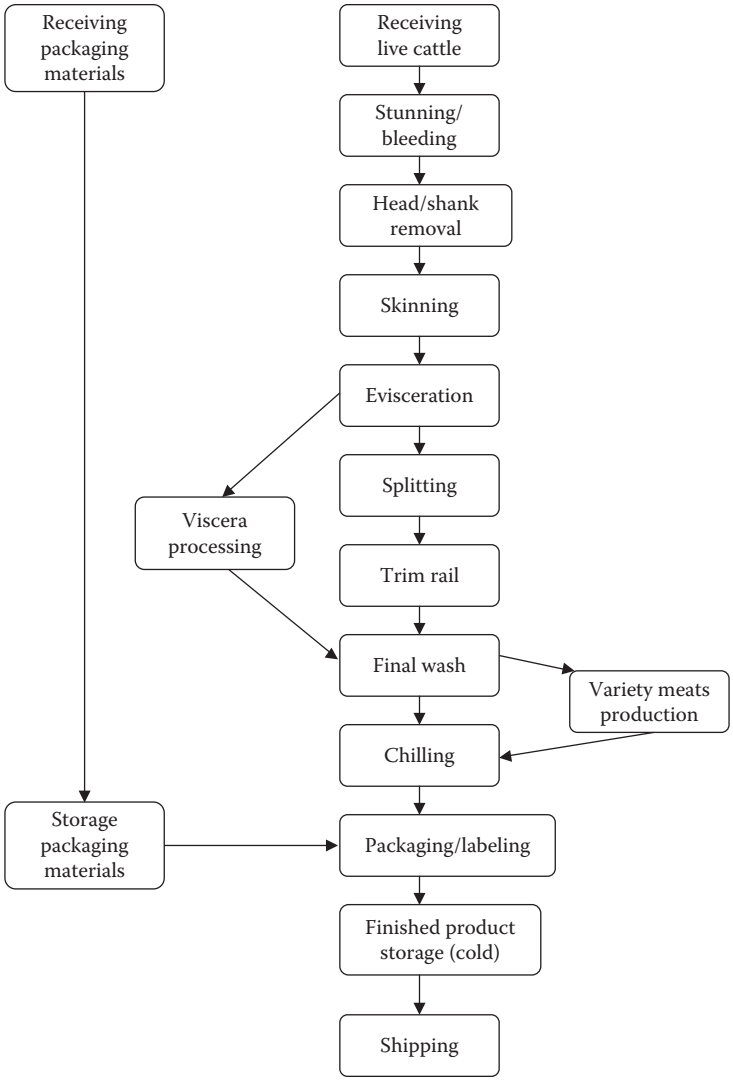

FIGURE 1.1 Flow chart for beef carcass slaughter. (Modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. Used with permission.)

There are a number of quality issues that are of concern to the producer, such as marbling, color, and texture of the meat, that do not affect the slaughter process.

Cattle are received, unloaded from trucks, and held in pens. Prior to slaughter, packers will usually require that animals be kept off feed to facilitate the dressing procedures. The amount of time animals are kept off feed will vary by establishment. The animals must have access to water in all holding pens. This is a requirement of the Humane Slaughter Act.

A thorough inspection of the live animals before slaughter is called *ante mortem inspection*. The inspection is to identify any disease conditions in the cattle. Some disease conditions are unacceptable because they may affect human health. Others are unacceptable from an aesthetic standpoint.

Stunning is the first step in the slaughter procedure. This must be done in a way that complies with the Humane Slaughter Act. Most establishments use a mechanical method, such as a captive bolt, to render each animal completely unconscious with a minimum of excitement and discomfort. Because of the positive finding of Bovine Spongiform Encephalopathy (BSE) in the United States, the method of air injection stunning is prohibited.

Sticking is the second step in the process. A sharp blade is inserted into the neck, severing the carotid arteries and jugular vein, resulting in exsanguination and death. Typically, this is done while the animal is hanging head down from the rail. These overhead rails or tracks move at a controlled speed so that the carcass advances through the various slaughter processing steps.

The next step is *removing the hide*. This may be achieved through various methods, either using mechanical equipment or by hand (at small operations). After the head and hide are removed, many establishments use *antimicrobial interventions*. Antimicrobial interventions include, but are not limited to, hot and/or ambient temperature water wash, organic acid wash, and steam vacuuming. The steam vacuuming system is used to remove contamination from the dehided carcass either before or after evisceration. Additionally, some of these interventions are used as a multihurdle methodology. These interventions can be done before or after evisceration of the carcass for the removal of visible contamination.

Bunging, when the rectum (bung) is secured to prevent contaminating the carcass with fecal material, happens prior to evisceration.

Evisceration is done to separate the internal organs from the carcass. Even in highly mechanized plants, this is still done by hand. It is important that evisceration is done properly so as not to *contaminate* the carcass with the contents of organs such as the stomach or intestines. Fecal material or stomach contents *(ingesta)* contain many bacteria, and may possibly harbor certain harmful bacteria *(pathogens)* such as *E. coli* O157:H7, *Salmonella*, *Campylobacter jejuni*, and so on.

At this point the carcass receives *post mortem inspection*. Similar to ante mortem inspection, the carcass is examined for disease conditions that cause the carcass or parts to be unacceptable for human consumption.

Next, the carcass is *split* with a saw. At the trim rail, an inspection reveals whether the carcass is free from contamination or quality concerns that can be removed by *trimming*.

In the next step, the carcasses are weighed, *marked* with an official United States Department of Agriculture (USDA)-inspected and passed brand, and washed. They are then moved to a chill box or cooler and *chilled* to a specified temperature. The chilling step helps inhibit the growth of spoilage and harmful bacteria. There are various methods and equipment used for chilling the carcass.

Carcasses are typically *stored* in large refrigerated warehouses called coolers until they are shipped. It is important that an appropriate temperature, humidity, and air flow be maintained in coolers. Generally, the colder the temperature in the cooler, the slower the bacteria grow. Proper temperature is essential for preserving the quality and maintaining the safety of the product.

From this point, the carcass halves are ready to be *fabricated*, which means cut into parts. This *further processing* may happen at the slaughter facility in a processing or fabrication department, or the carcasses may be shipped to another plant.

Edible and inedible by-products will be covered when we discuss the Raw Product—Not Ground processing category. Some establishments may include edible and inedible by-products in their Slaughter HACCP plan.

1.2.2 Pork Slaughter Process

Look at the process flow diagram for pork slaughter (Figure 1.2). We will discuss the process for the skin-on pork carcass, which is most common. There are other pork slaughter processes, such as skinned, or hot boned.

Hog slaughter is somewhat similar to beef slaughter, but there are some major differences; we will highlight only the differences. Hogs are usually stunned with an electrical shock or using CO_2 that renders them unconscious and insensitive to pain. Sometimes hogs will be hanging during the sticking and bleeding step. However, sticking and bleeding can also be done while the hog is lying on its side or being held on its back. Instead of removing the hide, usually hogs are scalded and *dehaired*. Then, they are quickly singed with a flame to remove any remaining hairs.

The step called "*gambrelling*" refers to slicing the tendons on the back of the hock so that the carcass can be hung on a gambrel or special type of hook.

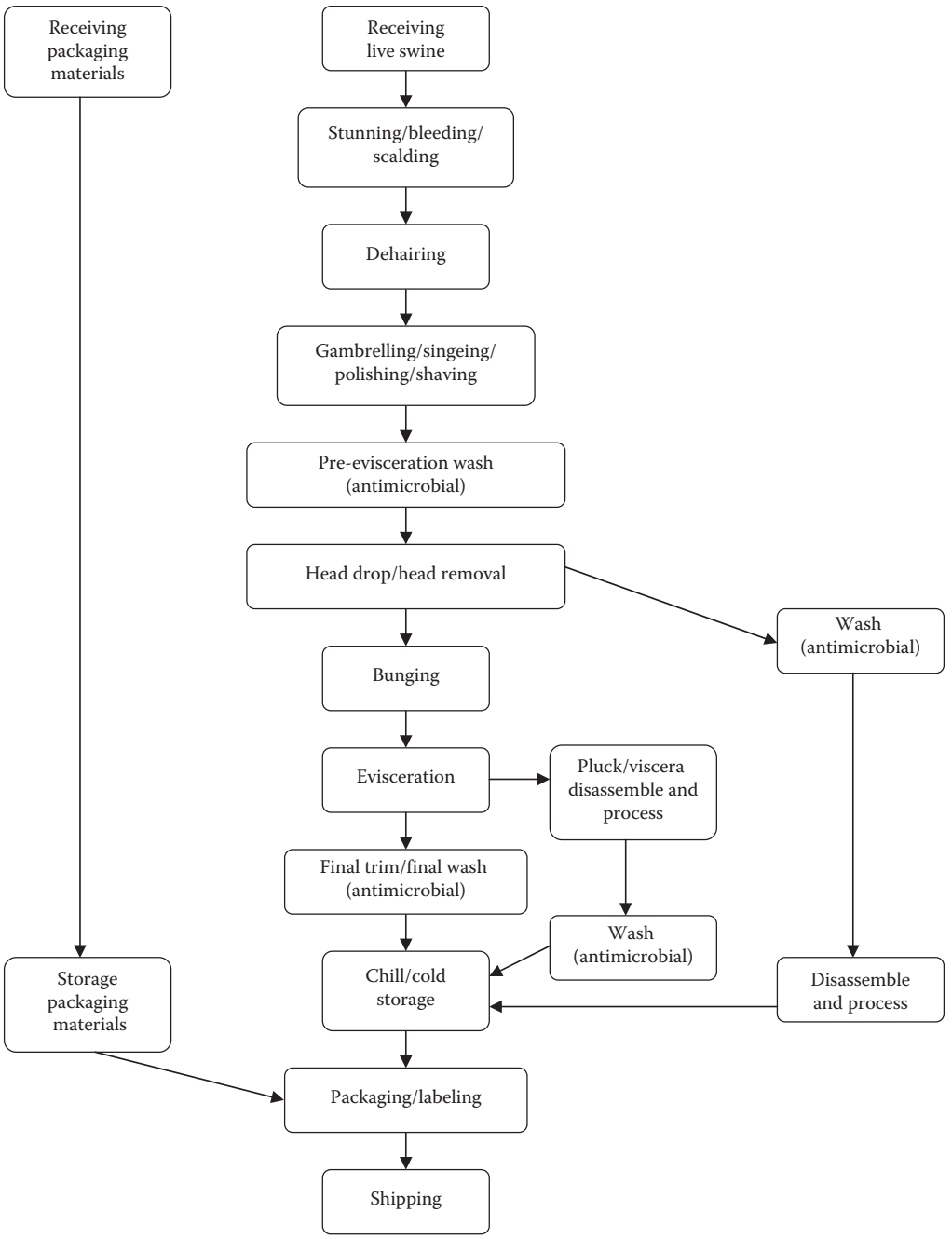

FIGURE 1.2 Flow chart for pork carcass slaughter. (Modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. Used with permission.)

1.3 Raw Product: Not Ground

This processing category includes all raw products which are not ground in their final form. Some examples are beef trimmings, tenderized cuts (e.g., injected or marinated), steaks, roasts, chops, poultry parts, fabricated products, and edible by-products (e.g., livers, gizzards).

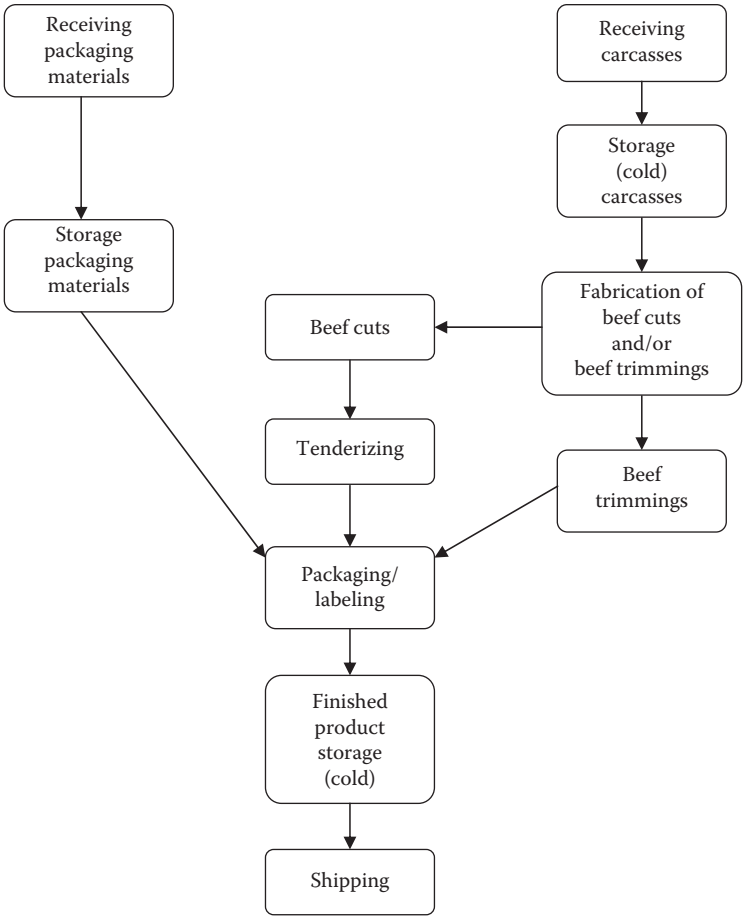

FIGURE 1.3 Flow chart for beef trimmings and tenderized beef cuts. (Modified from *Meat and Poultry Safety* Manual published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. Used with permission.)

The process flow diagram (Figure 1.3) for this category uses the example products beef trimmings and beef tenderized cuts. Notice that the first step is *carcass receiving.* Carcasses are chilled after slaughter for a specified period allowing them to become firm to facilitate a neat job of cutting.

1.3.1 Fabrication

Fabrication refers to creating the various cuts from the carcass to produce particular types of product. *Primal* or *wholesale* cuts are made first (Figure 1.4). Their names usually identify where the meat comes from on the animal, such as the loin, the shoulder, and so on. The plant typically uses large mechanized saws to fabricate the carcass into primal cuts.

As denoted in Figure 1.4, *retail cuts* describe what part of the primal cut the meat comes from, for example, rib roast or round steak. Retail cuts may be made with a saw, especially if they include bone. Often, primal parts are boned before cutting into retail cuts, in order to produce boneless items. Establishments that produce portion controlled retail cuts for hotels, restaurants, and institutions are often called *HRI* (Hotel, Restaurant, and Institution) operations.

Packaging materials (such as wax-treated paper or plastic film) protect the product from damage during refrigerated or frozen storage.

The final step is distribution, either to other departments in the same plant, other plants, or to retail markets.

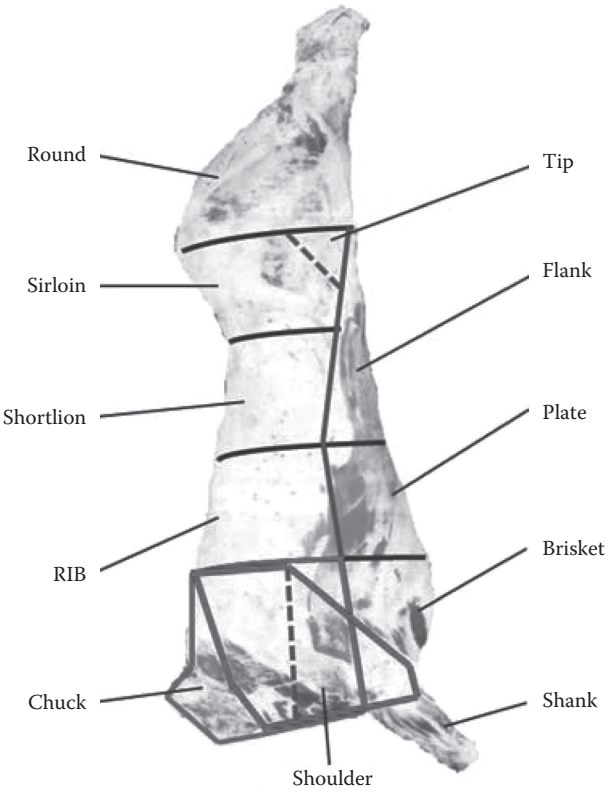

FIGURE 1.4 Beef retail cuts. (Modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. Used with permission.)

1.3.2 Tenderization

Tenderization is another procedure used in some plants. All cuts can be tenderized, but this is typically used on cuts from lower quality grades and less tender cuts of higher graded carcasses. There are several methods for tenderizing meat. They include aging (natural chemical process), the use of enzyme solutions (artificial chemical process), and use of mechanical tenderizers. Mechanical tenderizers typically press many thin blades through the meat pieces, cutting the muscle fibers.

1.3.3 By-Products

The processing category of Raw Product—Not Ground includes edible by-products. Consumer demand has had an effect on the production levels of various by-products. For example, vegetable oils have replaced animal fats like lard and tallow in the frying industry. Technological developments have also had an impact on the demand for inedible by-products. Synthetic materials have been developed to make many items that were once made of animal products.

Edible by-products: Some of the edible by-products include tongues, brains, sweetbreads, hearts, livers, and kidneys. These are called variety meats. Because variety meats are more perishable than carcass meat, they must be chilled quickly after slaughter and processed or moved quickly into retail trade. They may be sold as fresh or frozen items, or used to make other processed foods. There are exceptions to the use of edible by-products derived from cattle 30 months of age and older, including brain, skull, eyes, trigeminal ganglia, spinal cord tissue, and dorsal root ganglia. As of January 12, 2004, new regulations and policies prohibited the use of the aforementioned materials for human consumption as a result of a positive case of BSE.

Some descriptions of the sources and uses of various edible by-products are as follows:

- *Casings* for sausages are sometimes made from sheep or hog intestines. (Note: the distal ileum of beef small intestines are prohibited regardless of age.)
- *Chitterlings* are made from thoroughly cleaned and cooked intestines of pigs, and consumed as a variety meat.
- *Blood* is used as an ingredient of certain specialty products.
- *Tripe* is obtained from the first (rumen) and second (reticulum) stomach compartments of cattle. It is consumed as a variety meat and used in specialty products.
- *Sweetbreads* are thymus glands obtained from the ventral side of the neck and inside the chest cavity of young cattle. They are used fresh or frozen.

Inedible by-products: The uses for inedible by-products are constantly changing based on the available technology and consumer interests. Next are some examples of the ways in which inedible by-products are used:

- *Hides*, *skins*, and pelts are used to make leather goods and glue.
- *Fats* are used to produce industrial oils, lubricants, soap, glycerin, and other cosmetic ingredients. Most inedible fats are processed by dry rendering.
- *Bones* are used to produce animal feed (except ruminant bones to feed ruminants) and fertilizer.
- Some *glands* are used to produce pharmaceuticals. For example, bovine ovaries yield estrogen. The pancreas glands yield insulin, which is used to treat diabetes.
- *Lungs* are used to produce pet foods.

These lists are by no means complete but give a few examples of the uses of edible and inedible by-products.

1.4 Mechanically Separated Product

Often, the industry searches for ways to yield the maximum edible, wholesome product from the meat or poultry carcass. The mechanical separation process is a technology that industry uses to obtain more usable product from bones from which the muscle has been removed. Often, you will see these products referred to as *"mechanically separated (species) or MS (species)."* Any species can be used: veal, lamb, pork, chicken, or turkey. (Note: use of beef in MS product is prohibited.)

The process begins with bones (Figure 1.5). Bones for this process have usually already had most of the muscle tissues removed by hand boning, or they are bones, like neck bones, which are difficult to process. The bones are ground up, and the resulting mass is forced through a sieve. The softer muscle particles are thus separated from the hard bone particles, which remain behind the sieve. The resulting product has a paste-like consistency.

Great pressure is used to force the product through the sieve, and this results in a temperature rise in the product. Therefore, product must be processed quickly and the temperature immediately reduced, in order to prevent oxidation and microbial degradation of the product. Even with these precautions, this product will deteriorate quickly.

Although the MS product has many of the characteristics of meat and may be used as a meat ingredient in the formulation of quality meat food products, it is not meat, as defined in the regulations. In particular, the consistency of MS livestock product and its mineral content are different from those of meat. The bone marrow, spinal cord, and a certain amount of fine bone particles are included in the finished product. This actually provides a readily absorbed source of valuable nutrients like calcium and iron. However, there are specific limits on the quantity and size of the bone particles included in the final product. There are also limits on how much of the MS product can be used in meat or poultry products, and it must be identified in the ingredients statement of the label. As per regulations MS beef is prohibited for use as human food.

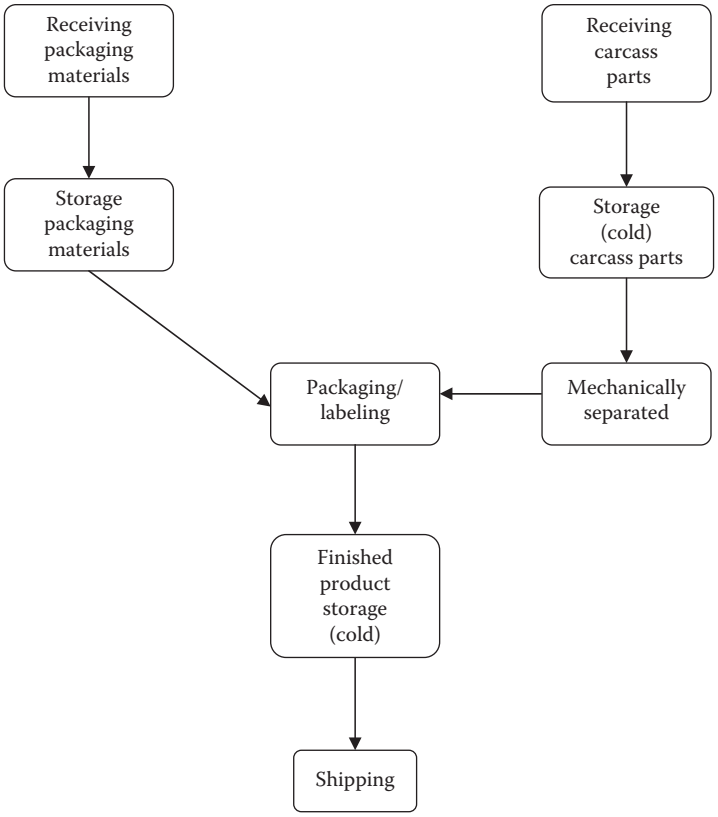

FIGURE 1.5 Flow chart for mechanically separated pork. (Modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. Used with permission.)

A similar technology used by industry is called *advanced meat recovery (AMR).* This process obtains the meat tissues from the bones without incorporating significant amounts of bone and bone products into the final product. The resulting product consists of distinct particles of meat, with the typical color and texture of the species used. There are no special limits on the use of this product. Nevertheless, the presence of central nervous system (CNS)-type tissue is evident in the product derived from AMR systems. Therefore, AMR-derived products from cattle that contain CNS tissue cannot be used as an ingredient of a meat food product. More specifically, Regulation 9 CFR 318.24 prohibits the use of skulls or vertebral columns (with exceptions) of cattle 30 months of age and older from use in AMR systems.

1.5 Raw Product: Ground

This processing category includes all raw products that are ground. Some of the common products are ground beef, hamburger, ground beef patties, ground pork, fresh sausage, Italian sausage, and ground poultry products. Beef, pork, veal, lamb, chicken, and turkey can all be ground and sold or used in other products. One of the favorite products served in this country is the hamburger patty, which is the example we will use in this section (Figure 1.6). However, the processing steps that are used to produce hamburger patties are also used for other products. Establishments differ in how they design their production processes, and you may see many variations of the basic processes that we illustrate.

Meat for use in ground products may come into the establishment from outside suppliers, or it may be produced within the establishment during fabrication and boning operations. Nonmeat ingredients and

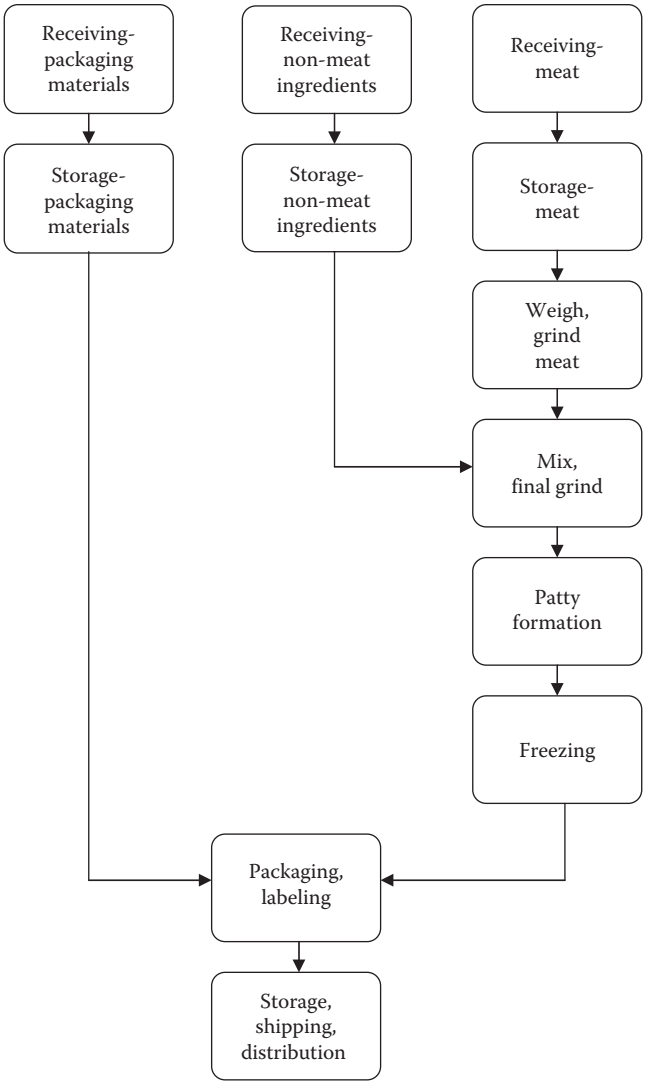

FIGURE 1.6 Flow chart for ground beef patties. (Modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. Used with permission.)

packaging materials will come from outside suppliers. Many establishments use a combination of suppliers, depending on the cost and type of product available from each.

Written purchase specifications are developed by some establishments to ensure that a consistent product is received. Specifications are formal agreements between the supplier and the purchaser, and may include quality aspects, such as portions of lean and fat, and safety factors such as laboratory testing for pathogens.

After meat ingredients are received, they are stored in freezers or coolers until use. Meat products must be maintained at refrigeration temperatures adequate to prevent spoilage and growth of pathogens. *Refrigeration* achieves several purposes: it slows the growth of microorganisms, including spoilage bacteria and pathogens; slows the metabolic and enzymatic activities within the meat tissues that would lead to product deterioration; and also reduces moisture loss from the product.

Chiller or cooler temperatures in the range of 38–45°F will substantially retard most pathogen growth. However, certain types of bacteria, like *Yersinia* and *Listeria*, can grow at these temperatures and may

be a significant hazard. Chiller storage is temporary because even at these temperatures, the spoilage organisms will continue to grow, although at a very slow rate. Freezers, generally maintained at –100°F or below, halt the growth of all bacteria. Products kept at these temperatures will maintain safety and quality for longer periods of time.

Dry ingredients and packaging materials are also received and stored prior to use.

Ground products are often made from *trimmings*. Trimmings are the pieces that are removed from carcasses while producing higher quality retail cuts of meat. Grinding is a way that establishments can use lower quality products that would not be saleable to a retail consumer. In addition to trimmings, ground beef is also commonly made from flanks, short plates, shank meat, briskets, chucks, rounds, or sirloins. Meat ingredients used may be fresh or frozen, or a combination.

Often products contain *nonmeat ingredients*. Ground products are often seasoned with salt, sugar, spices, or other flavorings. Depending on the product being made, water may be added, and some product formulations include binders and extenders such as soy flour or nonfat dry milk.

Establishments use a specified recipe, called a *formulation*, to create a consistent product batch after batch. The formula lists the weights or percentages of ingredients to be used. Meats and other ingredients are weighed before use to ensure that the proper amount of each is added to the batch.

Comminution is the process of reducing the particle size of meats. Several different machines are used, including the grinder, the bowl chopper, and the flaker. Some producers use a combination of several of these in the production of a product.

The *grinder* consists of a hopper into which the meat chunks are placed. The meat then moves along an auger or screw, through a cylinder, at the end of which is a grinding plate and a knife. As the meat is pressed up against the plate the knife turns and cuts off small bits of the meat. The size of meat particle produced is determined by the size of the holes in the grinding plate.

Another method of reducing particle size is the *bowl chopper.* This machine consists of a metal bowl that revolves and a metal knife that rotates, cutting through the meat pieces in the bowl. The bowl chopper also mixes product as it chops it.

The *flaker* is used on large frozen blocks of meat or meat trimmings. Product is pressed against the knife blades, which shave off pieces of the still-frozen meat, enabling it to be used in formulation without thawing.

Sometimes meat ingredients go through several grinding processes. Often, fat and lean meat ingredients are ground separately and then combined.

After comminuting, products are mixed thoroughly. Often products are transferred to a separate piece of equipment, called a mixer or blender, in order to mix it. The *mixer* consists of a chamber that the ingredients are placed into, and blades or paddles that turn and mix the product, resulting in a uniform distribution of fat and lean particles. Nonmeat ingredients, if used, are added at this stage.

After comminuting and mixing, the ground meat mixture is often *shaped* into different forms. Fresh sausage may be extruded into a casing. Hamburger or ground beef is often shaped into patties using a patty machine. After formation, the patties may be frozen.

Because of the moving metal parts common in these operations, there is a possibility of metal chipping or breaking. Proper maintenance of equipment is essential to reduce this possibility. Some establishments use a *metal detector* to identify product that may be contaminated with metal fragments.

The final step for ground products at the processing establishment is *packaging and labeling.* Product may be packaged into retail size packages, into larger containers for institutional use, or into bulk containers for sale to other establishments for further processing. Although there are many different combinations of packaging materials in use, plastic liners and cardboard boxes are some of the materials commonly used. Labels must accurately reflect the product.

After packaging and labeling, products must be held at proper refrigeration temperature during storage, and throughout distribution to the customer.

Many variations of these steps are used to produce the different products available in the marketplace. Ground products may be marketed in bulk or in patty form. Fresh sausage products are also included in this category, and these products may be sold in bulk or in casings. Poultry products are common today, and ground poultry products are available in bulk, packaged into chubs (short plastic casings), or formulated into a variety of sausage products.

1.6 Irradiation

Food irradiation is a technology that exposes food to radiant energy in order to reduce or eliminate bacteria. Ionizing radiation will reduce, and in some circumstances eliminate, pathogenic microorganisms in or on meat and poultry (Figure 1.7). FSIS recognizes irradiation as an important technology for helping to ensure the safety of meat and poultry. FSIS has included ionizing radiation as an approved additive in pork carcasses and fresh, or previously frozen, cuts of pork that have not been cured or heat processed for the control of *Trichinella spiralis*, which causes trichinosis. Ionizing irradiation is also recognized as an approved additive in fresh or frozen, uncooked, packaged meat or poultry products for the purpose of reducing pathogenic microorganisms and extending shelf life.

Radiation is broadly defined as energy moving through space in invisible waves. Radiant energy has differing wavelengths and, hence, degrees of power. Forms of radiant energy include: microwave and infrared radiation, which heat food during cooking; visible light or ultraviolet light, which are used to dry food or kill surface microorganisms; and ionizing radiation, which penetrates deeply into food, killing microorganisms without raising the temperature of the food significantly. Food is most often irradiated commercially to reduce the numbers of pathogenic microorganisms, to extend shelf life, or to prevent reproduction of insects. Food irradiation for these purposes is practiced in many countries, including the United States.

Treating product with irradiation could result in significant *reduction or even the elimination of pathogens*. Ionizing radiation has been shown to be effective at eliminating *Salmonella*, *E. coli* O157:H7, *Clostridium perfringens*, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Campylobacter jejuni*, among others. Irradiation also can significantly extend the shelf life of meat and poultry food products through the reduction of spoilage bacteria.

Irradiation *dose* is measured in kilo Gray (kGy); the maximum dose for use on meat products is 4.5 kGy. The radiation dose necessary to reduce the initial population of bacterial pathogens by 90% (the *D*-value, or 1-$\log_{10}$) range from 0.1 to 1 kGy. Higher radiation doses (above 1 kGy) are needed to accomplish the same antimicrobial effect in a frozen food versus a nonfrozen food of the same type.

Irradiation does not significantly increase the temperature or change the physical, sensory, or nutritional characteristics of foods. Irradiation does not make food radioactive. During irradiation, the energy waves affect unwanted organisms but are not retained in the food. This is similar to the way that food cooked in a microwave oven does not retain those microwaves. Because irradiation does not raise product temperature, product is still raw and requires refrigeration.

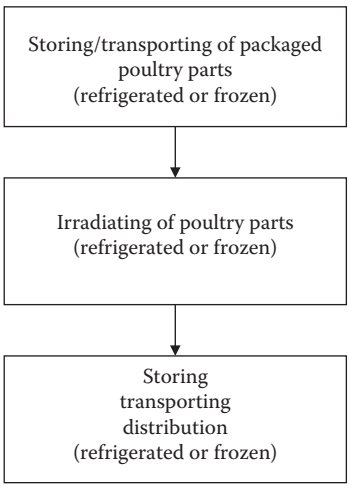

FIGURE 1.7 Flow chart for irradiation of poultry parts. (Modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. Used with permission.)

The irradiation process requires a source of energy. The two types are radioisotopes (radioactive materials such as cobalt or cesium) or machines that produce high-energy beams. Specially constructed facilities are used to confine the beams so that the personnel will not be exposed.

The Food and Drug Administration (FDA) regulates all aspects of irradiation: what products it can be used on, allowable dose, and how those products are labeled. The USDA is responsible for the inspection and monitoring of irradiated meat and poultry products and for the enforcement of FDA regulations concerning those products.

The "*radura*" is an internationally recognized symbol identifying irradiated food. The FDA requires that both logo and a statement ("Treated with irradiation" or "Treated by irradiation") must appear prominently on the label of packaged foods, and on bulk containers of unpackaged foods.

Irradiation can be used within an *HACCP system*. Establishments that irradiate product probably would establish critical limits such as radiation dosage. By ensuring that specific limits for these parameters are met, establishments could be reasonably sure that a predetermined reduction in pathogens has been achieved within the irradiated product.

1.7 Heat Treated but Not Fully Cooked: Not Shelf Stable

Up to now, all of the processing categories that we discussed dealt with raw product—product that had not been heat treated. This section covers a group of products that receive a heat treatment, but they are *not fully cooked*. These products still need to be thoroughly cooked in order to be safely consumed.

The products included in this category vary quite a bit from each other. What they have in common is that *they have received some type of heat treatment, but not sufficient heat treatment to result in a ready-to-eat (RTE) product*. One well-known product is bacon, a cured and smoked pork product. Another product is cold smoked sausage, a product that has been smoked to add flavor, but is still raw. Partially cooked battered and breaded poultry is included in this category; it has been cooked only enough to "set" the breading. Char-marked patties are similar; they have been cooked only enough to add distinctive char marks on the meat surface, but are still essentially raw. Low temperature rendered products are heat treated to melt and remove some of the fat in the meat tissues, but again, they are not fully cooked. As you can tell, there are many different types of products grouped into this category.

We are going to discuss the processes involved, focusing on some of the most common products. There will be many variations of these processes used by establishments, and this module will only provide an introduction to the procedures used.

1.7.1 Bacon

Bacon is an example of a product that is cured and smoked. Let us study the process flow chart (Figure 1.8) for this product. First, the raw meat ingredients are *received*, either from another establishment, or from the fabrication department within a large establishment. In this case, the raw meat ingredient used is the pork belly.

The nonmeat ingredients are weighed and combined. Bacon is a *cured* product, which means that *additives* are used to preserve the product and stabilize the color. Following are some of the most common additives:

- *Salt* is used for flavor and because it preserves the product by inhibiting bacterial growth.
- *Sugar* is sometimes used as a sweetener. It can counteract the harsh flavor of the high levels of salt used in some products.

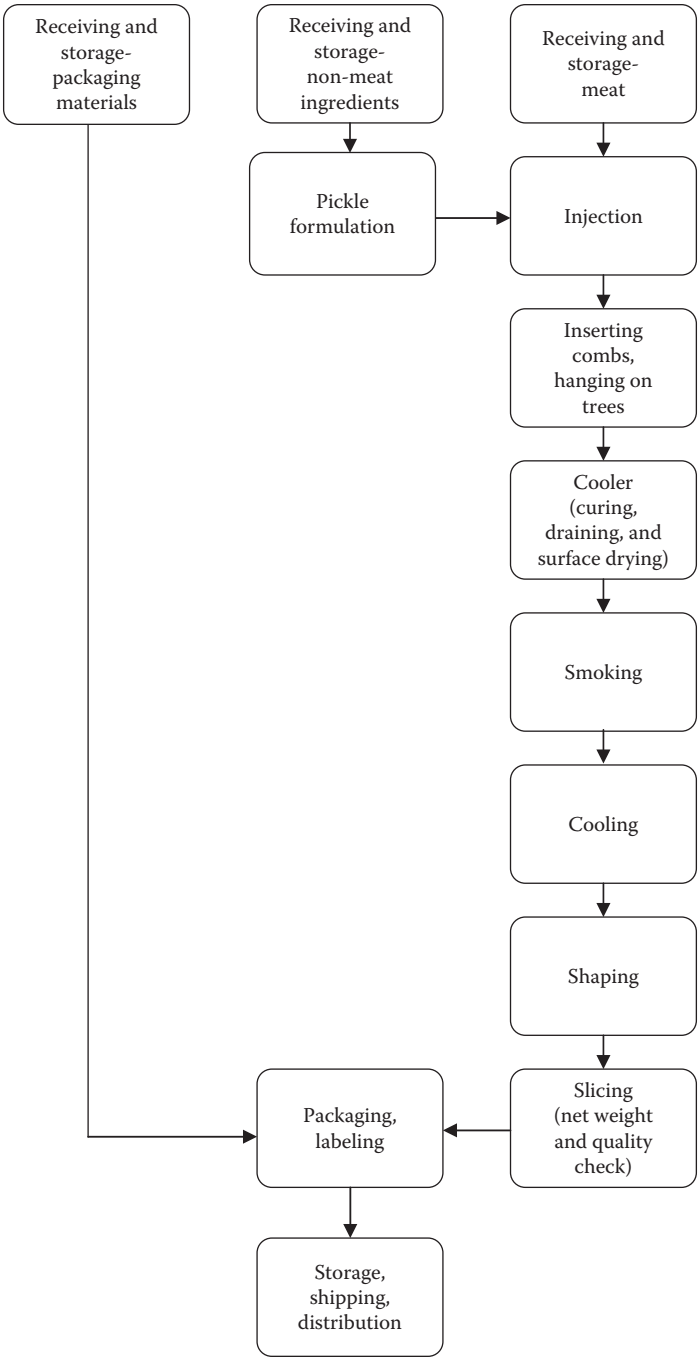

FIGURE 1.8 Flow chart for bacon. (Modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. Used with permission.)

- *Nitrite (or less commonly used nitrates)*—stabilizes the color of the meat, contributes to the characteristic flavor of cured meat, inhibits the growth of both pathogens and spoilage microorganisms, and retards rancidity (deterioration of the fat).

The amounts of nitrite and nitrate allowed are restricted by FSIS regulations. Additives that have regulatory limits are known as *restricted ingredients*. Because nitrates are reduced to nitrites and further

converted into nitric oxide which react with the amines present in muscle fibers to form *nitrosamines* (are known to cause cancer), the nitrite and nitrate levels must be closely monitored. Also, as nitrites can be very toxic to humans, the use of these ingredients is carefully controlled. Supplies of nitrites and mixtures containing them are kept securely under the care of a responsible employee of the establishment. Often, the nitrite is purchased premixed with salt and colored pink to prevent its accidental misuse.

Nitrite is most important because of its role in developing the cured meat color. Nitric oxide (the chemical reaction product of nitrite) reacts with *myoglobin*, a complex protein present in meat. Myoglobin is the pigment that is responsible for the red color of muscle tissue. A series of chemical reactions results in the formation of the stable pink color of cured meat.

These are just some of the most common curing ingredients. Many other ingredients are used by the industry, and will contribute to the variety of formulations that you may encounter.

These cure ingredients are sometimes mixed with water to form what is known as a *pickle*, or a curing solution. The solution is often injected into the meat using an *injector.* This equipment carries the meat past a series of needles that pierce it and force the pickle solution into the interior of the meat pieces. This process is called *pumping*. This results in a fast and even distribution of the pickle. There are many other means of introducing the pickle; sometimes meat pieces are simply placed into a barrel or vat of the pickle. This is a much slower process than injection.

After injection, the meat pieces are hung onto racks called *trees* or *cages*, which hold the meat while it is further processed. The meat hangs in a cooler for a period of time to ensure that the cure ingredients have time to react with the meat, and to allow some of the solution to drain out, if necessary.

The next step is the *smoking* process. The racks of meat are loaded into a smokehouse. The establishment operator carefully controls the smokehouse. Time, temperature, and humidity are parameters that affect the product. These parameters are usually carefully monitored to ensure that the smoking step proceeds as designed by the plant.

One common type of monitoring equipment used is the dry bulb/wet bulb thermometer. This device monitors the temperature inside the smokehouse with two thermometers set right next to each other, one dry, and one inside a moist piece of cloth. The difference between the two temperature readings is used to calculate the humidity of the environment.

The operator sets the smokehouse controls to run through a series of processes in which the addition of steam and smoke will change the conditions inside the smokehouse. Although bacon receives some heat treatment in the smokehouse, it is not fully cooked. The smokehouse treatment is primarily designed to deposit the smoke onto the surface of the meat.

Smoke has several important effects on the meat product:

- It develops the characteristic smoke flavor.
- It results in a color change (browning effect) on the surface of the meat.
- It has some preservative effect.
- It protects the meat from *oxidation*, which is the development of off-flavors.

Smoke is a complex mix of chemical compounds, including phenols, alcohols, organic acids, carbonyls, hydrocarbons, and gases. The phenols and carbonyls produce the color and flavor of smoke. Smoke has a *bactericidal* action; that is, it kills some of the bacteria present. This is due to the combined effects of heating, drying, and depositing the chemical components of the smoke. Smoke is often produced from hardwood sawdust in a *smoke generator.* Liquid smoke is also used.

After the product has been smoked according to the establishment's desired process standards, it must be cooled down to safe product storage temperatures. This is often done initially in a *blast cooler* for maximum cooling effect. This cooler forces cold air at a very high velocity around the bacon pieces, quickly cooling them.

After the product is properly chilled, it may be sold in the bulk form. Most bacon, however, is sold as sliced product. The meat pieces are usually shaped in a press, or *blocked*, in order to produce uniform slices. The product is sliced and packaged. *Net weights* are checked by establishment personnel to ensure that the net weight statement on the label is accurate. Other quality checks are often performed by the establishment on the finished product. The product is now ready for final distribution.

1.7.2 Other Products in this Category

Cold smoked sausage is similar to bacon in that the product is smoked primarily for appearance and flavor. The process is called *"cold-smoked"* because the smoking does not result in high enough temperatures to cook the product. The smoke used is not actually cold; it is usually 90–120°F. The product must be quickly cooled to prevent bacterial growth.

Partially cooked battered, breaded poultry products are another product in this category. The raw poultry pieces are coated with *batter*, a liquid mixture of flour, egg, milk, or water; or with *breading*, a powder or granular mixture of cereal products, like breadcrumbs; or they are both battered and breaded. The pieces are then heat treated to "set" or precook this coating, usually in hot oil. The poultry product inside is still uncooked. The products are cooled, usually in a special *IQF (individually quick frozen)* freezer, and packaged.

Char-marked patties are also included in this category. These products receive a heat treatment on the outside surface that produces a "char-mark" which imitates the marks created from cooking the product on a grill. The product is still essentially uncooked, and it is important that product labeling distinguish this product from RTE products. It is crucial that this product be fully cooked by the final user, to ensure safety.

Low-temperature rendered products are derived from the low-temperature rendering of fresh meat. The products are usually ground, heated, then treated to a process that separates some of the fat from the lean portion. The temperature used must not exceed 120°F. The product is then cooled quickly to limit potential growth of bacteria at these warm temperatures. The heat treatment is not sufficient to eliminate pathogens or to result in a cooked appearance. The rendered product is frozen and used in further processing operations. If the raw meat trimmings had at least 12% lean meat prior to rendering, the resulting product is called *Partially Defatted Chopped* (*species*). If the fatty trimmings used as raw materials contain less that 12% lean meat, the resulting products are called *Partially Defatted (species) Fatty Tissue*.

There are, of course, many other products that you may encounter that would fall into this category. The common characteristic is that these products receive some heat treatment, but not enough to result in a fully cooked, RTE product.

1.8 Fully Cooked: Not Shelf Stable

This processing category includes all food items that have been fully cooked, but are not shelf stable. *Fully cooked* means that these products have been sufficiently cooked so that they are safe to eat as they are, with no further preparation required by the consumer. This is also known as "*ready-to-eat*." Note, however, that many of these products are customarily eaten hot, and cooking instructions may be included on the label. This does not affect the classification of these products into this processing category. An example is the hot dog. This product receives sufficient heat treatment to be fully cooked, and does not necessarily need to be reheated by the customer. Most customers do, however, heat this product, and cooking instructions may be included on the label of the product. Another parameter that defines this category is that the products are not shelf stable. *Shelf stable* means that the product has received a treatment that renders it safe to store without refrigeration. This does not apply to this category. These products, although fully cooked, are not shelf stable, and must be kept refrigerated or frozen in order to maintain safety and quality. Again, the hot dog is a great example. It must be kept refrigerated by the consumer until it is eaten.

There are many different types of products that fall under this category. There are some major groups that we will closely examine, but there will be many other products that you will encounter in the industry. Keep in mind that what they all have in common is that they are fully cooked, but they are not shelf stable. Some examples that we will discuss are the cooked and smoked sausages, cooked deli meats such as ham, roast beef, pastrami, corned beef, cooked chicken roll, and smoked turkey breast. Other meat and poultry products that fall under this classification are salads, such as chicken or ham salad, and frozen entrees.

1.8.1 Cooked and Smoked Sausage

One of the major product groupings that fall under this category is the cooked and smoked sausage. There are many different types of these sausages made; some common examples are bologna, cooked salami, polish sausage, and hot dogs. Let us take a closer look at hot dogs as an example of how these products are produced (Figure 1.9).

The first steps are the same as we have previously covered: meat and/or poultry, other ingredients, and packaging materials are received and stored in the establishment until ready to use. Many establishments carefully control the quality of the incoming ingredients through purchasing specifications. Meat

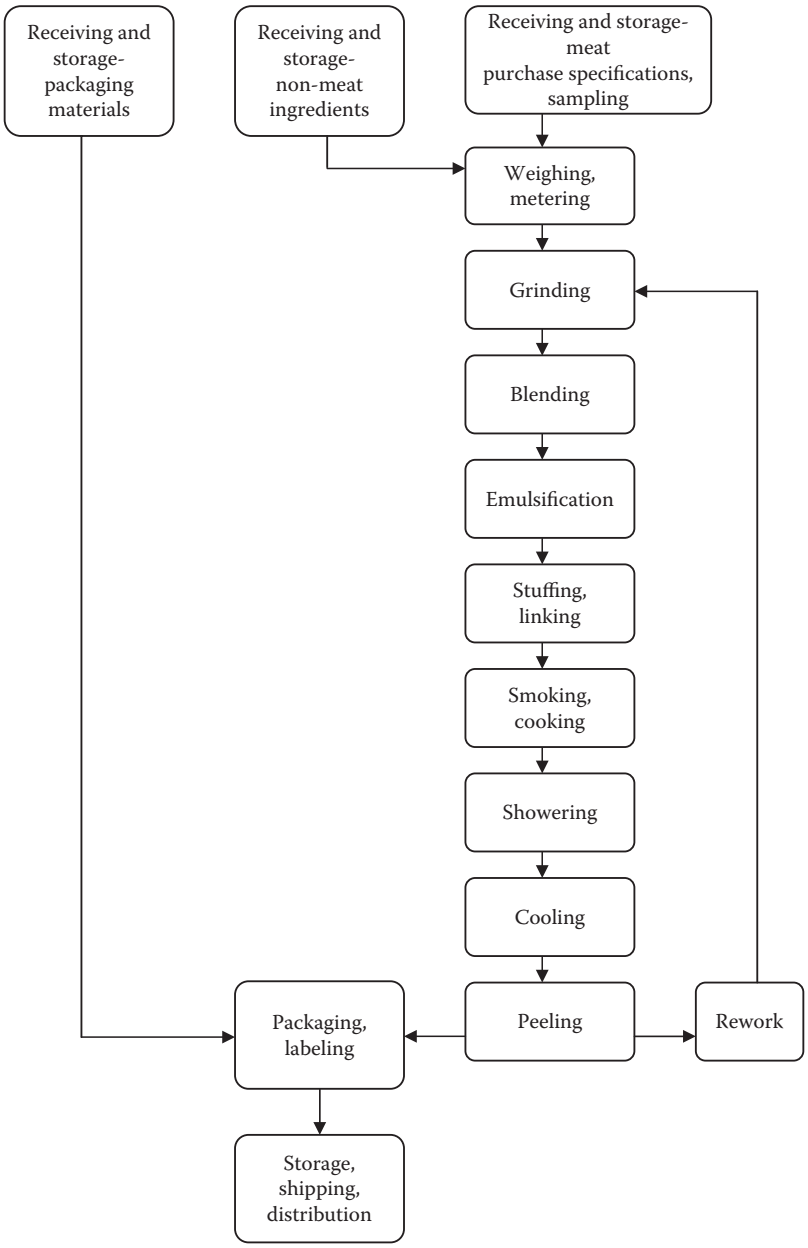

FIGURE 1.9 Flow chart for hot dogs. (Modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. Used with permission.)

ingredients may have quality specifications such as percent fat, moisture, and protein. These are parameters that will affect the final quality of the product.

Raw meat ingredients used in these products will depend on the type of finished product desired. Not long ago, most hot dogs were either a combination of pork and beef, or they were all beef. Today, establishments still make these products, but many more combinations of ingredients are used. Many formulations include at least some *poultry* products, (turkey or chicken), and some products are made exclusively with poultry.

Many larger volume establishments use a system called *least cost formulation*. This is a computerized program that allows the processor to determine the specific allocation of ingredients required for a given product at a minimum cost. The product can be manufactured subject to the ingredients available. These establishments carefully analyze samples of each batch of ingredients and enter the data into the computer program. The program determines how many pounds of each ingredient to use, in combination, to produce the desired product. Theoretically, each finished batch of product will then be identical to the other batch. Of course, the final retail label must have a list of ingredients in the correct *order of predominance*, despite any variations caused by the least cost formulation system.

The first step in the formulation process is weighing or measuring the meat and/or poultry ingredients. They are ground and mixed or blended with the nonmeat ingredients. Often establishments will *preblend*, that is, they will grind and mix the meats with water and salt, and sometimes with the nitrite, and let it stand for a period of time in a cooler.

We have already discussed the most common nonmeat ingredients used in hot dogs: water, salt, curing agents like sodium nitrite, and sugar. Let us take a look at some of the other ingredients that may be used, depending on the formulation.

Binders and extenders, such as dry milk powder, cereal flours, and soy protein, have a number of uses in a sausage formulation. They increase the overall yield, improve binding qualities, and add certain flavor characteristics.

Cure accelerators such as ascorbates and erythorbates are used to speed up the curing process. They also stabilize the color of the final product.

Phosphates are used to improve the water-binding capacity of the meat, and contribute to the flavor and color of the product.

Spices and flavorings are used to add flavor to the sausage. The wide range of available spices, seasonings, and flavorings is a primary reason for the variety available in sausages in the marketplace.

- *Spices* are any aromatic vegetable substance that is intended to function as contributing flavor to food, rather that as a nutritional substance. The active aromatic or pungent properties of spices that contribute the most to the flavoring effect are present in the volatile oils, resins, or oleoresins of the spice. Spices may be used whole or ground. White pepper, paprika, and nutmeg are common spices used to produce the characteristic flavor of the hot dog. Because paprika also adds color and makes meat look brighter red, it must be listed as "paprika" on labels.
- *Flavorings* are substances that are extracted from a food, and contribute flavoring, such as spice extracts.

After the nonmeat ingredients are blended with the ground meats, the mixture is *emulsified*. This is done in an emulsifier, which further reduces the size of the meat particles to achieve a very fine texture. Fat, protein, salt, and water are mixed and combined into a semifluid emulsion. The meat muscle protein, myosin, is *solubilized*, or released from the muscle fibers, by salt. The solubilized protein and water combine and surround the fat globules, and suspend the fat particles within the mixture.

Careful control of the amount of each ingredient is essential to the quality of the final product. The manufacturer must select a mix of raw meat materials with the appropriate binding characteristics. Different meats vary in their ability to bind. Lean beef, for example, bull, cow, and shank meat, has high binding ability. Regular pork or beef trimmings with more fat, and poultry, have medium binding ability. Low binding meats contain high levels of fat, such as jowls and briskets. Organ meats have no binding qualities. The binding capabilities are directly proportional to the myosin (red) in the muscles. Thus, the paler the muscle, the less bind it contributes to the mixture.

Control of the emulsification process is also essential. Product defects result from too much chopping or from an increase in temperature during the process. Over-chopping makes the protein fibers too short. It also creates heat from friction that melts fat. This results in product defects such as pockets of fat in the final product.

After emulsification, the mixture (or "batter") is *stuffed* into casings, usually artificial plastic casings that allow moisture to cook out and smoke flavors to penetrate. Natural casings such as sheep small intestines may also be used.

Following stuffing, the product is *linked* by pinching and twisting the casing to form separate units of sausage. The sausages are still held together by the casing. These lengths of casings are then placed on racks or trees, and are ready to be loaded into the smokehouse. Some establishments load trees into individual smokehouses, however, some large volume establishments use continuous smokehouses.

The smokehouse parameters that must be controlled are temperature, time, and humidity. The product must be exposed to a high enough temperature in order to produce a fully cooked, RTE product. The temperature inside the smokehouse, and the internal temperature of the sausage, may be monitored by the establishment in order to verify that the critical limits are met. Cooking is a very important step, because it is here that any pathogens that may be in the product will be eliminated and the numbers of spoilage bacteria will be lowered to an acceptable level.

After the product has reached the desired final temperature, the cooling process begins. This product is often showered with cold water inside the smokehouse. This removes some of the heat from the product, and immediately halts the cooking process. The shower is usually not sufficient to complete the cooling process. Usually the product is moved to another chiller or cooler to finish cooling. Some establishments use very cold water as a chilling medium, sometimes with salt added to lower the temperature below the normal freezing point of water. This is called a *brine chiller.* Other establishments may use cold air, and some use a combination of methods.

The cooling process is also known as *stabilization.* There are two types of bacterial contamination that must be addressed by the stabilization process:

- Spore-forming bacteria (*Clostridium perfringens* and *Clostridium botulinum*) can survive cooking when in the heat-resistant spore form, and these organisms need to be considered as the products are chilled. Growth (sometimes referred to as *"outgrowth"*) of these bacteria is slowed by rapid cooling. Cooling rates, or time/temperature relationships, must be carefully controlled in order to ensure that product does not remain at warm temperatures that would support the outgrowth.
- Recontamination with bacteria (e.g., *E. coli, Salmonella, L. monocytogenes*) must be considered as cooked products are exposed to the environment, food contact surfaces, or cross-contamination with raw product prior to final packaging. Proper chilling and cold storage temperatures are essential to limit the growth of these bacteria.

After product has been chilled to the desired temperature, it is removed from the artificial casing in a machine called a *peeler.* This equipment quickly runs the sausage through a tunnel that has a tiny blade that slices the casing. Steam or air is then used to blow the casing away from the sausage. The sausage links are now separate. If you closely examine the outside of a hot dog, you might see where the casing had been cut. This blade is a potential source of contamination, since it contacts every hot dog!

Sometimes a product that has partially or fully completed the production cycle is not sellable but is still wholesome, and can be used for food. For example, the casing of some sausages may split during the cooking or smoking cycle. Manufacturers may reuse these edible but unsaleable products by removing the casing and adding the contents to the grinder to include in another run of the same product. This is called *rework*. Since the proteins are coagulated from cooking, rework has no bind capabilities. Of course, the ingredients of the rework must be compatible with the ingredients of the batch to which they are added.

The final steps are packaging, labeling, and storage. The product is ready for distribution to retail stores, restaurants, or institutions.

1.8.2 Deli Meats

Deli meats such as ham, roast beef, and smoked turkey breast all have very similar processes. These products are produced by adding a solution of ingredients to the raw meat ingredient. Cured products, such as ham, turkey ham, and corned beef, have nitrite in the solution. Other products, like roast beef or chicken roll, may have only salt and seasonings used. The solution is often added with an injector, but products may also simply be immersed in the solution.

Traditionally, these products were produced with whole muscle pieces, such as bone-in hams, pieces of beef round, whole briskets, or whole boneless turkey breasts. Today, many products are made with chunks of meats of various sizes, to make chopped and formed products.

Products may be *tumbled* or *massaged*, which increases both yield and tenderness. In this procedure, meat and solution are added to a chamber with baffles. The chamber or the baffles rotate, which subjects the meat pieces to a gentle beating process. This produces muscle fiber disruption, with a corresponding release of salt-soluble protein, which in turn coats the meat pieces. The protein is then coagulated by cooking to form a matrix between the individual pieces, thus giving the product an intact muscle appearance.

The meat pieces are often formed into uniform shapes. This can be done by stuffing them into nets, casings, or molds. The product takes on the shape of the mold when cooked.

The cooking and cooling of these products is similar to the cooked sausage procedure. Some of these products, however, are cooked in a water bath or in a steam chamber.

After chilling, many of the products are packaged as whole roasts, for the retail deli market. Other products are sliced and packaged in retail consumer-sized portions. Most are vacuum packed, which helps to protect the product quality and increases the shelf life.

1.8.3 Salads

Another type of product in the fully cooked—not shelf stable category is the meat salad. Ham and chicken salad are some of the common salads produced. The establishment starts with fully cooked product. The fully cooked meat is chopped or ground, and mixed with other RTE ingredients such as mayonnaise, salt, spices, onions, celery, or pickle relish. The finished salad is packed into containers, and may be distributed fresh or frozen.

These products are rarely reheated; most consumers eat them cold. Therefore, there is no chance that any pathogens present will be eliminated by consumer reheating. Whatever bacteria are in the product when mixed, or contamination of the product during mixing, will remain in the product when eaten. The temperature of this product must be carefully controlled to ensure that bacteria in the mixture do not have a chance to grow.

1.8.4 Fresh or Frozen Entrees

Another group of products that fall within this category are the fresh and frozen entrées. These range from precooked chicken pieces and barbecue beef to prepared dinners with meat or poultry along with rice, pasta, sauce, and vegetables. There are many of these convenience-type items produced today, and new products are introduced almost daily.

The processing procedure for all of these products is very similar. Fully cooked meat or poultry portions are combined with sauces, vegetables, pasta, or other ingredients. Each of the ingredients is individually weighed or portioned, to result in the desired finished proportions.

Most of these products are designed to be reheated by the customer. Most packages will include instructions for reheating. This does offer some degree of safety to the consumer, in that the reheating may eliminate some bacteria if present. However, this reheating must not be depended on, as consumers may vary greatly, how well they reheat the product. These products are intended to be fully cooked and must be safe to eat without the reheating step.

These are some of the major product groups that fall within the Fully Cooked—Not Shelf Stable category. There are other products not mentioned here that you might encounter in the marketplace or being produced in an establishment. These products all have some things in common: they are fully cooked

and RTE by the consumer; and they require refrigeration or freezing in order to maintain product safety and quality.

1.9 Product with Secondary Inhibitors: Not Shelf Stable

Finished products produced under this regulatory processing category can be not-ready-to-eat (NRTE) or ready-to-eat (RTE) meat and poultry products that have been processed in a manner that utilizes strategies which produce results that will inhibit secondarily the growth of pathogenic bacteria. Finished products in this regulatory processing category may or may not have had heat applied to the product. The finished products in this category are *not shelf stable products* and require special handling to maintain their wholesome condition. In other words, the product may be heat treated, but not fully cooked, and a secondary inhibitor gives a cumulative effect (heat plus a food additive that affects the product) so that the product is RTE, yet it would *not* be RTE in the *absence* of the secondary inhibitor. The NRTE products must be kept refrigerated or frozen to maintain product quality and safety. Refrigeration, therefore, is still the primary inhibitor of the growth of pathogens and spoilage bacteria in NRTE products. RTE products need to be kept refrigerated to inhibit growth of spoilage organisms that are still present and capable of growth at ambient, nonrefrigerated temperatures.

Secondary inhibitors are usually ingredients or processes such as fermentation or drying that when used, in combination or alone, assists in inhibiting, or slowing the growth of possibly harmful bacteria. Primary microbial growth inhibitors include lowered water activity (a_w) and higher acidity. Salt or sugar in quantities that effectively lower the water activity of the finished product is an example of a secondary inhibitor. We will discuss each of the inhibitors and then concentrate on one product example—country-style ham.

Water activity (a_w): Microorganisms in food need water in order to live and grow. The water must be in a form that is available to the microorganisms. Water activity is a measurement of how much water is available in a product. The water activity can be reduced by removing water (drying) or by increasing the concentration of solutes dissolved in the water (adding salt or sugar).

Acidity: Most bacteria grow best in a medium that is neutral or slightly acidic, and the growth of most bacteria is significantly inhibited in very acidic foods. The ionic hydrogen concentration (pH) is measured on a scale from 1 to 14, with 7 being neutral; pH levels above 7 are basic or alkaline, while those below 7 are acidic. Foods that are highly acidic are seldom the vehicles for pathogens. Many foods are acidified to prevent the growth of undesirable microbes. This may be done by adding acidic ingredients, like tomatoes, or by adding the acid directly, like vinegar. The acidity of products may also be increased by the process of fermentation.

The process of using secondary inhibitors is a very complex system. Often several different inhibitors are used, each depending on the others in order to result in a safe product. You will learn more about this topic in the Food Safety Regulatory Essentials (FSRE) training.

Some examples of products that may fall into this processing category include products that are uncooked, cured, fermented, dried, salted, or brine treated, which are not shelf stable but can be RTE or NRTE, such as sliced country style ham, salt pork, and semidry fermented sausage. The product standards, processing methods, and labeling are all factors that must be considered in determining the regulatory processing category for the 03I products.

Let us look at the example of the perishable sliced country-style ham (Figure 1.10).

1.9.1 Country-Style Ham or Shoulder

Sliced country-style ham or shoulder is a cured, dried product, traditionally made from a single piece of raw meat from a pork shoulder. The example shown is for an NRTE, cured product (Figure 1.10).

Dried whole muscle products are mostly dry cured. An initial process for manufacturing whole muscle products consists of dry mixing the nonmeat ingredients with the meat. Curing is the addition of salt, saltpeter, nitrites, sugars, spices, and flavorings. Nitrate and nitrite contribute to the characteristic cured

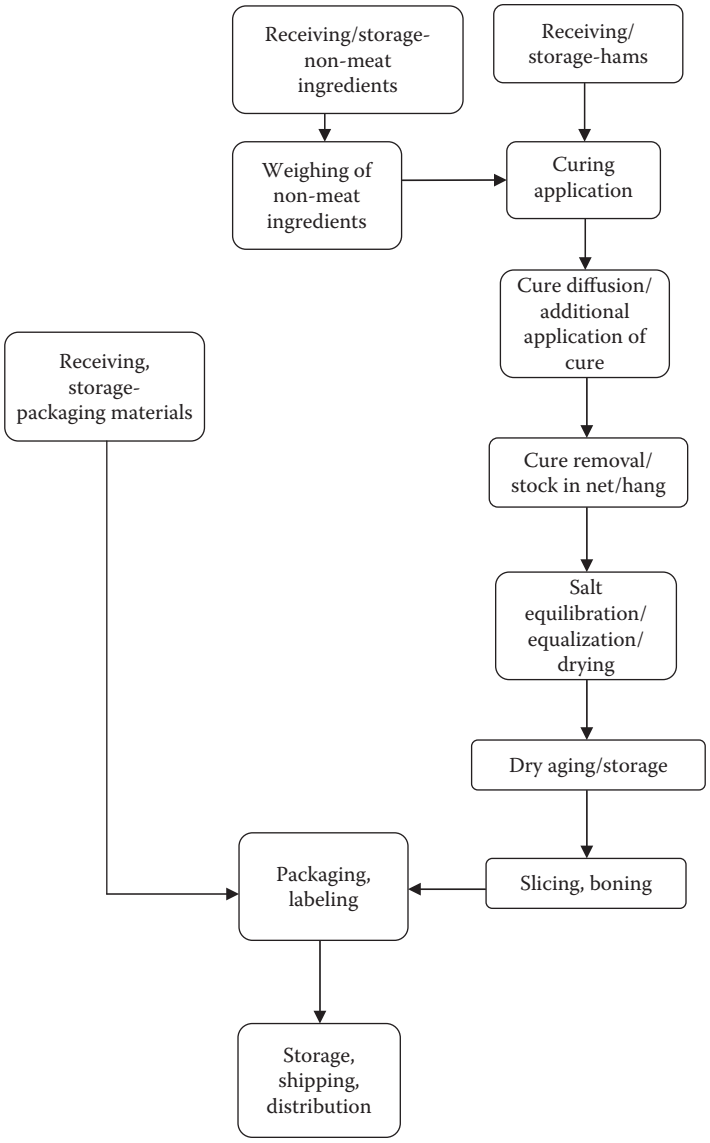

FIGURE 1.10 Flow chart for sliced country-style ham. (Modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. Used with permission.)

flavor and reddish-pink color of the cured pork. All ingredients added are carefully weighed in order to conform to the product formula.

The entire exterior of the ham or pork shoulder is coated or rubbed by the dry application of salt combined with the other ingredients. Additional salt or dry-cure mixture of salt may be reapplied to the product as necessary to insure complete penetration. The high salt level and the colder temperatures are the only measures protecting against the growth of spoilage and pathogenic microorganisms.

After the initial salting, the product is held for some period of time at refrigeration temperatures (at 40˚F) for the salt mix penetration and equilibration ("burning" period). This period often takes many weeks (at least 28 days) to achieve uniform salt distribution to greater than 4.5% with a water activity below 0.96. The goal is to eventually lower the water activity sufficiently to inhibit microorganisms to a point at which the temperature can be elevated. The product undergoes a maturation period (during this stage, the product is held at elevated temperatures for drying and flavor development), air drying, and

smoking (if desired), and storage. During these periods at higher temperatures, the humidity and air circulation is lowered, with further moisture loss. This final step in the process can be from 3 to 12 months in duration. The final product is then sliced and vacuum packaged for sale.

This is an example of a system of inhibitors. The nitrite and the salt are both inhibitors, they work together to achieve a certain amount of preservation of the product. This also extends the shelf life of the product, because spoilage organisms are inhibited. The lethality of the process for pathogens achieved in a salt-cured product will depend on the interaction of salt content, time and temperature of curing, drying, and aging. In addition, this product does not receive the amount of drying or reduction in water activity needed to make it shelf-stable. Therefore, the product is not completely preserved, and still requires refrigeration for safe storage. Products that are shelf stable will be covered in other sections.

1.10 Not Heat Treated: Shelf Stable

This processing category includes products controlled by water activity, pH, freeze dried, and dehydrated product, such as salami, pepperoni, or prosciutto. What defines this category is that the product is shelf stable, and while heat may be applied, it is not the *primary* means of achieving lethality. Many processors of products, such as pepperoni, include a low temperature heat treatment step in order to safely produce this type of product. We will examine the processing steps that are necessary for this type of product.

The process flow diagram (Figure 1.11) for this category represents salami/pepperoni. These are dry sausages that are RTE without any further cooking. Pepperoni was traditionally made with pork, while salami was made of pork along with some beef. Today, of course, many combinations of meat ingredients are used.

Many of the processing steps are similar to the processes we have already discussed. Raw meat ingredients are ground and mixed with nonmeat ingredients. The meat mixture is stuffed into casings. Let us take a look at some of the unique aspects of these processes.

Starter cultures: Bacterial fermentation is used to produce the lactic acid that results in the tangy flavor associated with this type of sausage. The acid inhibits bacterial growth. The resulting lower pH causes the proteins to release water, which assists in the drying process, and further inhibits bacterial growth in the finished product. Producers typically use a commercial lactic acid bacteria starter culture. Commercial starter cultures consist of a blend of harmless bacterial strains such as *Lactobacillus*. Simple sugars, such as dextrose or corn syrup, are added. They help promote lactic acid bacterial growth by serving as food to the bacteria during fermentation.

Fermentation: This step is an important one for pathogen control. During this step, the pH level of the product is reduced by the starter culture activity and by appropriate time/temperature factors. The starter culture is added to the meat mixture along with the sugar. The mixture is stuffed, and held in an environment optimum for their growth. These rooms are sometimes called *green rooms*. The temperature and humidity are carefully monitored. The starter culture bacteria actively reproduce, and as they do they give off lactic acid, which decreases the pH of the product. It is important that lactic acid is produced quickly, because it inhibits undesirable bacteria, like the toxin-producing *Staphylococcus*. The pH is monitored over time to determine when the process is complete. During fermenting, the establishment will probably want to achieve a pH of 5.0 or less within a certain time.

Most microorganisms thrive on pH near neutral (7.0) although there are exceptions. Meat processors can control pH to limit microbial growth and give meat a longer shelf life. Muscle tissues are close to 7 in the live animal. At slaughter, lactic acid builds up and the pH is lowered. The pH of fresh meat ranges between 5.3 and 6.4. At pH between 6.0–6.4 meat spoils faster than meat in the lower pH range (5.3–5.7), because the spoilage bacteria are more active at the higher pH.

Heating (optional): Both *Salmonella* and *E. coli* O157:H7 have been isolated from fermented sausage products. Consequently, many dry/semidry fermented sausages, particularly in the United States, have a significant "heat step" in the process to assure lethality of high numbers of bacterial pathogens.

Drying: At this stage, the salami/pepperoni is hung in a *dry room* to dry. Again, temperature and humidity are controlled. One of the factors that affect microbiological growth is moisture. Bacteria

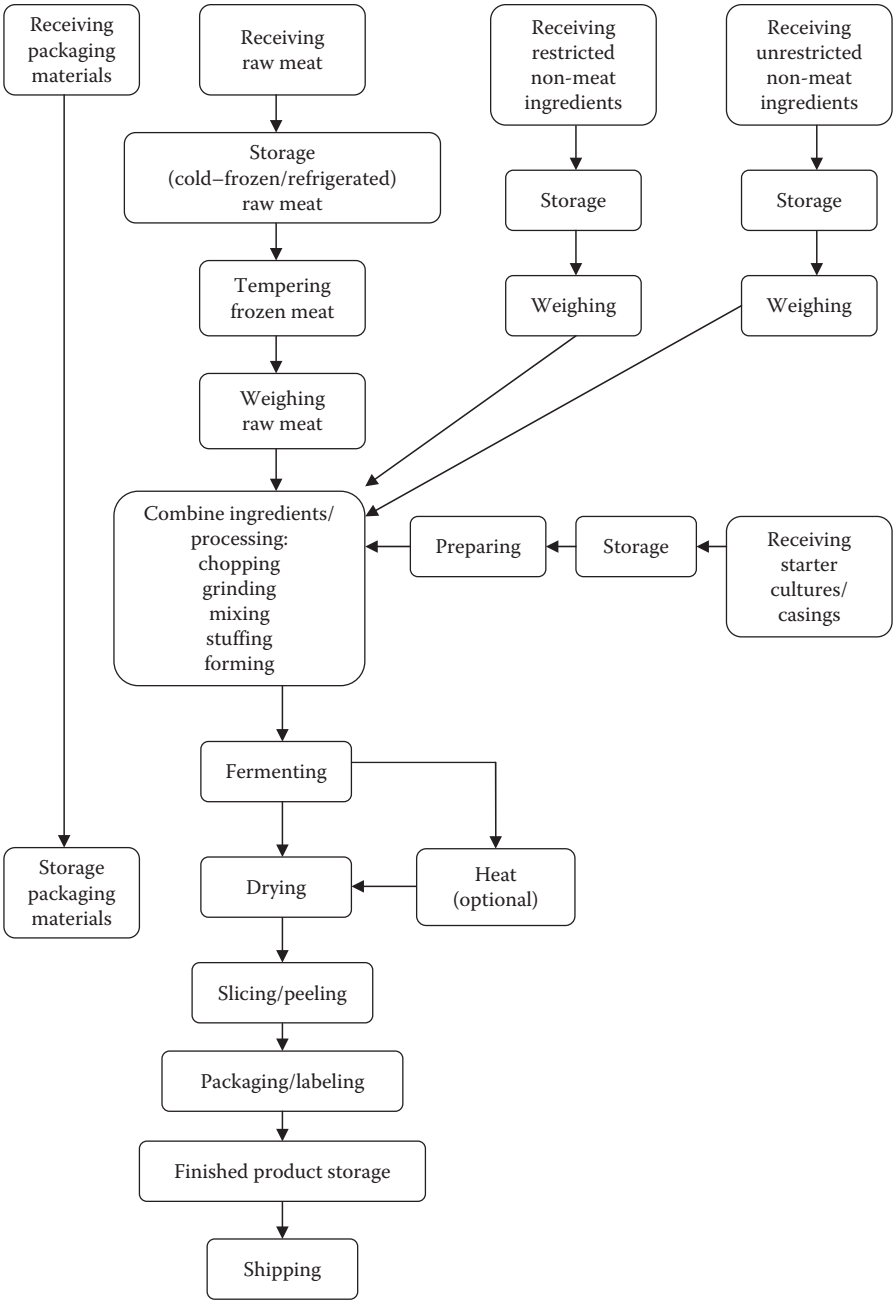

FIGURE 1.11 Flow chart for pepperoni. (Modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. Used with permission.)

need moisture to survive and grow. Drying is the simple process of dehydration in which osmosis withdraws water from the cell of the spoilage organisms, shriveling or inactivating the cells. The product must be dried to the point at which bacteria are inactive or destroyed in order to create a safe, shelf stable product.

FSIS product standards state that dry sausage must have a *Moisture Protein Ratio (MPR)* of 1.9:1 or less, in order to qualify as a shelf stable product. This is a calculation that compares the percentage of moisture to the percentage of protein. For comparison, fresh meat has an MPR of about 4.0:1.

At this point the finished sausage sticks are dry and ready to be packed for storage and distribution. Some product is *sliced* so that it can be used for purposes such as sandwiches, pizza, or salads. This step is another one where the potential for cross-contamination of product must be controlled by the establishment.

Validation for E. coli: In light of food-borne outbreaks of *E. coli* O157:H7 linked to dry fermented RTE beef sausage products, FSIS strongly recommends that all procedures for dry and semidry fermented sausages be validated to show a 5-log reduction of *E. coli* O157:H7. Full documentation is required.

This process category contains all products that are shelf stable, which may or may not have been heat treated. These products are rendered safe by a combination of processes, such as fermentation, heating, and drying.

1.11 Heat Treated: Shelf Stable

This processing category includes rendered products, popped pork skins, bacon bits, snack sticks or jerky, summer sausage, kippered beef, and pickled sausages. These products are considered RTE, meaning they can be consumed as packaged. This category contains products that are shelf stable, and have received a full lethality treatment. Cooking is generally the primary method for achieving all or most of the lethality in these products.

1.11.1 Rendering

Rendering refers to the extraction of edible and inedible fats and oils from meat after slaughter. The rendering process can be either wet (usually through steam) or dry. It yields products such as tallow and lard.

1.11.2 Summer Sausage

Summer sausage is a semidry sausage. It is not as dry as the "hard" sausages such as salami and pepperoni. This product is made RTE by a combination of fermentation (pH), smoking, cooking, and drying. Just one or two of these steps is not enough to produce a safe, shelf stable product.

The formulation for summer sausage typically includes lean beef, lean pork, sage or ground mustard seed, salt, sugar, pepper, and sodium nitrite. It may include a starter culture. The meat ingredients are ground, and seasonings and preservatives are added. Then the mixture is stuffed into casings. Next it is placed in a cooking chamber and heated at a low temperature (100–110°F) to quickly ferment the product. Often a smokehouse is used, although generally no smoke is applied. When the required pH is reached, the temperature is raised, and product is heated to a desired temperature for lethality. This also kills off the lactic acid bacteria and stops the fermentation. The product is then placed in a drying room until it dehydrates to the specified level. Sometimes a smokehouse is used as the drying room.

1.11.3 Snack Sticks and Jerky

Different types of snack sticks are made (Figure 1.12). Some are fermented, with either a low or high moisture level. Some are not fermented, with a very low moisture level. They are usually ground, and are similar to a dry sausage. Jerky is not fermented; it is often beef, but turkey and other products are also found in the marketplace. Jerky may be made from either solid pieces of muscle or chopped product. Processing steps are similar to dry sausage and summer sausage:

- *Acidifiers* such as citric acid, lactic acid, and glucono delta-lactone may be used to reduce the pH of the product.
- *Antioxidants* such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), or propyl gallate may be used to prevent oxidation and rancidity.

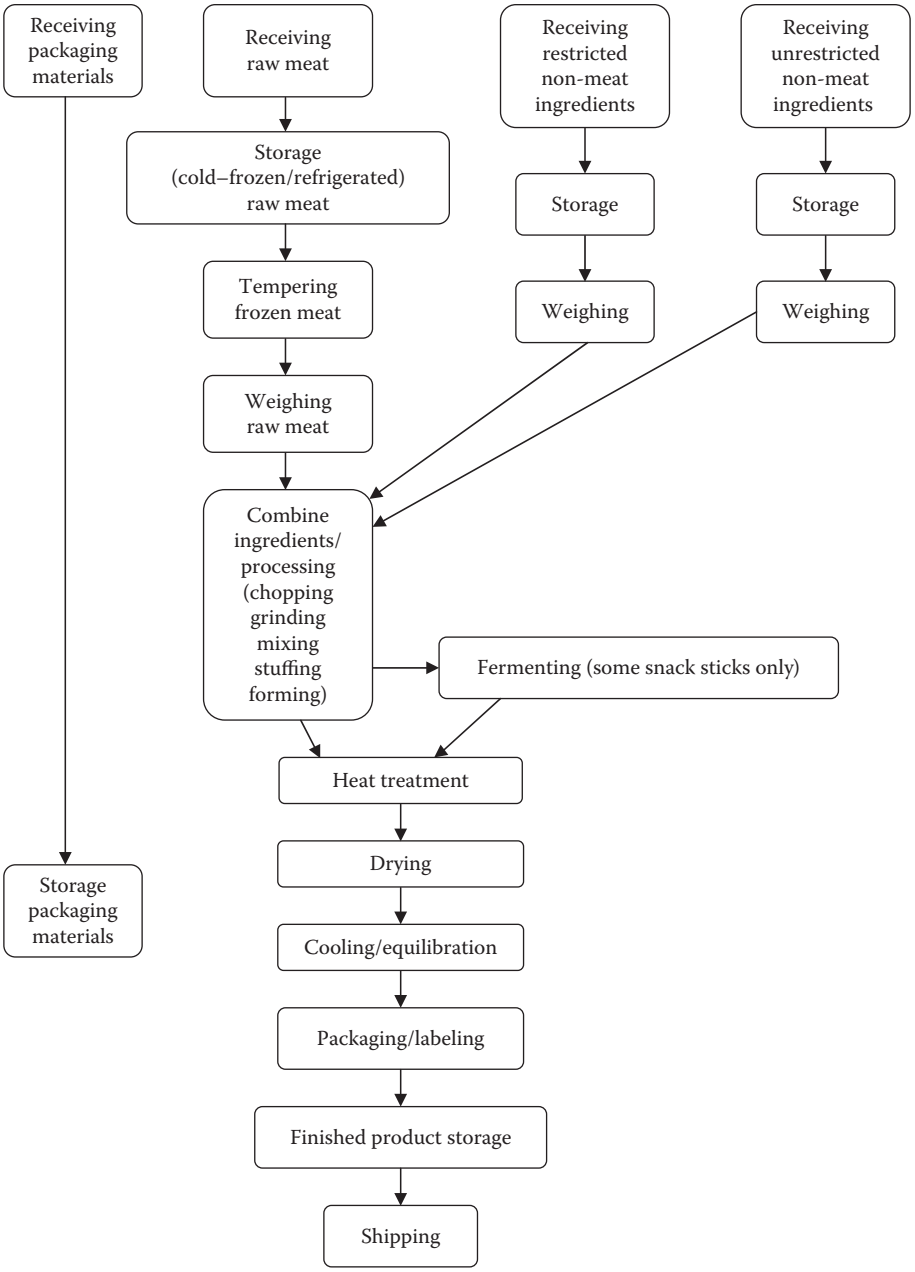

FIGURE 1.12 Flow chart for jerky or snack sticks. (Modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. Used with permission.)

- The *thermal processing* step, which involves cooking the product for a specified amount of time, is designed to control bacterial growth, both spoilage and pathogens. The cooking process also helps to shorten the drying time.
- The *drying* step helps reduce the moisture in the product to the desired level. Reducing the moisture helps control *Trichinae* and enteric pathogens such as *E. coli* O157:H7 and *Salmonella*.

FSIS has established *product standards* for certain dried products that specify an MPR needed to achieve shelf stability and ensure the product meets the standard established for that product. For example, a "dry sausage" must have an MPR of 1.9:1 or less, in order to be labeled as a "dry sausage" and also ensure shelf stability. Likewise, a nonrefrigerated, semidry, shelf stable sausage must have an MPR of 3.1:1 or less and a pH of 5.0 or less to ensure shelf stability. However, for most type of salt-cured dried products, the water activity (available moisture) of the product is the primary factor affecting shelf stability and safety.

These products may be stored and shipped at frozen, refrigerated, or ambient temperatures. Storing product below ambient temperature is usually done for quality reasons.

1.12 Thermally Processed: Commercially Sterile

This processing category includes canned meat products, products in reportable pouches and semirigid containers. Common examples are stew, chili, soup, canned hams, Vienna sausage, hash, potted meat product, and pasta sauce with meat. Although there are several types of packaging options available, such as pouches, plastic cups and plastic pans, the metal can is still the most common package used. For this reason, these products are usually referred to as *"canned."*

This category contains all products that have been thermally processed in order to achieve commercial sterility. The term *commercially sterile* does not mean that the product is completely sterile. Complete sterility is not achievable with canned products, because the *thermal processing* required to assure absolute sterility is so severe that the quality of the product would suffer. Certain types of microorganisms survive the thermal processing, but remain dormant or are inhibited from growth by some other factor. Thermally processed, commercially sterile product is RTE. It can be eaten directly from the container, although most consumers heat the product as a matter of personal taste.

1.12.1 Thermal Process

Our example product is pasta with meat sauce (Figure 1.13). You will notice some familiar processing steps, such as receiving, storage, assembling ingredients, and formulation. Because we have covered steps very similar to these earlier, we will not cover them now. Let us focus on some of the items that are unique to thermal processing:

- *Filling*: involves adding product, which has been mixed and formulated, to the product container. The typical container is a metal can. In most operations, filling is done with high-speed machines.
- *Types of cans*: There are several types of cans used most frequently in the meat industry. Three basic types of cans are round sanitary, drawn aluminum, and oblong. The *round sanitary* can holds a variety of meat-base products, such as pasta with meat sauce. *Drawn aluminum* cans are used most often for Vienna sausage and meat spreads. Their use is increasing because they use less metal and are more economical. *Oblong* cans are used for canned luncheon meats. To prevent an interaction between the meat product and the metal, cans are generally *coated* on the inside. The kind of coating that is typically used with meat products is sulfur-resistant. It prevents the sulfur released from meat proteins during retorting from staining the tinplate black.
- *Sealing*: Lids are placed on the filled cans and a *hermetic* seal is formed. This seal prevents air from getting in or out. It is this seal which will preserve the integrity of the can after it has been thermally processed, so it is critical that the seal is formed correctly.
- *Thermal processing*: involves placing the filled and sealed cans in a *retort* so that they can be thermally processed. A retort is a steel tank in which metal crates or baskets containing the cans are placed for subsequent cooking and cooling. The retort operates under pressures of 12–15 pounds per square inch (psi). This pressure cooking raises the cooking medium (usually steam) to temperatures above the normal boiling point of water, 212°F. For 3 min at 250°F is an example of a minimum process. The retort subjects product to a high temperature for a sufficient duration to destroy the organisms that might adversely affect consumer health, as well as more resistant organisms that

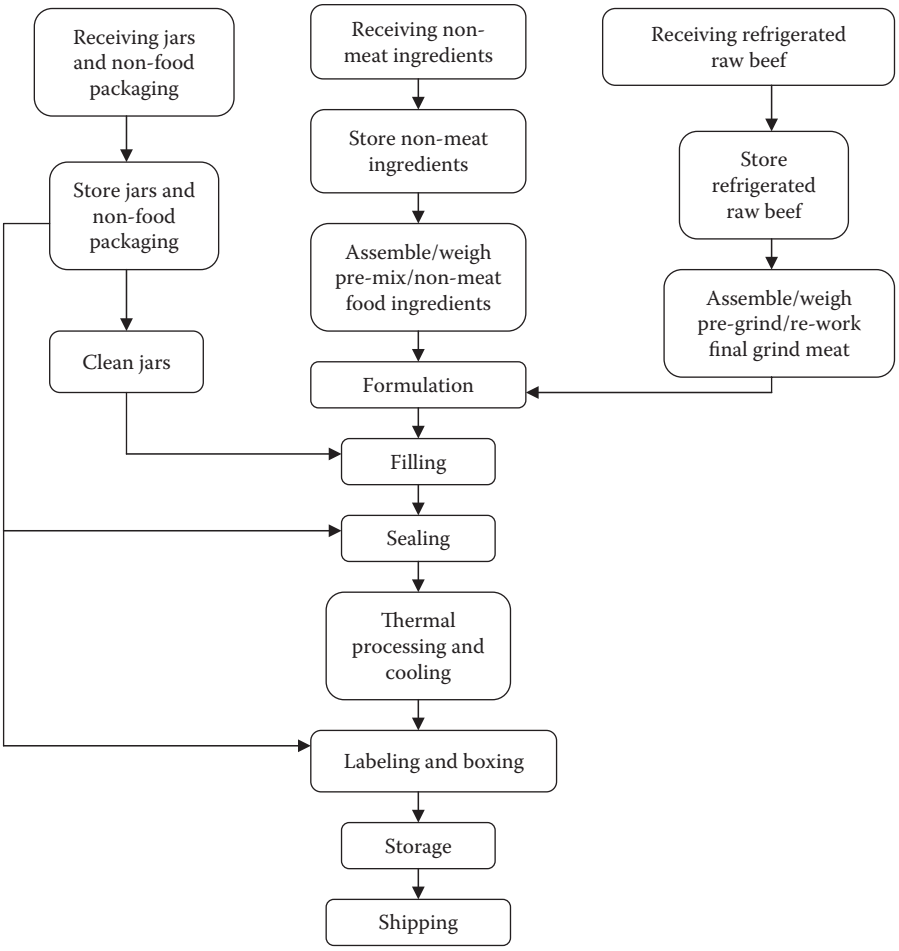

FIGURE 1.13 Flow chart for pasta sauce with meat. (Modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. Used with permission.)

cause spoilage under normal storage conditions. These procedures are based on the destruction of all vegetative cells and all spores of the deadly, toxin-producing bacteria *Clostridium botulinum*.

- *Cooling*: After the heat process has been completed, all canned meat products should be cooled as quickly as possible to stop the cooking process and to lower the temperature below the range at which any heat-tolerant bacteria can grow. Because the cans are wet, it is best to permit some heat to remain to evaporate any remaining moisture, in order to prevent rust. When cans are being cooled, they contract and even well-made seams may permit some inward leakage. Therefore, the water used for cooling must be as near sterile as possible. Canning cooling water is chlorinated, and rust inhibitors may be added.
- *Incubation*: After product is cooled, some establishments will hold it for 10–30 days before the cans leave the establishment. This holding time is used to test a sample of the retort load. A sample of each processing lot is held at a temperature that would promote bacterial growth. This is done in an *incubator*. At the end of this incubation period, cans are examined for evidence of spoilage. If none is found, canned products are shipped.
- *Storage*: Canned meats should be stored in a cool, dry place because relative humidity and temperature influence their shelf life. Canned meat and poultry will maintain quality 2–5 years if the can remains in good condition and has been stored in a cool, dry place.

Here are some terms you commonly hear with regard to thermal processing:

- *Critical Factor*: Any characteristic, condition, or aspect of a product, container, or procedure that affects the adequacy of the process schedule. Critical factors are established by processing authorities.
- *Processing authority*: The person(s) or organization(s) having expert knowledge of thermal processing requirements of canned foods and utilizing procedures recognized by the scientific community as being adequate to properly calculate and assign thermal processes.
- *Process calculation*: Scientifically defined procedures that determine the process time and temperature as adequate under specific conditions of manufacture for a given product.
- *Process deviation*: Any change in a critical factor of the scheduled process that reduces the sterilizing value of the process.
- *Scheduled process*: The time, temperature, and critical factor controlled process, selected by the process authority and scientifically determined to yield commercial sterility under conditions of manufacture for a given product.
- *Sterilizing value*: This is normally expressed as "F_0" and is the number of minutes required to destroy a given number of microorganisms at a given temperature. The F_0 value is used to compare the sterilizing values of different processes.
- *Vacuum*: Removal of air from the can to prevent oxidation of the product.

This category contains many different types of canned product. Although we discussed metal cans, you will also see plastic cups and flexible pouches on the grocery shelf. These items are processed in a similar fashion. This category groups all products that are thermally processed in order to achieve commercial sterility.

Part II

Meat Science

2

Muscle Biology

Amanda D. Weaver

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2.1 Introduction

Meat is comprised of numerous tissue types, including nervous, adipose, and connective tissue. Skeletal muscle tissue generally constitutes between 50% and 70% of carcass weight of meat animals, and subsequently, most of the value. Muscle, the living precursor to meat, is a uniquely complex tissue that assumes many forms in order to execute its various functions. Given that muscle is converted into meat postmortem, an understanding of the underlying biology that regulates this tissue antemortem is critical. This chapter will provide a brief introduction to muscle types and introduce the topics of muscle growth and development.

2.2 Types of Muscle

The majority of meat derived from carcasses of domestic livestock species is composed of striated skeletal muscle. However, the living animal depends on the functions of three distinct types of muscle to maintain life: smooth, cardiac, and skeletal muscles. These muscles can be distinguished by their primary functions, structures, and nervous control (Table 2.1). The following discussion will focus on the properties of skeletal muscle as it is used for food; however, a brief description of these three types of muscle will be given for comparison.

2.2.1 Smooth Muscle

Smooth muscle is a critical component of numerous animal tissues providing elasticity to the walls of arteries, linings of the gastrointestinal, reproductive, urinary and respiratory tracts, as well as the

TABLE 2.1

Characteristics of the Three Different Mammalian Muscle Types

Muscle Type	Striated	Nuclei per Muscle Fiber or Cell	Nervous Control
Skeletal	Yes	Many	Voluntary
Cardiac	Yes	1–2	Involuntary
Smooth	No	1	Involuntary

lymphatic system. These cells vary widely in their size and shape, but a basic spindle form is common. Smooth muscle cells contain only a single nucleus that is typically located near the center of the cell. As in skeletal muscle, the proteins actin and myosin in smooth muscle are the basis for contraction and movement; however, these proteins are not organized into an ordered, striated appearance as in skeletal muscle. Contractile action of smooth muscle is controlled involuntarily via the autonomic nervous system and therefore conscious thought is not required to generate movement.

2.2.2 Cardiac Muscle

Cardiac muscle is located only in the heart where it provides rhythmic contractions throughout an animal's life. In a similar way to skeletal muscle, cardiac muscle appears striated when viewed under a microscope. However, cardiac muscle contains structural features not observed in skeletal muscle tissue, including branched fibers and intercalated disks, which separate adjacent cardiac muscle cells, and provide the ability to physically communicate contractile force directionally throughout the heart. Similar to smooth muscle, cardiac muscle cells generally contain a centrally located nucleus and are controlled involuntarily by the autonomic nervous system.

2.2.3 Skeletal Muscle

Skeletal muscle is the most abundant type of muscle in the animal body. Contractile, structural, and regulatory proteins in this muscle type are highly organized into a distinct striated pattern. Skeletal muscle is so named as it is attached to the skeletal framework of the animal, in various configurations, producing different types of levers and ultimately movement. Skeletal muscle fibers (cells) can range in length from 10 μm to more than a few centimeters. To provide genetic material along the whole length of skeletal muscle fibers, these specialized cells are multinucleated. Nuclei in mature skeletal muscle fibers are located along the periphery of the fiber. Skeletal muscle contraction is directly stimulated by somatic efferent nerves and therefore this muscle type is often referred to as voluntary muscle (Goll and others 1984; Gerrard and Grant 2006a).

2.3 Muscle Growth and Development

Although skeletal, smooth, and cardiac muscles take on widely different characteristics and functions in their mature form, early events leading to their development are similar. The development and subsequent growth of muscle is the product of complex cellular changes that accompany myogenesis and the growth of this dynamic tissue pre- and postnatally. To more efficiently produce muscle that results in high-quality meat products, it helps to have an understanding of these events and the processes by which they can be manipulated.

2.3.1 Myogenesis

The embryonic cells that ultimately become muscle, with few exceptions, are derived from the mesodermal layer of the developing embryo, a layer that also gives rise to fat and bone tissue. As mesodermal cells become more prominent, they begin to organize into cuboidal clusters known as somites. Further

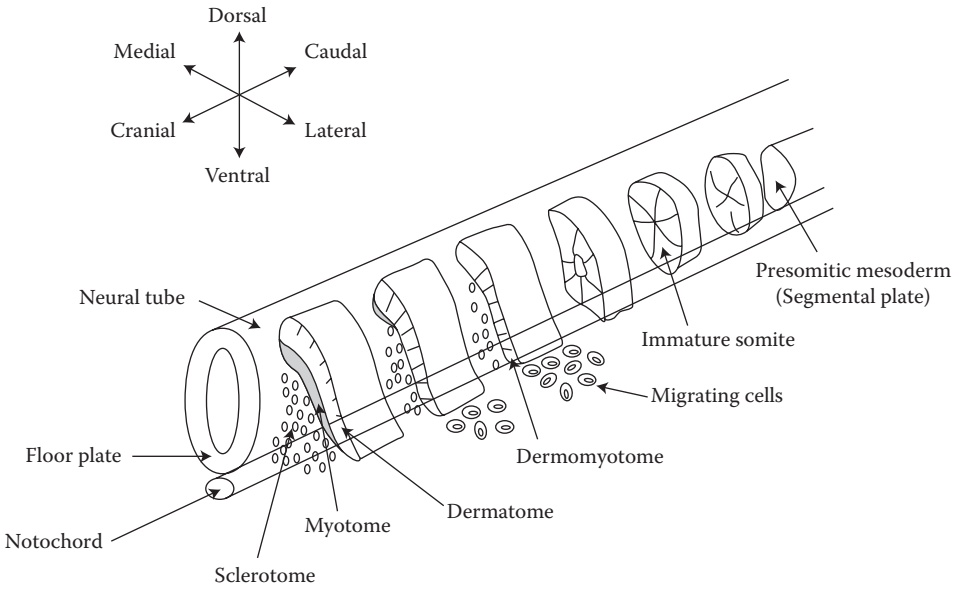

FIGURE 2.1 Formation and organization of somites begins as cells of the mesoderm organize into somites along the nerural tube. Somites further mature into myotome, dermatome, and sclerotome layers which delaminate and migrate to become muscle, skin, and the vertebral column, respectively. This embryonic event occurs in a cranial to caudal fashion. (Reprinted from *Cell*, 78/1, Buckingham M., Molecular biology of muscle development, p. 16, Copyright (1994), with permission from Elsevier.)

organization of the somites gives rise to the dermomyotome and the sclerotome (Figure 2.1). Cells of the sclerotome eventually migrate and become the vertebral column while the dermomyotome cells separate to form the dermatome and the myotome, with cells of the dermatome becoming the dermis while cells of the myotome migrate to different regions of the body and give rise to muscle tissue.

The expression of one or more genes of a family of transcription factors, referred to as myogenic regulatory factors (MRFs) which are members of the basic helix–loop–helix (bHLH) family of proteins, initiates transcription of muscle-specific genes. This process ultimately results in committing mesodermal cells to the myogenic lineage. As cells express these key genes and become committed to the muscle cell lineage, they are termed myoblasts. Myoblasts are bipolar, spindle-shaped, mononucleated cells that are capable of progressing into the cell cycle and increasing in number through proliferation. However, to complete the developmental process and ultimately produce functioning muscle, cell differentiation must occur for myogenic cells to attain the level of specialization and organization required for contraction. At this point in development, myoblasts are signaled to cease dividing, align with one another, and begin to fuse together to form multinucleated myotubes (immature muscle fibers). Simultaneously muscle-specific genes, such as those encoding myosin and actin, are upregulated (Stromer and others 1974; Hawke and Garry 2001; Buckingham and others 2003; Biressi and others 2007). Figure 2.2 illustrates the progress of muscle fiber development.

Four key MRFs responsible for regulating myogenesis are Myf5, MyoD, myogenin, and MRF4 (Figure 2.2). Myf5 and MyoD possess key regulatory functions in the process of myoblast proliferation and differentiation and are more highly expressed in undifferentiated myoblasts. Myf5 is the initial MRF to be expressed in the developing embryo, and initiates early myoblast differentiation followed closely by expression of MyoD. Myogenin and MRF4 are considered secondary MRFs as they are responsible for terminal differentiation and maintenance of differentiated myofibrils. Differentiation must occur for the developing cell to become a specialized muscle cell. MRF4 expression occurs later during myogenesis and is the most predominant MRF to be identified in adult skeletal muscle (for reviews, see, Weintraub 1993; Perry and Rudnicki 2000; Pownall and others 2002; Sabourin and Rudnicki 2000; Houba and tePas 2004).

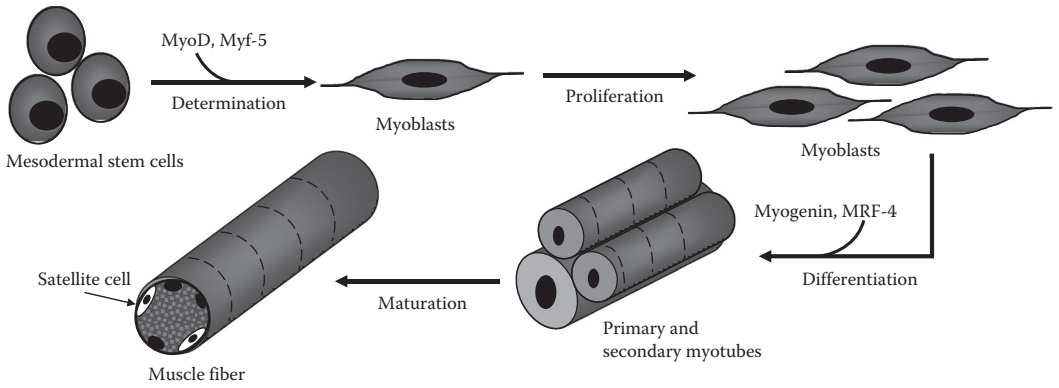

FIGURE 2.2 Events of myogenesis.

2.3.2 Muscle Fiber Formation

Muscle fibers express a biphasic developmental pattern in most domestic livestock species, resulting in two distinct populations of fibers, primary and secondary, in the developing fetus. Primary myofibers are generated first, via the fusion of myoblasts, into a structure known as a myotube. Primary myotubes then function as a structure for the organization, alignment, and fusion of other myoblasts into a larger population of secondary myofibers. Primary myofibers form during the initial stage of myogenesis (from approximately months 1–3 postconception in bovine fetuses) while secondary myofibers form during the second wave of myogenesis in the fetal stage (approximately months 3–7 postconception in bovine fetuses Du and others 2010). Histologically, primary myofibers are larger in diameter than secondary myofibers. Both structures initially possess centrally located nuclei; however, in contrast to secondary fibers, primary myofibers express slow myosin ATPase activity. Secondary myofibers maintain communication with their "parent" primary myotubes through membrane-associated proteins that form gap junctions between the structures. However, this association is lost following the innervation and contraction of primary myotubes which elicits the detachment of secondary fibers from the primary. Following separation, a new population of myoblasts can utilize the primary myotube as a template for fusion and development of additional secondary fibers (Figure 2.3). This well-orchestrated production of secondary myofibers gives rise to approximately 90% of the muscle fibers in most domestic livestock species. It is important to note the role of innervation in this process. Innervation is not required for the formation of primary myotubes. However, because contraction of primary myotubes is required for the development of secondary myofibers, innervation is a controlling factor modulating overall myogenesis (Ashmore and others 1972; Swatland and Cassens 1973; Swatland 1994; Gerrard and Grant 2006b; Du and others 2010).

Collectively, these biochemical and morphological events of fetal muscle development occur primarily during the first two-thirds of gestation and for most species are completed by birth. In other words, fiber number is essentially set at birth, with few exceptions. Therefore, events or conditions that enhance or impede embryonic or fetal development can dramatically impact fiber number and ultimate muscle mass of the individual.

2.3.3 Postnatal Muscle Growth

Muscle can increase in size by one of three processes: hypertrophy, hyperplasia, or accretion. Hyperplasia refers to an increase in fiber number resulting in an increase in muscle size, while hypertrophy is defined as an increase in fiber size, also resulting in an increase in muscle size. Muscle can also increase in size by the accretion of material, such as fat and connective tissue, between cells; however, this discussion will focus on the mechanisms of hypertrophy and hyperplasia.

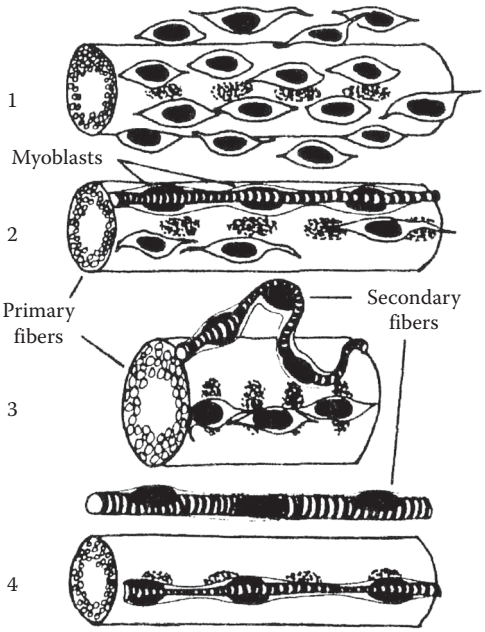

FIGURE 2.3 Stages in the development of secondary muscle fibers. (1) Proliferating myoblasts surrounding a primary fiber, (2) myoblasts fuse together to form a secondary fiber on the surface of the primary fiber, (3) contraction of the primary fiber causes detachment of the secondary fiber, and (4) secondary fiber is released and a new secondary fiber is forming on the surface of the primary fiber. (Reprinted from Swatland HJ. 1994. *Structure and Development of Meat Animals and Poultry.* Boca Raton, FL: CRC Press. pp. 287–363. With permission.)

In most domestic livestock species, muscle fiber formation is completed by birth or shortly thereafter and remains essentially stable. Therefore, achieving muscle growth postnatally is dependent on the enlargement of fibers developed prenatally. This hypertrophic growth of muscle fibers is mainly a function of protein synthesis and protein degradation. The difference between the amount of protein synthesized and the amount degraded is termed net protein accretion. During growth, the rate of protein synthesis must exceed the rate of degradation to generate a net accumulation of protein and ultimately an increase in muscle mass. In contrast, when protein degradation exceeds the amount of protein synthesized, muscle atrophy results. The relative rates of synthesis and degradation of muscle proteins influences the longitudinal and radial growth of muscle fibers (Beitz 1985; Young 1985; Trenkle 1986; Marple 2003).

Muscle fibers have a diameter of 10–20 μm soon after formation, and grow to a normal mature size of 50–80 μm in diameter. To increase in diameter, muscle fibers go through a process known as longitudinal splitting of myofibrils. Myofibrils are the filamentous organelles within muscle fibers, composed of contractile units known as sarcomeres. These organelles split longitudinally to form two new myofibrils which in turn enlarge and become functional via the synthesis of additional contractile, regulatory, and structural proteins. This process of producing new myofibrils is known as myofibrillogenesis. Radial growth of muscle fibers is stimulated by exercise or work and is commonly termed exercise- or work-induced hypertrophy. Conversely, longitudinal growth of muscle fibers is advanced by the stretching of muscles, generally through associated bone lengthening. This type of growth is referred to as stretch-induced hypertrophy and is accomplished through the process of sarcomere addition. Sarcomeres are the contractile units of muscle and are arranged end to end in a series or "box-car fashion." Newly synthesized proteins are organized into new sarcomeres and generally added to the end of existing myofibrils resulting in an increase in fiber length (Williams and Goldspink 1971; Stromer and others 1974; Gerrard and Grant 2006a,c).

Both types of muscle fiber hypertrophy require synthesis of new proteins. This process involves transcribing DNA into mRNA within the nucleus, transporting mRNA out of the nucleus and into the

sarcoplasm for translation into protein, and finally arranging the newly synthesized protein into all the structures within the muscle fiber. It is important to note that this process not only occurs in the case of muscle hypertrophy. In fact, a critical and continuous balance exists between protein synthesis and protein degradation in muscle cells. Proteins are regularly assembled, positioned in the cell, and eventually replaced with a newly synthesized protein, in a process known as protein turnover.

The disassembly of proteins from muscle fibers is mediated by protein degradation pathways that involve the systematic proteolysis of proteins into peptides and ultimately into amino acids by proteases. The three main endogenous protease systems in skeletal muscle are the ubiquitin proteosome, the lysosomal system, and the calcium-dependent protease (calpain) system. Of these, the calpain system is responsible for a majority of protein turnover in living muscle and is also the most accepted candidate for postmortem tenderization (proteolysis) of meat. The best-characterized members of the calpain family include μ-calpain and m-calpain and their specific inhibitor calpastatin. The μ and m forms of the enzyme are cytosolic, ubiquitously expressed, and require micromolar and millimolar calcium concentrations, respectively, for activation (Goll and others 2003).

Lysosomes are cellular organelles containing proteolytic enzymes, such as the cathepsins, that function best in a low-pH environment such as that of a lysosome. These enzymes are capable of degrading proteins into small peptides and amino acids, but are responsible for relatively little protein turnover in muscle due, in part, to the limited number of lysosomes present in muscle. In addition, the role of this enzyme system in muscle is further hampered by the fact that cathepsins cannot degrade muscle proteins outside the lysosome and lysosomes cannot engulf intact myofibrils. Therefore, degradation by the lysosomal system in muscle is limited primarily to sarcoplasmic proteins (Goll and others 1983; Allen and Goll 2003).

The third protease system is the ubiquitin proteosome. The proteosome is a large protein complex that resembles a hollow cylinder with the catalytic site residing inside the cylinder. In contrast to the lysosomal system, the proteosome is highly concentrated within muscle cells, which means that its regulation is critical to prevent unintended degradation. The first line of regulation lies with the requirement of ubiquination. In this process, ubiquitin is covalently attached to proteins indicating they are to be targeted for degradation. Polyubiquinated proteins and/or polypeptides are then transported to the proteosome and preferentially degraded. The second control point for this system is the entrance to the cylinder itself, which is very small (1–1.3 nm) in diameter restricting the entrance of large proteins or protein complexes. Because of this restriction, large proteins or complexes must first be degraded into smaller polypeptides by another protease system, such as the calpain system. Research has shown that the proteosome and the calpain system work in cooperation to identify (target) and degrade myofibrillar proteins (Robert and others 1999; Allen and Goll 2003; Houbak and others 2008).

2.4 Regulation of Muscle Mass

In addition to understanding the processes involved in muscle fiber development and growth, researchers in the field of livestock production should also be aware of the numerous mechanisms by which these processes can be manipulated. Some of the most important factors affecting muscle fiber development and growth processes are discussed below.

2.4.1 Factors Influencing Muscle Fiber Hyperplasia

As aforementioned, muscle fiber number is essentially set at birth; therefore, manipulation of fiber number must occur prenatally. One exception to this rule is the case of muscle fiber death as new muscle fibers may be generated postnatally following severe injury resulting in fiber loss. This regeneration is achieved by the activation of satellite cells (quiescent, mononucleated muscle cells located between the basil lamina and sarcolemma), which proliferate and fuse with other satellite cells to form new fibers. These specialized cells can also contribute to muscle growth when a hypertrophic stimuli is applied such as resistance training. During such a challenge, satellite cells are activated, proliferate,

and fuse to existing myofibers resulting in growth. For a more detailed description and discussion of satellite cells, readers are referred to Mauro (1961), Hawke and Garry (2001), Cornelison (2008), and Relaix and Marcelle (2009). Although the ability to regenerate muscle fibers in this way is notable, as a mechanism for growth, this process does not contribute significantly to variation in the mass of muscle grown.

A number of intrinsic and extrinsic factors have been demonstrated to have an impact on the number of muscle fibers formed during the prenatal period. For example, larger species by nature express increased numbers of muscle fibers. Excessively large fibers pose challenges for nutrient and metabolite diffusion so that, by necessity, muscle fiber diameter is relatively constant between species and the difference in muscle mass between species is due primarily to variation in muscle fiber number. Research has also indicated that within species, different breeds express variation in fiber number, and gender differences sometimes exist for this trait as well (Table 2.2; Rehfeldt and others 1999; Wegner and others 2000; Gerrard and Grant 2006c). Also, gene mutations have been reported to result in hyperplasia and extreme muscularity.

The myostatin gene mutation, responsible for the "double-muscling" phenotype in cattle, is one such example. This member of the transforming growth factor family is expressed throughout myogenesis and is also detectable in postnatal skeletal muscle (Kambadur and others 1997; McPherron and others 1997). Mutations in the gene can vary, but ultimately result in a gene product (protein) with altered function. Myostatin appears to modulate myoblast proliferation and differentiation. Therefore, when myostatin expression is mutated in certain ways a substantial increase in muscle fiber number, and consequent muscle mass, is manifested. Belgian Blue cattle exhibit a high frequency of a myostatin deletion that results in an extremely muscular phenotype. In fact, Belgian Blue cattle with the mutated gene have an approximately 20% increase in skeletal muscle as compared with normal cattle (Shahin and Berg 1985; Muroya and others 2009).

Another key factor in determining muscle fiber number is maternal nutrition. This area of study was recognized after the Dutch Famine of World War II and is again receiving much attention. The concept of fetal programming asserts that nutritional status of the dam can influence the offspring during fetal development and throughout the subsequent postnatal period (Du and Zhu 2009). Nutrition during gestation programs fetal muscle development, along with other tissues and health parameters (Barker and others 2002). Furthermore, it has been demonstrated that the genetic potential for muscle growth cannot be fully realized if maternal nutrition is restricted beyond a certain level (Du and others 2009). Therefore, much of the current research in this area is focused on the use of this concept as a management tool for maximizing muscle yields of livestock species. The influence of maternal nutrition on muscle fiber number has been demonstrated in several livestock species (Dwyer and others 1994; Zhu and others 2004; Quigley and others 2005; Du and Zhu 2009) and while collectively these studies show a tendency for decreased muscle fiber number (specifically secondary muscle fiber number) more research is necessary to fully understand this area of growth biology.

TABLE 2.2

Comparison of Muscle Fiber Number between Genders of Various Species

Species	Muscle	Effect
Mouse	*Soleus*, *Tibialis anterior*, *Biceps brachii*	None
Rat	*Soleus*	None
Human	*Tibialis anterior*	Male > Female
Chicken	*Extensor longus*	Male > Female
Pig	*Longissimus*	None
Cattle	*Longissimus*	Male > Female

Source: From Rehfeldt C. et al. *Basic Appl Myol* 9:235–253. With permission.

2.4.2 Factors Influencing Muscle Fiber Hypertrophy

As discussed previously, muscle fiber size increases either by radial or longitudinal growth or both. Similar to muscle fiber hyperplasia, numerous factors can influence this type of growth, including sex, nutritional status, growth promoters, and genetic mutations.

Intact males demonstrate larger muscle fiber cross-sectional areas than females or castrated counterparts. This response is largely attributed to high circulating levels of the hormone testosterone which enhances both protein synthesis and degradation (Rehfeldt and others 2004; Therkildsen and Oksbjerg 2009). Although both processes are higher in intact males, protein synthesis differences are greater, resulting in net protein accretion. Specific muscles are also more sensitive to the influence of testosterone, such as the muscles of the crest of the neck in cattle.

Muscle fiber size is also responsive to nutritional status of the animal. Typically individuals placed on a high plane of nutrition develop muscles with larger diameters than those on a low or restricted plane of nutrition as nutrients are available for the synthesis of new proteins. It is also important to state the influence of nutrient partitioning as it influences the order of nutrient utilization by various body systems. The endocrine system coordinates the distribution of nutrients to physiological systems and tissues. Of the body systems, the highest priority during growth is given to the brain and central nervous system followed by the circulatory, respiratory, digestive, and reproductive systems. Hierarchy is also established for tissues, with the bone receiving first priority for nutrients during growth followed by muscle and adipose tissues (Owens and others 1993; Gerrard and Grant 2006d).

The callipyge mutation in sheep is a genetic condition resulting in extreme muscularity, primarily in the hind legs. This mutation is markedly different from the myostatin mutation in cattle. Sheep expressing this genotype develop fibers with much larger diameters than normal sheep after birth. Although these lambs demonstrate increased feed efficiency, muscle mass and lean product yield without increasing the risk of dystocia at birth, their use commercially is not widespread as the resulting meat is inherently tough when compared to normal lamb (Koohmaraie and others 1995; Jackson and others 1997a,b). Inadequate tenderness in this case is associated with elevated levels of calpastatin in the hypertrophied muscles. As indicated above, calpastatin is the endogenous inhibitor of the calpains which are credited with degrading structural proteins postmortem during the aging process. Therefore, increased levels of calpastatin limits the ability of the calpains to degrade proteins and improve tenderness during aging. However, in the living tissue, the increased protein synthesis and decreased degradation result in a net increase in protein accretion and ultimately larger muscle fibers and muscles (Koohmaraie and others 1995; Duckett and others 1998).

Finally, growth promoters such as β-adrenergic agonist and steroid implants have garnered much research focus since their introduction. These compounds work through various pathways to accomplish the common goal of increased muscle fiber size (Clancy and others 1986; Fritsche and others 2000; Gonzalez and others 2007; Kellermeier 2009) and will be further discussed in later chapters of this volume.

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3

Meat Composition

Robert G. Kauffman

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3.1 Preface

Anyone who has an interest in meat should know something about what it consists of, that is, what the pieces are, how much of the whole each piece represents, and how to measure each piece. Furthermore, the interested and educated person really should know from where meat originates and how to measure its composition and what causes it to vary. This is what this chapter is about. When you have finished reading it, I hope you will have a better understanding of where meat comes from, what it consists of, why its consistency varies, and how to assess it. This information should help provide a better understanding of how to use meat as a food, how to make it taste better and safer for

consumption, and how its properties can be best utilized for further processing, storage, and distribution. Specific references are given for further details on specific topics (Allen and others, 1976, Hedrick and others, 1993, Kauffman, 1971, Kauffman and St. Clair, 1978, Kauffman and others, 1986, Kinsman and others, 1994).

3.2 Introduction

3.2.1 Definitions and Measurements

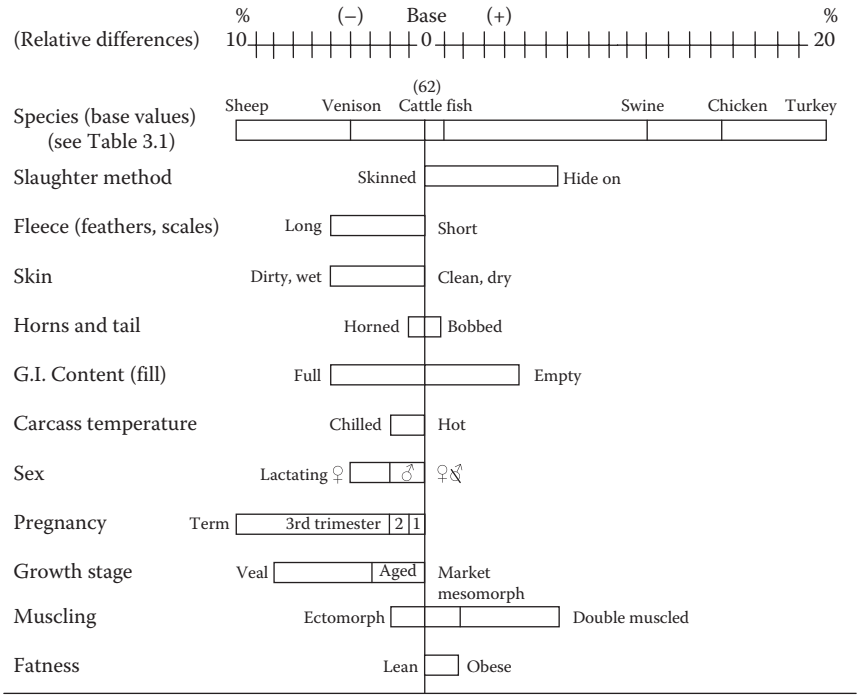
In the broadest sense, meat is the edible postmortem component originating from live animals. For the purposes of this text, these animals include domesticated cattle, hogs, sheep, goats, and poultry, as well as wildlife such as deer, rabbit, and fish. It is reasonable for the definition of meat to include such organs as heart and liver (often defined as variety meats), but the focus of this chapter is on meat defined as those tissues exclusively originating from an animal's carcass, a proportion amounting to about one-half to three-fourths of the animal's live weight. This carcass proportion of the live animal weight is classically calculated as dressing percentage and can vary considerably. Some species, such as the turkey, can yield a carcass weighing about 80% of the live weight, whereas a market lamb's yield is closer to 50%. Animals with small and empty gastrointestinal tracts (such as hogs or poultry rather than ruminants) that are not pregnant, that are more heavily muscled and fatter, that do not have long fleeces or dirty hides, and that have been slaughtered in a manner that leaves the skin and feet intact with the carcasses (hogs) will have higher dressing percentages (see Figure 3.1).

Excluding the skin, the carcass component of live animals basically consists of three parts: muscle, fat, and bone. Of these, muscle is the most important, constitutes the majority of the weight, and often is considered unequivocally synonymous with "meat." This can be a reasonable assumption, but fat deposits and some bones are often processed, merchandized, and used along with muscle and must be included in the broader definition of meat. Figure 3.2 is included as an example of the relative composition (in specific detail) of market animals and is representative of a live mature beef steer. From this information, one can calculate the proportions of any one part to the various larger component parts. For instance, the longissimus muscle represents (approximately) 51% of the back muscles, 12% of all carcass muscles, 7% of the carcass, and 4% of the live animal. These values can vary depending on species, degree of fatness, and other similar factors affecting dressing percentage. However, it provides a relative guide that reflects the composition of live animals and how it is related to the meat component. Furthermore, Figure 3.2 indicates that "meat" has its origin in many muscles of the carcass. In closer observation, one can deduce that some muscles contribute considerably more to meat than others, and that is because they vary in size and shape, dependent directly on the biological functionality.

Composition is defined as the aggregate of ingredients, their arrangement, and the integrated interrelationship that forms a unified, harmonious whole. Figure 3.2 is an example of this. For market animals raised to produce meat for human consumption, the greatest emphasis is on the musculature and its relationship to everything else. The proportion of the animal's musculature is related to several criteria, the three most important being dressing yield, fatness, and muscling (expressed in terms of ratio of muscle to bone). Realistic averages of composition for most meat animals are included in Table 3.1. Muscle varies from 25% (lamb) to 50% (turkey) of the live weight and muscle to bone ratio varies from 1.8 (chicken) to 5.0 (venison).

There are several arithmetic approaches to expressing quantitative composition, but the one most commonly used is the weight of a part expressed as a percentage of a larger part, such as % muscle of a retail cut of meat, or % protein of a muscle. Another less-used technique is to express the part and the whole as logarithmic functions of each other.

Measuring composition can vary from subjective techniques to ones precisely objective. Even when a technique is considered objective, at least some subjectivity inadvertently prevails. Here are some of the more commonly used approaches for determining gross composition (% lean) of meat cuts.



[1] Some factors are interrelated such as species, slaughter method, and fleece.

[2] Condemnations (due to bruising, disease, contamination, etc.) and weighing errors are not included but could change yields slightly to excessively.

[3] Interpreting Figure 3.1:

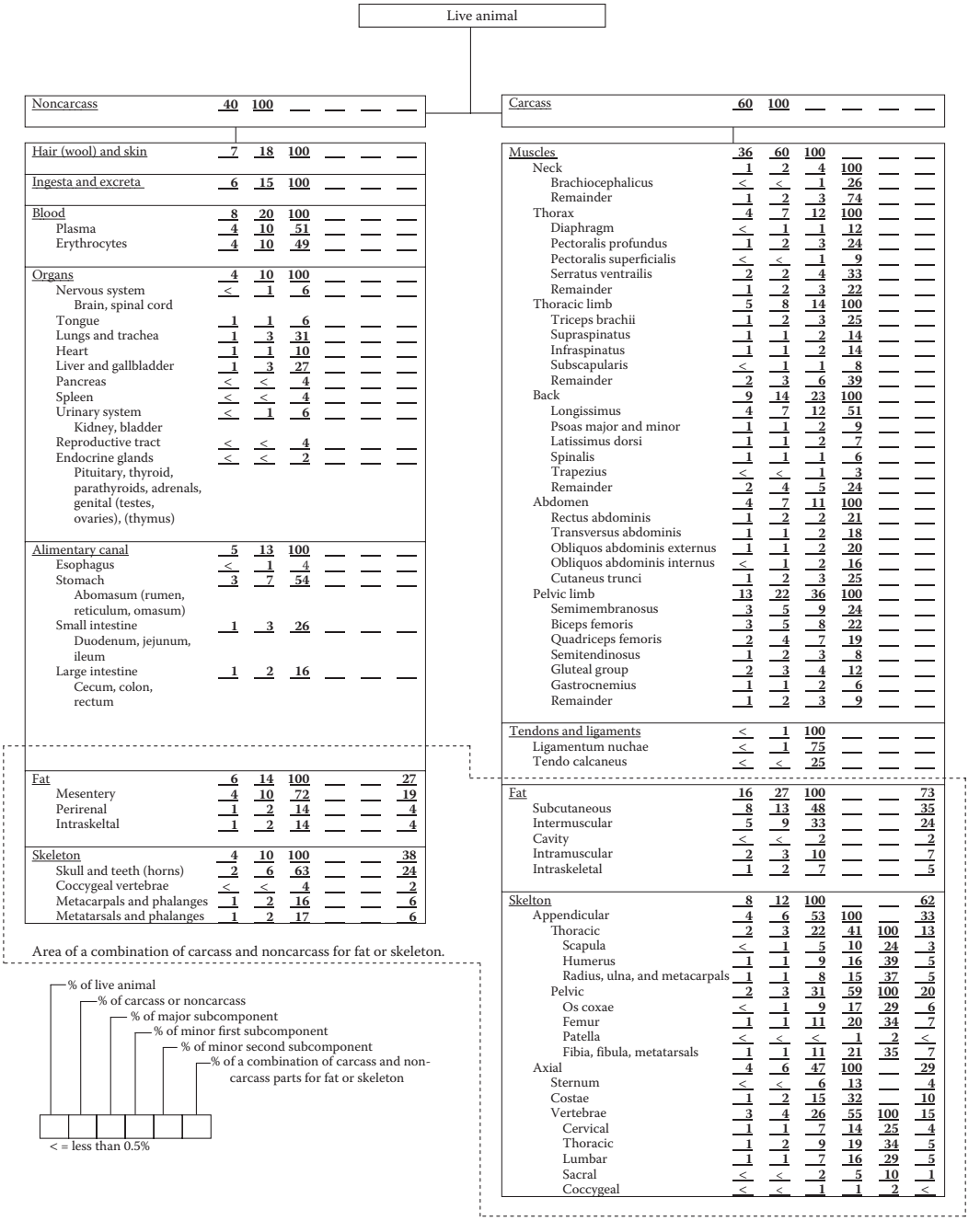
A. For a skinned, hot, lean carcass of a market weight, polled heifer, the base dressing percent would be 62%. The heifer had an empty GI tract so add 5% to base. She was 8 months pregnant so subtract 8%. Her hide was wet and dirty so subtract 5%. She was heavy muscled (mesomorph) so add 2%.
   Approximate net dressing % = 62 + 5 – 8 – 5 + 2 = 56%

B. For a lean carcass of a market weight wether lamb, the base dressing percent would be 52%. The wether had a full GI tract so subtract 5% from the base. He had a long fleece so subtract 5%. The wether was a double muscled Texel so add 7%. The carcass was chilled so subtract 2%.
   Approximate net dressing % = 52 – 5 – 5 + 7 – 2 = 47%

FIGURE 3.1 Twelve factors affecting dressing percentage of meat animals.

3.2.1.1 Visual Appraisal

Every time consumers purchase cuts of meat, they often select them on the basis of their lean/fat/bone ratios as estimated by visual inspection. This is simply accomplished through visual comparisons. Quantitatively, the method lacks accuracy, but for practical purposes in meat selection, it is effective, especially when compositional variations are large. On a more detailed basis, visual scores can be established with photographs and then the meat cuts scored on proportions of lean. However, scoring is too subjective to reflect quantitative differences and is too difficult to standardize to be consistently applied over time. Perhaps the one greatest value of visual inspection is in the estimation of proportions of intramuscular fat (marbling) when determining gross composition by dissection (in which dissecting marbling is essentially impossible).



Key

The data represents cattle, swine, and sheep. Prototype for this figure was cattle and appropriate carcass proportion of all components for swine and sheep should be 70% and 50%, respectively, and then adjusted proportionately. Each number following a part is its percent of larger parts by weight. Example: scapula is <0.5% of live animal, 1% of carcass, 5% of carcass skeleton, 10% of appendicular skeleton, 24% of thoracic limb skeleton, and 3% of combined skeleton parts. Sum of percents within a group = 100%. Parts in parenthesis may be absent due to species, sex, or stage of growth.

FIGURE 3.2 Approximate proportion of components of meat animals.

TABLE 3.1

Gross Compositional Variations among Animal Species

Live Weight, kg	Beef 550	Veal 160	Pork 110	Venison 70	Lamb 50	TomTurkey 16	Broiler Chicken 2	Farm-Raised Catfish 0.7
Average Proportions of Live Weight								
Noncarcass (%)	38	46	27	42	48	18	23	37
Carcass skin (%)	[a]	[a]	5	[a]	[a]	9	9	[a]
Carcass fat (%)	17	7	23	10	17	6	7	[a]
Carcass bone (%)	10	15	9	8	10	17	22	12
Carcass muscle (%)	35	32	36	40	25	50	39	51
Total	100	100	100	100	100	100	100	100
Dressing yield (%)	62	54	73	58	52	82	77	63
Carcass muscle/ bone ratio	3.5	2.1	4.0	5.0	2.5	2.9	1.8	4.3

[a] Included with noncarcass component.

3.2.1.2 Linear Measurements

A simple, inexpensive ruler can be used to measure subcutaneous fat thickness, muscle depth and width, and bone length and thickness. From these measurements, areas of each component can be estimated and then expressed as a proportion of the whole. Unfortunately, the areas are not exactly accurate, nor are unexposed bones and seam fat of different dimensions (as well as marbling) included in the estimate.

3.2.1.3 Area Measurements

By tracing the areas of the exposed muscles, bones, and fat on acetate paper and then measuring the exact areas of each component with a compensating polar planimeter, composition can be estimated. Also, such areas can be assessed using photometric, electronic, and computerized imaging techniques. Even though this is more accurate in determining the exposed areas, it has the same limitations as visual appraisal or linear measures because unexposed bone and seam fat as well as marbling cannot be accounted for.

3.2.1.4 Density

The Archimedean principle suggests that cuts of meat displace a volume equal to their own. Because the density of fat is less than that of muscle, such a technique, even though destructive if water displacement is used and expensive if gas displacement is used, would provide an accurate estimate of lean. However, for meat cuts containing bones, the technique would not be satisfactory. The density of bone is nearly twice that of muscle and would bias the estimate of muscle.

3.2.1.5 Anyl-Ray

This is based on x-ray attenuation as an index of tissue fatness. It generates electromagnetic waves of a character sensitive to absorption and reflection or back scatter by the elements in ground meat. The radiation is directed through a sample where size, shape, compaction, and weight must be constant while sample composition varies. When calibrated intensity of radiation is directed through a sample, this energy intensity is directly proportional to sample composition. A carefully mixed and selected ground meat sample is subjected to a minute amount of carefully controlled x-rays. Because lean absorbs more x-rays than fat, there is a difference in energy transmitted. The penetrating rays are collected by a radiation-measuring device, which in turn energizes a calibrated digital percentage fat meter. In the past,

it has been used to determine fat content in ground meat used for processing. The method is fast, requires a sample that can be reutilized for processing after measurement, and has a high degree of accuracy. The instrument correctly evaluates meat at any temperature, provided it can be properly compacted in the container. Further grinding or the addition of warm water may be necessary to achieve proper compaction for frozen samples. However, it is a relatively expensive method for determining the composition, and it cannot be used for small meat cuts or ones containing bone, and it estimates only fat and no other specific chemical components.

3.2.1.6 Dissection

This is the one most effective method of determining the gross composition of whole carcasses or individual wholesale or retail cuts. The method can be standardized and is highly repeatable in application. The method requires knowledge of anatomy and the patience and care to separate each component, preventing weight loss through evaporation and drip, and weighing and recording accurately. However, it does not account for variations of marbling, which would have to be assessed visually or subjected to chemical evaluation.

3.2.1.7 Proximate Analysis

For animal tissues, the primary chemical components used as a follow-up to or an alternative for physical dissection includes moisture, protein, lipid, and ash. The procedures for chemically analyzing each of these are described in the association of official analytical chemists (AOAC 1999). A major concern in using this method is adequate mixing and sampling of the tissues to be analyzed. Another limitation is in chemically analyzing bone because of the difficulty in grinding and sampling. (This does not apply for the ash determined in muscle.) Also, mixing ground components of soft tissues creates problems of fat collecting on the sides of the mixer. Finally, when moisture is being assessed, large errors occur when muscles are soft and watery or when excessive evaporation of moisture is lost from the surfaces of unprotected samples.

To determine the detailed chemical composition of muscle, fat, or bone, such as specific minerals, myofibrilar proteins, fatty acids, individual vitamins, and bound versus free water, numerous detailed and often extremely difficult, expensive, and sensitive chemical and spectrophotometric procedures are required. These procedures are not identified and described here because of their complexities and the need to maintain brevity.

3.3 Description and Composition of Muscle and Its Modifiers

3.3.1 Description

Meat animals contain, as a majority of their carcass weight, many muscles distributed in an unusually designed pattern to move the skeleton, for posture control, and for more specialized functions such as respiration, swallowing, and peristalsis. This musculature is categorized into two major types: striated and nonstriated. The less voluminous nonstriated or smooth muscles have some similar functions as striated muscles but possess different histological structures. Smooth muscles are primarily found in the linings of the gastrointestinal tract and the circulatory system as well as in specialized organs such as the gizzard of birds.

Striated muscles are categorized as either cardiac or skeletal. Cardiac muscles are confined to the heart and have the continuous responsibility of distributing and collecting blood throughout the body. Structurally, they are similar to skeletal muscles, except that they are more highly aerobic in their metabolic properties and therefore require higher concentrations of oxygen for their rhythmic contractions. Skeletal muscles are, as the name implies, associated with the skeleton; they either lie next to a bone or are attached to various bones, either closely or indirectly through their connective tissue fascia that may

attach directly or indirectly by tendons to distant bones. Depending on function and needs, skeletal muscles contract and relax and have very exacting cross-banding patterns.

Skeletal muscles play a major role in locomotion and posture control as well as in protecting vital organs. On average, the meat animal carcass contains about 100 bilaterally symmetrical pairs of individually structured muscles. There are large ones and small ones, depending on function and location. They have different shapes, colors, and concentrations of tendons. Many have a fusiform, multipennate shape, having a large middle portion that tapers at the ends. The attachments contain large quantities of tendinous connective tissue that attaches to bone. The long head of the triceps brachii would be an example of a fusiform-shaped muscle. Other shapes include flat or sheet-like muscles such as the cutaneus trunci, round-shaped muscles such as the quadriceps femoris, and irregular shapes such as the tensor fasciae latae, which has more than two attachments and is somewhat triangular shaped with thick and thin portions. In the more distal portions of the limbs, small muscles are uniquely attached to tendons for the specific purpose of either flexing or extending the feet and legs. In the more proximal locations, the muscles are larger and primarily serve as major sources of power. This is particularly true of the pelvic limb musculature. There are less than ten major pelvic muscles, whereas there are twice as many of smaller size in the thoracic limb. The longissimus thoracis et lumborum is the longest and largest muscle in many species and is located in the back to support the axial skeleton and to extend and erect the vertebral column. The flat muscles, generally located in the abdominal wall, support the abdominal cavity and its contents. Commercially, the flat muscles have less economic importance but they are the only ones found in bacon.

Skeletal muscles have a complex composition because they contain, in addition to muscle fibers, large quantities of supportive connective tissue, a complete vascular supply, and a nerve supply controlling each of the billions of muscle fibers. Also, skeletal muscles serve as storage depots for lipids and contain considerable quantities of extracellular fluids, primarily consisting of water.

Postmortem muscles vary in color, ranging from a dark purplish-red to a pale, light gray. This variation is primarily the result of myoglobin concentration as well as other biological factors such as pH. Myoglobin is a protein physiologically important in the transfer of oxygen and carbon dioxide to and from muscles during their normal metabolic activities. Breast muscles of poultry (pectorales superficiales) are very pale or white in color and contain low quantities of myoglobin, whereas leg muscles of venison are extremely dark purple and contain more than twice as much myoglobin. Striated muscles are multinucleated, distinguishing them from smooth muscles, which are mononucleated. These nuclei are near the sarcolemma cell wall whereas in smooth muscles, the nuclei are more centrally positioned. Skeletal muscles contain mitochondria, but not as many as are found in cardiac muscle. Other organelles such as ribosomes and the Golgi apparatus are also found in muscle fibers. Each fiber is surrounded by an intricate membrane, the sarcolemma, which surrounds the sarcoplasm that bathes the myofibrils, which are the contractile units of the fiber. Lipid particles in the form of neutral droplets and free fatty acids as well as glycogen granules are distributed throughout the sarcoplasm. (In postmortem muscles, glycogen is metabolized to lactic acid.) Enzymes are located in mitochondria and in other portions of the sarcoplasm. The sarcoplasmic reticulum and transverse tubules are responsible for the storage and transportation of calcium for contraction and relaxation.

To permit muscles to function properly as moving forces, they are harnessed to the skeleton through a unique set of connective tissue structures. This connective tissue "harness" circumvents the entire muscle and is called the epimysium; it winds its way through each muscle, dividing fibers into groups called fascicular bundles. The connective tissue at this level is called perimysium. The perimysium subdivides further into endomysium, which lines each fiber. The vascular system, which winds its way through muscles to supply the nutrients and remove toxic wastes, is closely related to individual fibers. In both the extracellular spaces and within fibers, there are fluids high in water content. In addition to the water, there are minerals, some water-soluble proteins, nonprotein nitrogenous materials, and other organic entities. Lipid in the form of neutral triglycerides is stored in the adipose tissue cells, which accumulate around venules and arterioles in the interfascicular spaces. This fat, when visible, is called marbling. Excluding water, the major components in muscle are the contractile proteins, which make up the myofibrils.

3.3.2 Gross Composition

A simpler approach to assessing the composition of muscles is to use proximate analyses to quantitate moisture, protein, lipid, ash, and carbohydrate. Muscle composition varies considerably and the accumulation of lipid is the most influential on this variation. On average, most muscles should contain about 1% ash (primarily represented by the elements potassium, phosphorus, sodium, chlorine, magnesium, calcium, and iron), 1% carbohydrate (primarily glycogen antemortem and lactic acid postmortem), 5% lipid, 21% nitrogenous compounds (predominantly proteins), and the rest (72%) as water. These values are compared to the composition of fat and bone as shown in Table 3.2. Some muscles may contain as much as 15% lipid (fresh weight basis), whereas others may contain <2%. Regardless of the lipid content, the protein/moisture ratio of about 0.3 remains quite constant for mature muscles. If time and expenses are limited, one may quickly, easily, and somewhat accurately assess proximate composition of muscles by making a few assumptions, using moisture analysis for the only determination. If it is assumed that ash and carbohydrate will not vary greatly and that their sum contribution is estimated at 2%, and if it is assumed that the protein/water relationship is 0.3, then if water is determined by homogenizing the sample and drying it, the only unknown left to be estimated is lipid content. This is calculated by difference. For example, if a sample (analyzed for moisture content) contained 70% moisture (M), then protein (P) content would be equal to $P/M = 0.3$, or $P/70 = 0.3$. Therefore, $P = 21$ or 21% protein. By subtracting the sum [2% (ash and carbohydrate) + 70% (M) + 21% (P)] from 100%, then lipid would be 7% or [100 – (2 + 70 + 21)].

3.3.3 Molecular Composition

There are a host of chemical compounds in muscle. They include free fatty acids, glycerol, triglycerides, phospholipids, nonprotein nitrogenous components such as DNA, RNA, ammonia, amine groups, and vitamins. There are glycogen granules and ATP. Myoglobin is present. Several minerals are present in minute quantities. Most important from a quantitative perspective are the various proteins of each fiber. These proteins are classified into four groups, the largest of which is myofibrillar. Myofibrillar proteins represent about 60% of the total proteins, whereas sarcoplasmic proteins represent 29%, stroma proteins 6%, and granular proteins 5%. Figure 3.3 is included to provide a detailed overview of the complexity of muscle composition. It is not intended to be precisely accurate nor to be memorized, but to serve as a reference to identify the various components of muscle and their quantitative contributions to its mass. It is assumed that these values represent mature, postrigor muscles of various species. Of all information presented in this chapter, Figures 3.2 and 3.3 should receive special attention because they are a detailed summary of the most important features of meat composition. (It required more time and effort to construct these figures than everything else combined in this chapter!) It should be understood that the methods of analysis used to determine most of the components of Figure 3.3 greatly affect the quantities reported.

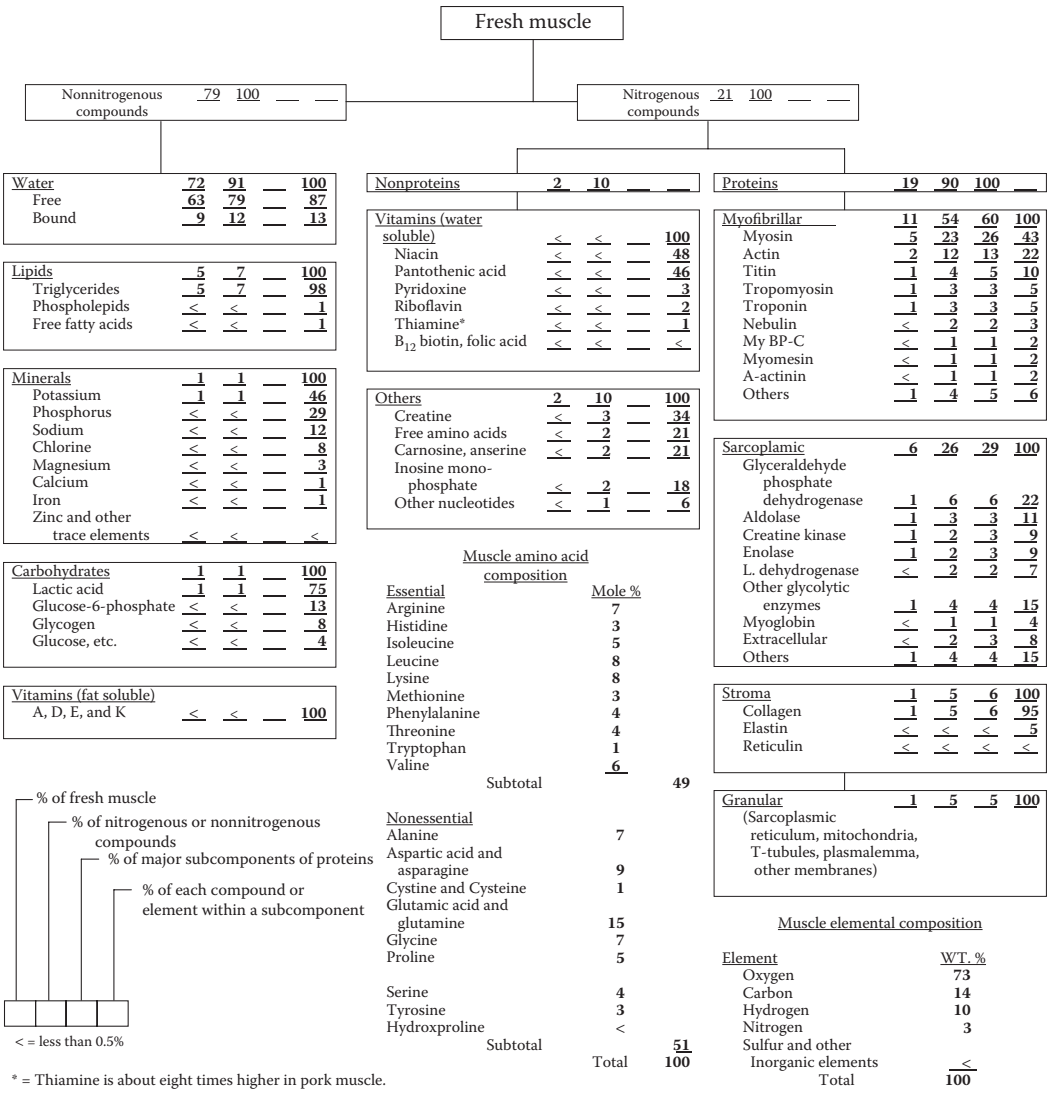
TABLE 3.2

Comparative and Approximate Gross Compositions of Muscle, Fat, and Bone[a]

	Muscle	Fat	Bone
Moisture (%)	72	9	25
Nitrogenous compounds (%) (primarily proteins)	21	1	10
Lipid (%)	5	90	20
Ash (%)	1	<[b]	45
Carbohydrate (%)	1	<[b]	<[b]
Total	100	100	100

[a] Proximate analysis expressed on a fresh basis for mature, postmortem tissues representing various anatomical locations.

[b] <0.5%.



Data are for mature, postrigor muscles representing various species. Each number following a part is its approximate percentage of larger parts by weight. For example: Myosin represents about 5% of fresh muscle, 23% of nitrogenous compounds in muscle, 26% of all proteins and 43% of myofibrillar proteins.

FIGURE 3.3 Fresh muscle composition.

The myofibrillar proteins are responsible for the contractile mechanisms and thus shorten or lengthen the muscle for movement and support functions. Sarcoplasmic proteins are primarily represented by enzymes and myoglobin. Stroma proteins originate from the connective tissue structure found as a part of muscle, the most important quantitatively being collagen. Collagen is resistant to most enzymatic reactions except collagenase. When heated in water, collagen is converted into gelatin, which is readily hydrolyzed by several enzymes. About one-third of collagen's amino acid residues consist of glycine, whereas another one-fifth is proline and hydroxyproline. It is the only protein known which contains hydroxyproline, with the possible exception of reticulin. Hydroxyproline analysis is often used as a measure for determining the total connective tissue in muscles. Another stroma protein of less concentration is elastin. It is even more resistant to degradation: to degrade, it must be subjected to high temperatures in the presence of strong bases or acids. Elastin contains about one-third of its amino acid residues as

glycine and over one-tenth as proline. Reticulin is the other major stroma protein. Its amino acid composition is similar to that of collagen, and it is often considered a form of collagen that contains lipids and carbohydrates.

There are nine known major myofibrillar proteins, as illustrated in Figure 3.3. Quantitatively, the one most important protein is myosin. In referring to Figure 3.3, myosin represents 43% of the myofibrillar proteins, 26% of all muscle proteins, 23% of all nitrogenous compounds, and 5% of the fresh muscle mass. Myosin is the thick strand of protein that appears in the sarcomere structure. Actin represents about 22% of myofibrillar proteins and is the thin filament within this same contractile formation. The other seven proteins represent smaller compositional fractions, but play equally important roles in contraction. Titin represents 10% and has by far the largest molecular weight and is considered more structural than metabolic in function. Tropomyosin and troponin each contribute about 5% and can be found attached to the actin molecule and are primarily responsible for initiating contraction after calcium has been released by the sarcoplasmic reticulum. All the other proteins combined represent <20% of the weight.

All the above mentioned proteins are composed of the 22 amino acids shown in Figure 3.3. Each amino acid is different according to the molecular characteristics of its side chain. The 10 essential and 12 nonessential amino acids and their mole contributions to muscle mass are included in Figure 3.3.

In addition to the proteins, there are other important nitrogenous constituents in muscle. First are the vitamins, which are divided into two classes based on their solubility in either aqueous or nonaqueous solutions. The lipid-soluble vitamins are minimal because of the small quantities of fat normally deposited in most muscles. However, water-soluble vitamins, primarily the B vitamins, are present in substantive enough quantities to serve as appropriate sources to meet daily dietary requirements for humans. They include thiamin, riboflavin, niacin, pyridoxine, pantothenic acid, biotin, folic acid, and B12. Ascorbic acid (vitamin C) and calcium are essentially absent in muscles, and because of this, muscles are not considered a perfect food from a nutritional perspective. The nitrogenous, nonprotein extractives include creatine, nucleotides, ammonia, methylamines, free amino acids, and other derivatives of proteins. Two of the components in highest concentrations are carnosine and anserine. Other extractives include volatile organic carbonyls, such as acetyl aldehyde, acetone, carbon dioxide, and formaldehyde, all of which have been found in muscles. Various sulfur compounds include hydrogen sulfide, methylmercaptans, and methyl sulfides.

The elemental components include carbon, hydrogen, and oxygen in great abundance either because of their molecular weight or number of molecules and are listed in Figure 3.3. In addition, nitrogen is abundant because it is a component of all proteins. Some minute quantities of sulfur are present in the form of the amino acids cystine, cysteine, and methionine. Inorganic minerals include calcium, magnesium, sodium, potassium, chlorine, phosphorus, and iron, but their contributions to mass are minimal. In assessing the various elements in the various components of muscles, in most instances—whether proteins, lipids, carbohydrates, vitamins, or nucleic acids—the elements carbon, hydrogen, and oxygen are always present. The unique compositional difference among proteins, nucleic acids, and vitamins is that in proteins, nitrogen molecules are in the side chains; in the other two groups, nitrogen molecules are incorporated into the ring structures. The protein myoglobin is somewhat of an exception in structure in that it contains a heme group as well as a globular protein fraction and contains iron as its central ion in the heme ring. The iron element in myoglobin is paralleled by the cobalt element in vitamin B-12.

3.3.4 Modifiers of Muscle Composition

General fatness of the animal influences the composition of muscles. Individual muscle fibers remain constant in their composition, but fresh muscle may vary from 1% to 15% in lipid content. This variation is due to such factors as genetics, stage of growth, sex, and the amount of physical exercise. As animals mature and muscles stop growing, intramuscular fat may accumulate around the vascular system, thus decreasing the relative mass of other components. The nature of the connective tissue matrix also affects the accumulation of fat. Loosely arranged muscles such as the latissimus dorsi, having parallel connective tissue strands, contain more fat than tightly compacted muscles such as the peroneus longus. The latter's connective tissue strands are thicker and more tightly structured, thus physically preventing excess fat accumulation.

Nutrition affects muscle composition simply by controlling the total lipid accumulation, depending on the total caloric intake and expenditure. In submaintenance diets, fat is mobilized (rather than deposited) from muscles. Quality of nutrition can also affect the mineral and vitamin content of muscles, but not to the extent that fat deposition is affected.

Stage of growth affects the protein/moisture relationship of muscles. In very young animals, this ratio is low (~0.1), whereas at maturity, the relationship is about 0.3. As already indicated, this remains reasonably constant throughout the animal's life and serves as a reliable guide in estimating the composition.

In addition to the structural differences in connective tissue, anatomical location of muscles affects composition because some muscles contain higher concentrations of tendon and epimysial sheaths of connective tissue. Because of this, there is a difference in the quantity of stroma proteins as compared to myofibrillar, sarcoplasmic, and granular proteins. For example, lower limb muscles have higher concentrations of connective tissue proteins than do supportive back muscles. Even though the molecular nature of stroma proteins changes during growth, the absolute quantities do not change. Some muscles such as the gluteus medius and longissimus have proportionately more white fibers requiring less oxygen. Therefore, their energy needs for muscle contraction are more anaerobic than that of muscles containing more red fibers. Consequently myoglobin concentration is lower and this may be true for fat content as well. An exception to this is the trapezius. It contains over 60% red fibers but also contains high amounts of lipid. The semitendinosus contains two clearly defined portions, one having predominantly red fibers and the other predominantly white fibers. As a result, molecular composition within this muscle varies considerably. However, in this example, the white fiber portion contains considerably more lipid than the red fiber portion, suggesting that muscle location and function affect composition more than fiber type per se. Perhaps fiber type affects composition primarily by its effect on postmortem tissue characteristics. The postmortem musculature originating from short-term stressed animals (especially those genetically susceptible to stress) become soft and watery and are much more susceptible to exudation during processing. Therefore, composition is readily affected if processing is considered. Dark, firm, and dry (DFD) muscles that contain high concentrations of red fibers (often the result of long-term antemortem stress) are less susceptible to such abnormal postmortem shrinkage.

Disease influences composition. Portions of muscles may be eroded away by muscular dystrophy and replaced with fat. Certain inorganic elements are lost from the tissues during stressful conditions related to disease. Certain central nervous system diseases also affect the general composition of muscle, primarily affecting the fat component. Injury to muscles affects composition. When major nerves are severed (accidentally or experimentally) the muscle atrophies and fat accumulates in the vacated spaces.

Exercise stimulates fiber hypertrophy and mobilization of lipids within muscles. However, there is little evidence suggesting changes in other chemical components.

Genetics affects fatty accumulation in muscles because of its relation to rate of maturity. Certain species of animals, such as the domestic duck, deposit very little fat in muscles. The rabbit has similar tendencies, whereas certain breeds of pigs, cattle, and sheep deposit large quantities of intramuscular fat. Within species, some breeds have greater tendencies to deposit intramuscular fat. Duroc swine appear to contain more intramuscular fat for a given degree of body fatness and age. Hereford and Charolais cattle do not deposit as much intramuscular fat at a given physiological state of maturity as do Angus cattle.

Fat in muscles is related to the total fatness of the body. When carcasses from obese animals are examined, there is generally more intramuscular fat than from those possessing leaner carcasses as compared to total body fat per se (e.g., Japanese Wygue cattle producing highly marbled Kobe beef), whereas other animals deposit large quantities of subcutaneous fat but deposit very little intramuscular fat.

Muscles grow at different rates and mature at different physiological times. This in itself affects composition. These differences are small, but if a muscle matures earlier and also has the structural potential for accumulating fat, then it will have a higher fat content at a given age than another muscle that matures at a later stage. This variation is also responsible for differences in protein/moisture ratios among muscles.

Control of various body processes by the endocrine system affects fat deposition in muscles. Thus by the presence or absence of testosterone, fat deposition is regulated in muscles. Bulls, rams, and boars possess muscles containing less intramuscular fat than male castrates (steers, wethers, and barrows). When cattle are fed diethylstilbestrol (a synthetically produced hormone that is currently banned from

use), it changes the composition of muscles simply by slowing down the animal's physiological time clock. The supplementation of certain hormones will increase fat mobilization in muscles; however, most of these changes are small.

3.4 Description and Composition of Fat and Its Modifiers

3.4.1 Description

Fat is often associated with such words as obese, plump, oily, wasty, greasy, big, and thick. Also it has been defined as the excess "white stuff" that is trimmed from a piece of meat, or the grease that lubricates a skillet, or some of the juices that flow out of hamburgers during cooking, or the foodstuff that is responsible for making foods "rich" because of its high caloric content. Fat has probably received more attention from consumers, and has been referred to more often, than any other single biological substance.

Epithelial, nervous, muscular, and connective are the four basic tissues involved in the processes of postnatal growth. Connective tissue's primary function is structural support: it is responsible for the physical shape of such biological substances as bone, cartilage, muscle, and fat. Adipose tissue is a type of connective tissue that surrounds synthesized lipids, which serve as heat–cold insulators and as reserve supplies of body energy. Therefore, fat is defined as a collection of adipose cells suspended in a matrix of connective tissue distended with cytoplasmic lipids, water, and other constituents.

Often the words fat and lipid are used interchangeably. Generally this is appropriate, but specifically it is an incorrect concept. Adipose or fatty tissue contains lipids, but lipids per se do not contain connective tissue, water, enzymes, and other constituents present in fat. However, because lipids are the major components of fat, it is important to describe these lipids in greater detail. Lipids include that group of nonpolar compounds soluble in organic solvents but insoluble in water. Pure lipids are colorless, odorless, and flavorless and can be classified as follows.

3.4.1.1 Simple Lipids

Simple lipids are esters of fatty acids with certain alcohols such as glycerol. If lipids are solid at room temperature, they are called fats; if liquid, oils. Waxes are simple lipids that are esters of fatty acids with long-chain aliphatic alcohols or with cyclic alcohols. Examples of waxes include esters of cholesterol as well as the vitamins A and D.

3.4.1.2 Compound or Conjugate Lipids

Compound or conjugate lipids are esters of fatty acids that, on hydrolysis, yield such substances as phosphoric acid, amino acids, choline, carbohydrates, and sulfuric acid, in addition to fatty acids and an alcohol. Examples include phospholipids, glycolipids, sulfolipids, and lipoproteins [such as low-density lipoprotein (LDL) found in blood].

3.4.1.3 Derived Lipids

Derived lipids are formed in the hydrolysis of simple or compound lipids. Examples include saturated and unsaturated fatty acids, aliphatic alcohols, sterols, alcohols containing the β-ionone ring, aliphatic hydrocarbons, carotenoids, squalene, and the vitamins D, E, and K. Fat is found in nearly every anatomical location imaginable, but the great majority of it occurs subcutaneously, inter- and intramuscularly, in the mesentery, on the walls of the thoracic, abdominal and pelvic cavities, and in bone marrow (intraskeletal). Fat is deposited in the udders of females and in the scrotal sacs of male castrates. Fat is deposited in brain, liver, and kidney, and the quantity may be excessive under abnormal conditions (diseases).

Lipids are found in some form in all body cells because phospholipids contribute to the structure of every cell wall. Blood and lymph contain lipids, the quantity varying greatly with time after an animal consumes a fatty meal. All dietary fats are transported to body tissues via one of these routes. Although

adipose tissue is ubiquitous, it is not evenly and universally distributed in obesity, but is deposited in certain preferential sites while other sites are spared. For example, feet, eyelids, nose, ears, and genitalia seldom accumulate excess fat.

3.4.2 Gross and Molecular Composition

Quantitatively, Table 3.3 represents an example of variations in proportionality of fat in different anatomical locations and it is expressed on both a dissectable and an extractable basis. For this example, as the animal matures, the total fat increases when compared to other body tissues, but for the most part the proportionate distribution remains reasonably constant. On an extractable basis, the internal cavity fats (cavity wall and mesentary) represent about one-tenth, intermuscular about one-fifth, intramuscular and intraskeletal about one-fourth, and subcutaneous over one-half of the total lipids deposited, regardless of stage of growth. Even though intramuscular and intraskeletal lipids are not included in the dissectable allocation, the total representation of extractable lipids (as a percentage of empty body weight) are lower than for the dissectable fat values because water is not included when extractable portions are expressed. One must remember that fat contains significant quantities of water. Table 3.2 includes the gross composition of fat and how it compares to that of muscle and bone.

Adipose cells vary in size depending on such factors as age, species, and state of nutrition. The increase in numbers of adipose cells does not necessarily dictate the quantitative amount of fat deposited. For mature pigs, about 45% of the total adipose cells are in intramuscular fat but this fat component represents <15% of the volume of extractable lipid. This is verification that cell hypertrophy contributes more to volume than does cell hyperplasia.

Lipids dominate in their contribution to adipose volume and weight. However, other constituents are also present. In immature tissues, there is a significant quantity of water. Also, because adipose tissue is structurally supported by a connective tissue matrix, the extracellular protein collagen is present. Other substances include enzymes responsible for lipogenesis and lipolysis, traces of certain minerals and minute quantities of glycerol, glucose, and glycogen as well as nervous tissue.

TABLE 3.3

Distribution of Pig Body Fat or Lipid During Growth

	Stage of Growth (Days of Age)		
	21–43	**64–106**	**149–213**
Dissectable Fat Basis			
Total fat as % of empty body	15	20	25
Distribution by Location			
Cavity wall (%)	5	5	7
Mesentary (%)	8	13	8
Intermuscular (%)	29	25	28
Subcutaneous (%)	58	57	57
Sum	100	100	100
Extractable Lipid Basis			
Total lipid as % of empty body	7	10	16
Distribution by Location			
Cavity wall (%)	2	3	6
Mesentery (%)	2	4	4
Intermuscular (%)	18	16	18
Subcutaneous (%)	51	54	52
Intramuscular (%)	20	14	11
Intraskeletal (%)	7	9	9
Sum	100	100	100

Adipose tissue lipids are primarily present as mono-, di-, or triglycerides. Each is composed of a molecule of glycerol bonded to one, two, or three fatty acids. These acids are either synthesized in the adipose cell or are synthesized in the liver and subsequently transported to the adipose cell via the circulatory system. Fatty acids in adipose tissue usually contain 16 or more carbon atoms, but there are a few that are shorter. The carbon chain may be completely saturated with hydrogen atoms, or there may be some double bonds, and these are called unsaturated fatty acids. The dietary origin of the fat dictates the variation expected and the iodine and saponification values reflect such variations. Oleic, palmitic, and stearic acids represent over 80% of the composition of meat animal lipids. However, the primary difference in physical properties of lard as compared to beef tallow is the higher quantity of linoleic acid that is more unsaturated and occurring in higher proportions in lard, giving it a softer structure at room temperature. Also, this higher degree of unsaturation results in a fat more susceptible to oxidative rancidity.

3.4.3 Modifiers of Fat Composition

The discussions that follow will parallel some of those already covered in the muscle section and thus will be limited to unique differences pertaining exclusively to fat. When compared with mature animals, young ones contain adipose tissue having considerably more water. Also, the phospholipid component is proportionally higher in young animals as compared with their triglyceride content.

For ruminants, most dietary fats are digested in the rumen where ingested unsaturated fatty acids are hydrogenated and then absorbed into the circulatory system. However, for monogastric animals, ingested unsaturated fatty acids are not hydrogenated and are absorbed and deposited in adipose tissue in their original structures. Therefore, this explains why pork fat is softer than beef tallow. The release of some hormones will stimulate mobilization of fatty acids. If this persists, the triglyceride fraction will be significantly reduced.

Anatomical location of fat is important when determining its composition. The number of intramuscular adipose cells represents nearly half the total adipose cell population of the body, yet the amount of extractable lipid is <10%. These cells are smaller and contain more water than do cells located subcutaneously. Also, within subcutaneous tissue, that portion located at the base of the pelvic limb contains less extractable lipid than the cells located over the back. Furthermore, the three distinct layers of subcutaneous fat over the back of pigs vary in fatty acid composition. The outer layer contains greater proportions of unsaturated fatty acids as compared with the other layers. Mesentary adipose tissue contains more saturated fatty acids than subcutaneous tissue; udder fatty tissues contain more fluid, nonlipid material, and less extractable lipids than other adipose tissues. Finally, certain organs such as the bovine kidney contains 30% oleic acid and 33% stearic acid as compared with bovine subcutaneous fat, which contains 40% oleic acid and <20% stearic acid.

Genetic variables influence the quantity of fat and its composition. Some examples include (a) more hydrogenated fatty acids in ruminants than monogastric species, (b) double-muscled cattle do not deposit fat as quickly as normal cattle, (c) some breeds of sheep accumulate greater quantities of fat over the rump, and (d) pigs contain more subcutaneous and less intermuscular fat than sheep or cattle. Females have the capacity to lactate, which is a unique process of fat accumulation in milk. Intact males of most species contain less fat than castrate males or females of similar chronological ages. Heifers contain more fat than steers at a given age whereas gilts contain less fat than barrows at a similar age. This observation may simply reflect variations in stages of compositional, physiological, and sexual maturity.

Atypical conditions such as obesity and steatosis (excessive fat deposition in muscles) increases the quantity of lipids deposited. Conversely, exercise and various environmental stresses reduce lipid deposition.

3.5 Description and Composition of Bone and Its Modifiers

3.5.1 Description

Bone is a complex tissue and subject to continual metabolic activity. A most obvious difference, when compared to muscle and fat, is its dense, hard, mineralized, cellular type tissue. The three cellular

components of bone are of one cell type and may change in morphological characteristics directly according to specific functional needs of the tissue. The cells involved include osteocytes which are responsible for maintenance; osteoplasts, which are involved in formation of new bone; and osteoclasts, which are responsible for mobilization and reabsorption of bone material. Histologically bone is characterized by its branching lacunae, which are cavity-like membranous materials, and by canaliculi, which are fine-structured canals. Bone contains a dense matrix of collagenous fibrous bundles in a ground substance encased with calcium and phosphorus. Bone is capable of structural alterations to accommodate stresses due to mechanical changes and biological demands incurred by pressure and by vascular, nerve, endocrine, and nutritional influences. Most of the rigid material in the skeletons of meat animals is either compact or cancellous bone. This indicates that there are different degrees of mineral density in the bone including available porous spaces which provide for the accumulation and maintenance of the marrow.

Another method of classifying bone is on the basis of bone formation. Some bones develop within mesenchymal tissue such as the skull, which is known as membrane bone. Other bones depend on prior cartilaginous scaffolding, such as the vertebrae, and this is called cartilage bone. This cartilage-type bone contains collagen and polysaccharides.

There are more than 200 individual bones in meat animals, and they are either on the axial skeleton or the appendicular skeletons (limbs). Figure 3.2 includes the major bones and their proportionate masses in the live animal. They all include bone marrow, which produces the majority of the red blood cells. They store minerals and mobilize them as needed for other body tissues. They repair themselves after an injury and are designed to provide the greatest support with a minimum amount of weight; this is why most bones have hollow structures.

3.5.2 Gross and Molecular Composition of Bone and Its Modifiers

Bone basically contains mineral deposited in an organic matrix. The matrix includes not only calcium, phosphorus, and carbonate, but also citrate, water, and small amounts of sodium, magnesium, potassium, fluorine, and chlorine. The crystals of bone mineral have a chemical composition similar to that of fluoral apatite. Fibers of collagen run throughout the matrix. The remaining space in the bone "mortar" is filled with a semiliquid substance that exchanges materials to and from the bone mineral via the circulating blood.

More than 99% of the body calcium is in bones. Collagen is about 93% of the total organic portion of bone. There are small amounts of insoluble sclera proteins and ground substance that are composed of mucopolysaccharides and mucoproteins. In fat-free analyzed bone, the mineral content accounts for about two-thirds of the mass, whereas in fresh bone it is about two-fifths. As included in Table 3.2, water represents about one-fourth the mass, protein about one-tenth, and the remaining one-fifth portion (which is the most variable) is lipid. As indicated, type of bone, age of the animal, and species are three factors that most affect bone composition.

Bone ash is composed mostly of calcium and phosphorus and much lesser quantities of magnesium, sodium, potassium, chlorine, and fluorine. When comparing bone to fat and muscle as shown in Table 3.2, the average composition is considerably different. If bone is compared to the Achilles tendon (almost entirely connective tissue), the tendon consists of two-thirds water and one-third organic solids with very little inorganic material. This compositional profile is quite similar to muscle. For the ligamentum nuchae, which is slightly more similar to bone, water content is about 57% and organic solids make up most of the remainder, but the elastin content is considerably higher than that for tendon.

The factors modifying the composition of bone are quite similar to that of muscle and fat and will not be repeated here. As illustrated in Table 3.4, age, species, and type of bone are three major modifiers of bone composition. Other unique factors modifying bone composition are (a) absence of vitamins D and A from the diet, (b) abnormalities in endocrine secretions (both low and high quantities), (c) lack of mineral supplementation in the diet (especially calcium and phosphorus), and (d) wasting-type diseases that mobilize mineral content from the bone, creating brittle and friable structures that have been significantly altered in composition.

TABLE 3.4

Variation of Bone Composition When Comparing Species, Age, and Bone Type

		Moisture (%)	Lipid (%)	Ash (%)	Protein (%)	Sum (%)
Cattle	2–3 months	55	7	19	19	100
	48–96 months	27	17	47	9	100
	Costae	37	9	37	17	100
	Femur	34	21	39	6	100
Pigs	6–8 months	37	18	33	12	100
	24–48 months	25	23	43	9	100
	Costae	33	18	37	12	100
	Femur	26	28	43	3	100
Sheep	5–6 months	39	11	33	17	100
	48–96 months	23	16	49	12	100
	Costae	32	9	39	20	100
	Femur	24	23	47	6	100
Chickens	2–3 months	50	11	24	15	100
	12–13 months	39	9	34	18	100
	Costae	42	10	29	19	100
	Femur	44	12	30	14	100

Source: Data extrapolated from Field, RA. et al. 1974. *J Anim Sci* 39:493–499.

3.6 The Composition–Quality Paradox of Meat

When evaluating meat, both composition and quality are important. Leanness (as contrasted to fatness) is virtuous, but by itself, it fails to meet ultimate expectations of consumers. The nutrient density of muscle is higher in lean meat and nutritive value is a part of quality. Therefore, from this perspective, composition affects quality. However, quality is more than just nutrient density. Wholesomeness, appearance, water-holding capacity, and palatability are quality virtues too! Marbling contributes to juiciness and flavor, however, more marbling reduces nutrient density. Furthermore, the exterior fat covering of fresh meat cuts is related to marbling. The association is not strong, but fatter cuts usually exhibit muscles containing more marbling. The paradox is that meat animals are fed to heavier weights, for longer times, and to ultimately less favorable "feed-to-meat" ratios so that muscles will contain more marbling to ultimately satisfy the consumer demands. This negative relationship between marbling and leanness is difficult to compromise and is one of the reasons why beef and lamb cuts may be too fat.

In pork and turkey, trim and heavily muscled carcasses appear to be more susceptible to the pale, soft, and exudative (PSE) condition. Meat cuts from such carcasses possess lean, heavily muscled cuts containing minimum quantities of fat. Nevertheless, the muscles often shrink excessively during processing. Fresh cuts of pork (loin and ham) and turkey (breast) that are exceptionally lean may be pale in color, soft in texture, and watery, all of which detract from appearance and ultimately their acceptance by consumers.

Therefore, quality of all meat products must be considered along with composition when assessing the overall value. The conflict between composition and quality continues to challenge scientists to discover new genetic combinations, different feeding and management programs, and more satisfactory postmortem processing technologies to ensure an ideal meat product that meets consumer demands.

ACKNOWLEDGMENTS

The author appreciated being invited to prepare this chapter. He thanks the dedicated scientists and teachers that contributed resources and advice and is especially indebted to the Department of Animal Sciences and the College of Agricultural and Life Sciences, University of Wisconsin-Madison, for

providing financial assistance, support, and encouragement. Finally, the reader is encouraged to carefully reexamine Figures 3.2 and 3.3. They summarize this chapter and serve as foundations for those that follow!

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4

Postmortem Muscle Chemistry

Marion L. Greaser and Wei Guo

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4.1 Introduction

The conversion of muscle into meat is a complex process involving many biochemical and physical changes. Muscle tissue is converted from an extensible, metabolically active system into one that is inextensible and quiescent with regard to its biochemical reactions. The speed and extent of postmortem metabolism has a profound effect on the properties of the muscle and its subsequent use for food. Also the extent of shortening during the postmortem period affects the meat's textural properties. This chapter summarizes our understanding of muscle's structural components, its metabolic pathways, and its behavior postmortem. Several books[1–5] and reviews[6–13] may be consulted for more extensive coverage of these topics.

4.2 Structure and Function of Living Muscle

4.2.1 Microstructure

4.2.1.1 Light Microscope Level

Skeletal muscle is the largest tissue component of meat animal species. A diagram showing the organization of muscle at various levels is shown in Figure 4.1. Whole muscle is usually connected to bone by tendons attached to a tough, nearly inextensible connective tissue layer called the epimysium. This layer is composed primarily of the protein collagen. The muscle is divided into bundles by thinner layers called the perimysium. Finally, each muscle cell or fiber is encased in a thin layer referred to as the endomysium.

The muscle cells when viewed longitudinally in the microscope have a striped or striated appearance. They were formed during embryonic development by the fusion of many precursor cells. The resulting muscle cells are typically long and cylindrical and contain numerous nuclei. The number of muscle cells remains relatively constant after birth. The increase in muscle mass during growth is due to large increases in cell length and diameter. Some of these precursor-type cells persist in the adult and are referred to as satellite cells. The satellite cells lie on the surface of the true muscle cells. A longitudinal view of a single muscle cell (double-headed arrow) is shown in Figure 4.2; this photograph is from muscle that has been fixed, paraffin embedded, sectioned, and stained. Note the nuclei (N) and the stripes that run perpendicular to the fiber long axis. Figure 4.2 also shows an adjacent capillary and blood cells (arrowhead).

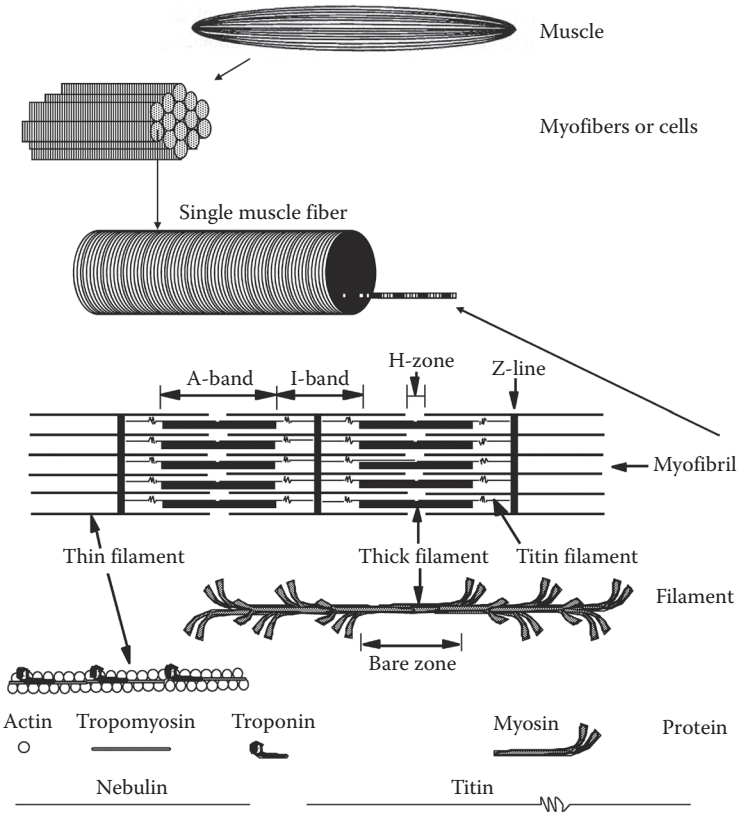

FIGURE 4.1 Diagram showing the levels of organization of muscle. (From Greaser, M.L. and A.M. Pearson. 1999. *Food Texture: Perception and Measurement*. pp. 228–258. Aspen Publishers, Inc., Gaithersburg, MD. With permission.)

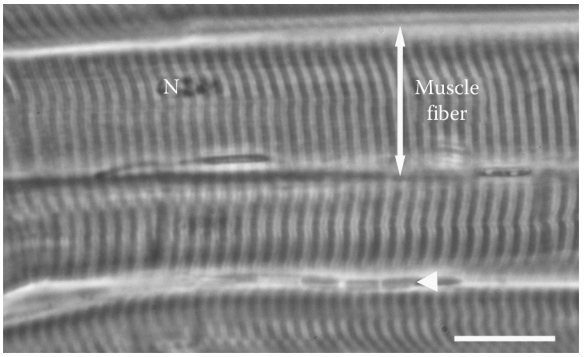

FIGURE 4.2 Light micrograph of a longitudinal view of several muscle fibers. This micrograph was prepared from muscle tissue that had been fixed, paraffin embedded, sectioned, and stained with hematoxylin and eosin. The double-headed arrow demarcates a single muscle fiber. The typical striped or striated appearance is visible. Each muscle fiber has multiple nuclei; one is shown with an N. Each muscle cell is adjacent to one or more capillaries (arrowhead) that supply oxygen and substrates for metabolism. Scale bar = 25 μm.

The alternating dark and light stripes are due to the presence of myofibrils inside the fiber. The individual myofibrils also have alternating stripes, and the striations in the fiber occur because the adjacent myofibrils have their respective light and dark bands aligned. The dark bands are called the A-bands and the light bands the I-bands (Figure 4.1). A thin perpendicular line referred to as the Z line bisects the I-bands. The banding structure is somewhat obscure in whole fibers and sections because the cell is too thick to have everything in focus. However, the myofibrils can be separately observed after disrupting muscle by homogenization and their band patterns are much clearer (Figure 4.3). The region between successive Z lines is called a sarcomere and it is the smallest functional unit of the myofibril. The lengths of the sarcomeres vary in suspensions of myofibrils due to their being derived from muscle cells which have varying degrees of shortening. The A-bands, if visible, always have the same length, but the I-bands decrease in length in myofibrils with shorter sarcomeres. Myofibrils with long sarcomeres have a zone in their middle that has somewhat lower intensity; this region is called the H zone.

4.2.1.2 Electron Microscope Level

An understanding of the filament arrangement that is responsible for these patterns became apparent with the advent of electron microscopy. The elegant work of Hugh Huxley showed that the sarcomere is composed of two major types of filaments.[14] The thick filaments (about 1.6 μm in length) are found in the A-band and they interdigitate with thinner filaments (about 1 μm in length) that attach to the Z lines (Figures 4.1 and 4.4). Muscle contracts by a relative sliding of these two filaments over one another. The filaments are in turn composed of proteins; myosin is the major constituent of the thick filaments and actin, troponin, and tropomyosin make up the bulk of the thin filaments. Two other narrow, filamentous proteins are present in the sarcomere but are not typically visible by electron microscopy of intact muscle. Titin, a giant protein that extends from the middle of the sarcomere to the Z line (Figure 4.1), is elastic in nature and believed to be important for myofibril assembly and for protecting the muscle from over-stretch.

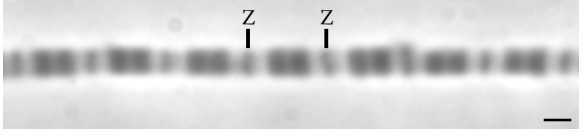

FIGURE 4.3 Light micrograph of a bovine psoas myofibril. The banding patterns visible in the intact cells are also seen in the myofibril. Alternating dark A-bands and light I-bands can be observed. The I-band is bisected by a thin line called the Z line. The section of a myofibril between a pair of Z lines is called a sarcomere. Scale bar = 1 μm.

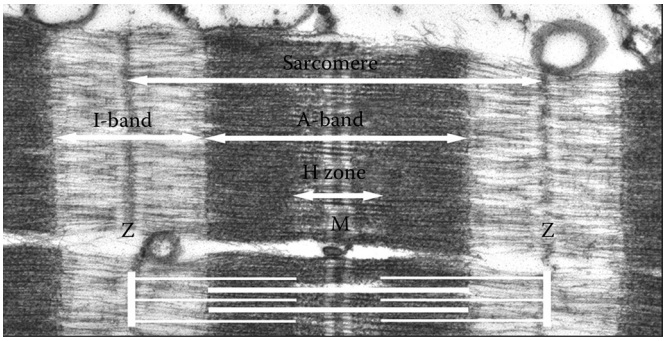

FIGURE 4.4 Electron micrograph of a longitudinal view of muscle. The banding pattern can be explained by the positions of the thick and thin filaments. The thin filament length is approximately 1 μm.

Nebulin is attached to the Z lines and is postulated to regulate the length of the thin filaments. These latter two proteins are sometime referred to as "cytoskeletal" proteins.

The sarcomere and its filaments are highly ordered in cross-section as well as longitudinally. The thick filaments are arranged in a hexagonal pattern. Six thin filaments surround each thick filament, and each thin filament is centered between three thick filaments.

4.2.1.3 Muscle Cell Heterogeneity

In spite of the high degree of filament order in muscle, there is considerable heterogeneity in the properties of the individual muscle cells. The striking color difference between the breast muscle of a chicken or turkey compared to the muscle of the thigh or leg is readily apparent. Early classification work grouped muscle cells into two groups, red and white.[15] The red muscle cells have more myoglobin (responsible for the color), have slower contraction speed, and typically rely on oxidative metabolism for ATP generation. The white muscles have more glycolytic enzyme content, have a faster contraction speed, and have an energy metabolism that depends on glycolysis. However, it soon became apparent that this simple two-type system for muscle fiber classification was inadequate. The best current fiber-type classification system is based on myosin isoforms (either using type-specific antibodies or histochemistry after various acid or alkaline pretreatment of muscle cross-sections). Type I fibers belong to the red group; type IIA, type IIB, and type IIX (or IID) constitute the white group. However, even this nomenclature is muddied by the fact that many fibers contain more than one myosin type. In most mammalian muscles, the fiber types are mixed even down to the fiber bundle level.

An example of the fiber type heterogeneity is shown with cross-sections from pig muscle (Figure 4.5). In Figure 4.5a, a frozen muscle section has been stained for the mitochondrial enzyme succinic dehydrogenase. The red, oxidative type I fibers having a darker appearance while the type II fibers are more lightly stained. In Figure 4.5b, the muscle section has been incubated at pH 4.7 prior to exposure to ATP and a phosphate-precipitating agent. The lighter fibers (in this case also the type II fibers) have had their myosin inactivated by the low pH treatment, while the darker staining fibers (type I) have stronger ATPase activity remaining. The proportions of the different fiber types are different between muscles, between different regions of the same muscle, and different between species. These divergent properties need to be kept in mind in the ingredient mixtures used in meat processing.

4.2.2 Contraction Mechanism

The primary function of muscle is locomotion. The muscle cell is packed full of the contractile myofibrils. Contraction is activated by a nerve impulse that passes from the spinal cord to a specialized region on the muscle cell termed the motor end plate. A single axon connects with several hundred muscle fibers in a group called a motor unit. At the end plate, a small amount of acetyl choline is released from the end of the axon, and this acetyl choline diffuses to the cell surface and binds to acetyl choline receptors embedded in

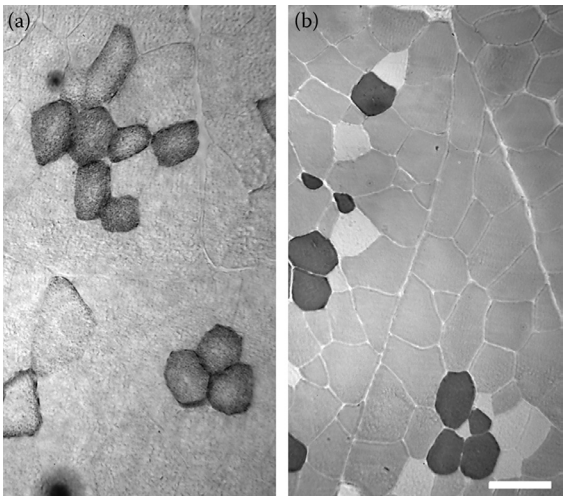

FIGURE 4.5 Light micrograph of pig muscle in cross-section. (a) Succinic dehydrogenase stained. (b) Myosin ATPase after preincubation at pH 4.7. In pig muscle, the dark type I fibers tend to be more clustered together than with muscles from other species. Scale bar = 100 μm.

the muscle cell membrane. The receptors cause a local depolarization of the cell membrane, and this depolarization wave spreads over the surface of the muscle cell. In addition, the depolarization wave passes down special perpendicular invaginations called T tubules that penetrate to the center of the muscle cell. The T tubules are attached to a specialized intracellular membrane system termed the sarcoplasmic reticulum. The attachment involves a protein called the ryanodine receptor. (This protein is so named because of its affinity for the plant alkaloid ryanodine.) When the depolarization reaches the T tubule—sarcoplasmic reticulum junction—the ryanodine receptor opens and calcium is released into the cell cytosol. The calcium diffuses to the myofibrils and binds to troponin on the thin filaments. Calcium causes a shape change in the troponin; this in turn causes tropomyosin to move deeper into the groove of the actin. With the tropomyosin movement, a binding site for the heads of the myosin on the surface of the actin is exposed, and the myosin binds and pulls or pushes the actin a small distance (about 10 nm). The myosin head then releases and it can reattach to another actin. This sliding of the filaments requires energy that is provided by ATP. The terminal phosphate bond can store this energy and release it to do the mechanical work. The myosin head is believed to bend in its middle region, with the portion near the thick filament shaft acting as a lever arm. The calcium required for activation is pumped back inside the sarcoplasmic reticulum by an ATP powered process. The cell membrane polarity is reestablished by the sodium—potassium pump found in the outer cell membrane. It also requires ATP to move the sodium and potassium against their concentration gradients. When the calcium and ion gradients are reestablished, the muscle is relaxed. A single muscle contraction is called a twitch, and it only requires about 200 ms to complete.

4.2.3 Muscle Metabolism

An overview of the major metabolic pathways involved in muscle energy conversions is shown in Figure 4.6. The pathways illustrated all revolve around the production and utilization of ATP. The major fuels include glycogen, glucose, and fatty acids. In addition, small amounts of amino acids can be metabolized under certain conditions. Glucose and fatty acids enter muscle cells by escape from the capillaries, diffusion through the extracellular space, and active transport across the muscle cell membrane. Glycogen is a polymer of glucose that is stored in the muscle in preparation for ATP generation. Resting muscle or muscle undergoing mild activity relies primarily on fatty acid metabolism for ATP synthesis. However, intense work may require more ATP-generating capacity than can be provided by lipolysis and fatty acid transport from the blood stream.[8] There is a small supply of triglycerides in the muscle cell that could liberate fatty acids, but this pathway does not appear to be significant for producing ATP.

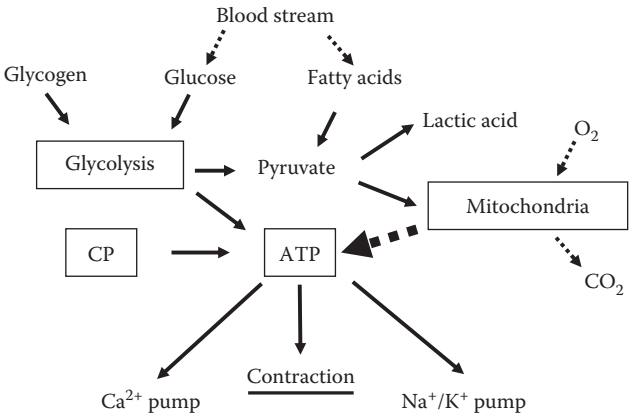

FIGURE 4.6 Overview of metabolism of muscle.

Both the glycolysis from glycogen and glucose as well as degradation of fatty acids result in the generation of pyruvate. In living muscle, pyruvate is usually transported to the mitochondria for further oxidation to carbon dioxide and water. The mitochondrial pathway yields a much larger amount of ATP than the glycolysis steps (indicated by the dense arrow in Figure 4.6). The greatest rate of ATP utilization in the muscle cell occurs during contraction. However, there is a continual need for ATP to power the calcium and sodium–potassium pumps in the cell.

An additional backup source of high-energy phosphate bonds can be provided by creatine phosphate (CP) (Figure 4.6). The enzyme creatine phosphokinase catalyzes the reaction of ADP plus CP to generate ATP plus creatine. When the ATP and C levels are high, the enzyme operates in the reverse direction to generate new CP. ATP levels are typically about 5 mM in the muscle cell, but CP levels may reach 20–30 mM in quiescent muscle. The transfer of the phosphate from CP to ATP is very rapid. Historically, there was much controversy about whether ATP really was required for muscle contraction because no decline in ATP levels could be detected during a twitch—the creatine phosphokinase reaction restores ATP to resting levels so quickly that the ATP level stays essentially constant. However, the limited quantity of CP (probably no more than fivefold greater than the ATP levels) means that it rapidly becomes depleted during short periods of intense work.

The steps in the glycolysis pathway are illustrated in Figure 4.7. The enzymes catalyzing the various steps are shown in italics. This is a remarkable pathway since the flux of metabolites can be varied nearly 100-fold with little or no change in the concentrations of the various intermediates.[11] Intense effort for many years has been exerted to determine what controls the rate of glycolysis.

It is now clear that stress can activate the glycolytic rate by a cascade of steps involving formation of cyclic AMP and ultimately the conversion of the relatively inactive phosphorylase *a* into the much more active phosphorylase *b* (Figure 4.8). The enzyme phosphofructokinase is also activated and inhibited by various metabolites that change in concentration between active and inactive cells. However, the key control of the glycolytic pathway is the supply of ADP and phosphate.[6] Thus glycolysis will proceed rapidly when ATP breakdown rates are high and virtually stop when the ATP requirements are minimal.

4.3 Postmortem Changes in Muscle

4.3.1 Biochemical Alterations

4.3.1.1 Small Molecule Changes

The muscle does not cease to function at the time an animal dies. However, the metabolic functions are markedly altered. The dashed arrows in Figure 4.6 indicate the reaction pathways that become blocked within a few minutes postmortem. First, the cessation of the blood supply means that there is no longer

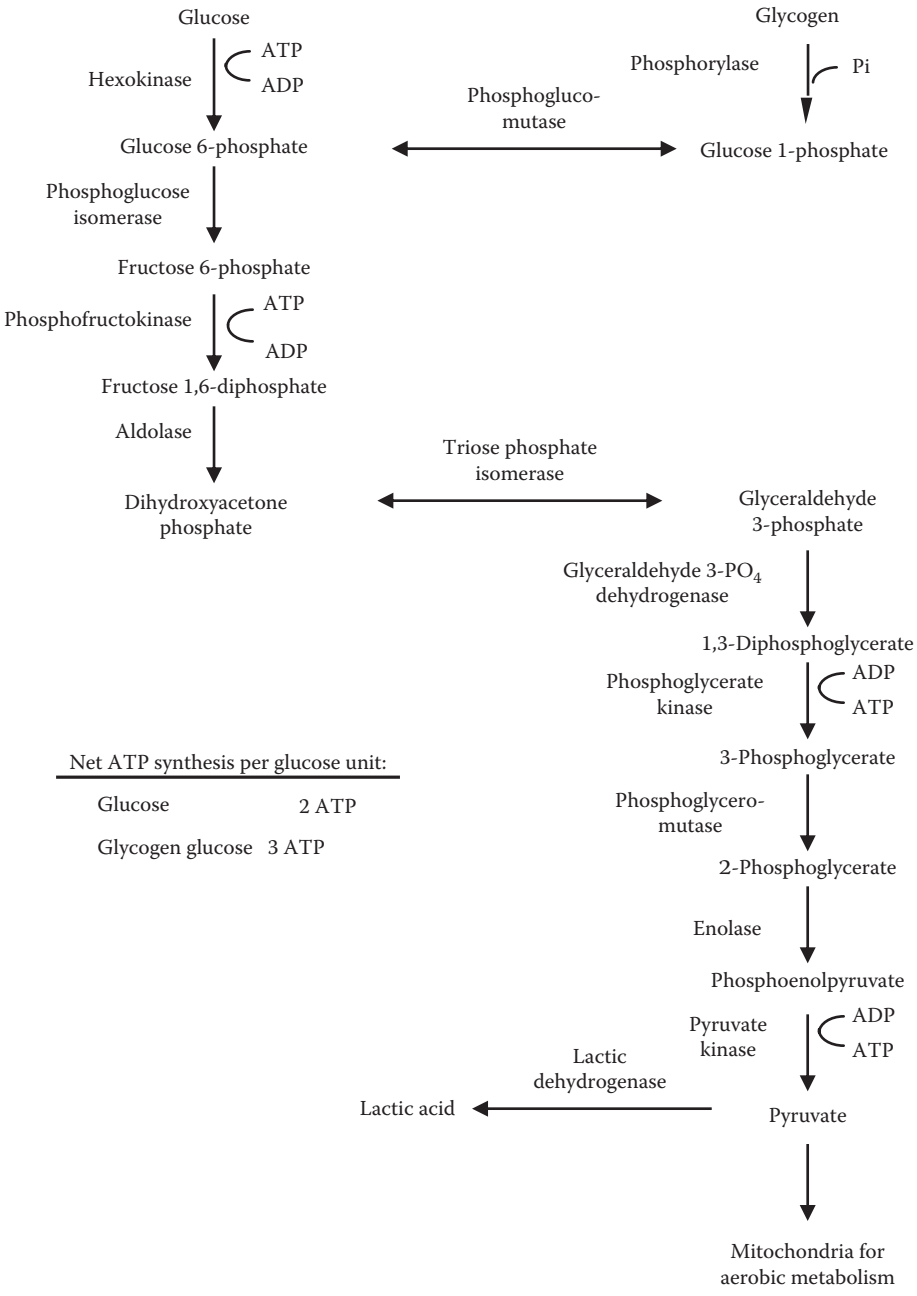

FIGURE 4.7 Glycolysis pathway.

a renewable supply of glucose or fatty acids from the blood stream. Second, the supply of oxygen is cut off. Thus, the major ATP-generating source for muscle is abolished after death. The pyruvate that is generated as an end product of glycolysis is converted into lactic acid, and, since metabolic waste products cannot be removed without a blood stream, the lactic acid accumulates in the muscle. Typically, there is a burst of muscular activity at the time of death due to trauma of the brain and spinal cord. Thus, the measured levels of creatine phosphate obtained within 5 min after death are usually depressed from those values found with biopsies or by nuclear magnetic resonance measurements on living muscle.

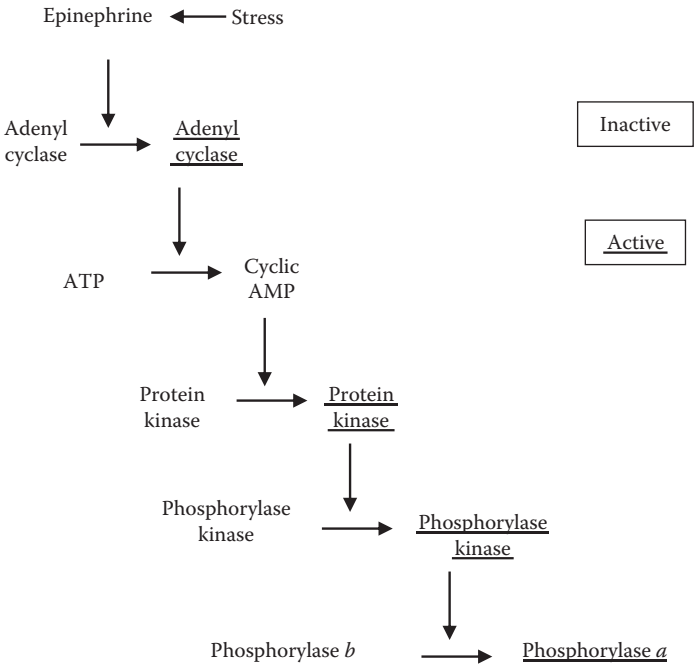

FIGURE 4.8 Stress activation of the glycolysis pathway.

Although the muscular activity rapidly subsides within a few minutes after death, the sarcoplasmic reticulum calcium pump and the cell membrane sodium–potassium pump continue to function to move their respective ions against the concentration gradients. The glycolysis pathway is the only source for this ongoing requirement of ATP. However, since there is a fixed supply of glycogen at the time of death, glycolysis can only continue for some limited time period postmortem. Usually glycolysis ceases before all the glycogen is depleted. Although the reasons for this cessation are not completely understood, possibilities include [1] the low pH that develops may inactivate one of the glycolytic enzymes and/or [2] the conversion of adenine nucleotides into inosine derivatives may halt the glycolytic flux. Some ATP is regenerated by the myokinase-catalyzed reaction of two moles of ADP to form one mole of ATP and one mole of AMP. The AMP is converted into IMP and ammonia by the enzyme AMP deaminase.

A graphical example of postmortem changes in a variety of muscle properties is shown in Figure 4.9. The time scale would be appropriate for pig muscle undergoing typical postmortem processing procedures (i.e., transfer of the dressed carcass to a 0–4°C chiller at 30 min after death). Note that the pH decline is fairly linear and is paralleled by the increase in lactic acid concentration. The latter often reaches values in excess of 100 mM. ATP concentrations remain fairly stable for the first couple of hours postmortem and then begin a linear decline. The commencement of this decline coincides with the depletion of the creatine phosphate. Creatine phosphate is usually depleted before the pH reaches 6.0. The overall shapes of the curves and relationships between the different parameters shown are consistent between different animals and species. The major differences will be in the time axis. The approximately 6 h time course shown for the pig would be more like 18–24 h in beef, 6–12 h in lamb, and <3 h for poultry. These are average times for muscles left on the carcass after slaughter. It should be noted that there is considerable variation in the cooling rate at different locations in the carcass. Thus, the surface will cool more rapidly than the deeper portions of the muscle tissue. One would therefore expect that the deeper portions of the round and chuck would reach the ultimate pH and go into rigor mortis before muscle that is closer to the surface. Likewise, in muscles susceptible to cold shortening, those regions within a couple centimeters of the surface that cool very rapidly may go into rigor earlier than muscle 5–10 cm below the surface. The size of the carcass, the amount of fat cover, the temperature of the chiller, and the air velocity will all have a profound effect on rate of postmortem metabolism and

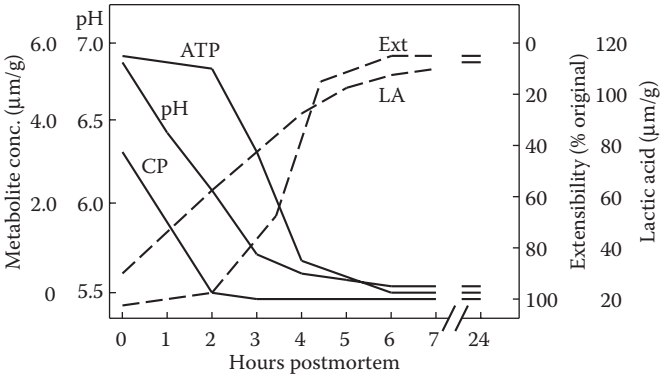

FIGURE 4.9 Chemical and physical changes in muscle postmortem. The pattern shown would be typical for pig muscle undergoing normal postmortem metabolism. Abbreviations: ATP, adenosine triphosphate; CP, creatine phosphate; LA, lactic acid; Ext, extensibility.

development of rigor mortis. In addition, the use of electrical stimulation to speed postmortem glycogen and high energy metabolite depletion will also markedly affect the time course of muscle metabolism.

4.3.1.2 Protein Changes

A number of postmortem changes in the muscle proteins have been identified. The myofibrillar proteins desmin, troponin T, titin, nebulin, and vinculin all become partially or completely degraded during the first week postmortem.[12,16] Although the proteolytic enzymes responsible for this degradation have not been unequivocally identified, the patterns of fragments generated and the proteolytic susceptibility *in vitro* all suggest that the calpains are involved. Calpains are calcium-activated proteases originally described by Dayton and others.[17] Three different isoforms of the calpains have been identified in muscle—μ-calpain (calpain 1), m-calpain (calpain 2), and P94-calpain (calpain 3). Calpain 1 requires micromolar concentrations for activity, while calpain 2 requires millimolar levels of calcium.[18–20] Calpain 1 degrades several proteins in skeletal muscle which are related to drip loss (discussed below). The calpain 3 is slightly larger than the other two isoforms; it binds to titin but is unstable when released. The current hypothesis regarding postmortem protein degradation suggests that the calcium in the sarcoplasmic reticulum leaks into the cytosol and activates the calpains after the muscle ATP is depleted.[21] A number of pieces of evidence support this hypothesis. First, soaking muscle strips in calcium solutions or injecting muscle with calcium results in increased proteolytic degradation. Second, animals such as the callipyge lamb that have higher muscle levels of calpastatin (the natural inhibitor of the calpains) have lower rates of postmortem protein degradation.[22] Third, the proteins that are degraded by the calpains in the test tube are the same ones that are broken down in postmortem muscle. Fourth, an early decrease in pH is accompanied by an early increase in intracellular calcium concentration[23] which results in a higher calpain 1 activity.[24] Rapid postmortem pH decline and lower ultimate pH lead to greater activity of calpain 1 and reduced calpastatin activity.[25–27] The calpains are maximally active near pH 7, so they would be expected to have much lower activity at the usual ultimate pH of around 5.5. The activity of calpain 1 declines rapidly postmortem; calpain 2 is more stable.[16] Calpastatin activity also declines after death, so it remains unclear which of these components are most important in controlling postmortem protein degradation.

4.3.2 Physical Alterations

4.3.2.1 Rigor Mortis

The major physicochemical change that occurs in postmortem muscle is the development of rigor mortis which results in an increasing toughness of meat.[28] The term comes from Latin and means the stiffness

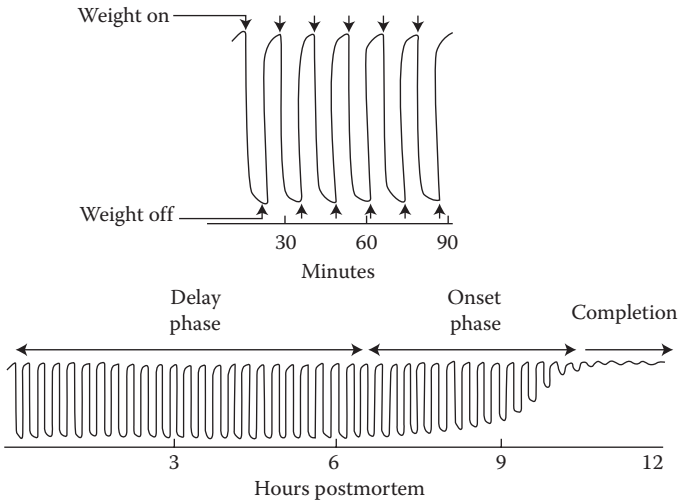

FIGURE 4.10 Rigor measurement and pattern. The upper trace demonstrates the pattern of weight addition and removal and the resulting changes in muscle length immediately postmortem. The lower trace shows the changes in extensibility from soon after death until the muscle is in full rigor. The pattern shown was obtained from a rabbit that had been anesthetized before death. (Modified from Bendall, J.R. 1973. *The Structure and Function of Muscle*, 2nd ed. Vol. II, pp. 243–309. Academic Press, New York, NY. With permission.)

of death. The time for rigor mortis development can be estimated by alternately loading and unloading a muscle strip and recording the changes over time.[29,30] Such a trace is illustrated in the upper part of Figure 4.10. The extensibility remains relatively constant for some time postmortem (Figure 4.10 bottom); this period is called the "delay" phase.[6] Subsequently, the extensibility declines rapidly during the "onset" phase. Finally, the muscle reaches a stage where there is no further decline in extensibility, and this is referred to as the "completion" of rigor.

The time course of rigor mortis is linked to metabolite changes in the muscle. The completion of rigor corresponds to the point where the ATP has been depleted (Figure 4.9). The onset period appears to start when the ATP levels begin to decline (note that the time courses shown in Figures 4.9 and 4.10 are not for the same species or muscle). The loss of extensibility is due to the firm attachment of the myosin heads to actin. In the normal contraction cycle, ATP is required to dissociate these two proteins and allow the filaments to slide over one another. In postrigor muscle, the supply of ATP is depleted leading to strong actin–myosin bonding so muscle shortening or extension is no longer allowed. The process is somewhat complicated by the fact that not all fibers in a muscle strip deplete their ATP at the same time because of biological variation and the difference in fiber types.

The pattern shown in Figure 4.10 (bottom) was produced from an animal that had been anesthetized at the time of death. Animals slaughtered under commercial conditions will typically have a much shorter delay phase or even the absence of delay. The time course of rigor mortis is extended two- to threefold at 10°C versus 37°C.[31]

4.3.2.2 Shortening

Unrestrained muscle may also shorten as it goes into rigor mortis. This shortening can be as high as 25–30% of the initial length when the muscle is maintained at 37°C.[32] Minimum shortening occurs at temperatures near 15°C. Similarly if muscle is held isotonic, it will develop force during the onset of rigor mortis. The force developed is much weaker than that produced by living muscle during contraction (estimated to be <5%),[6] but is sufficient to significantly change the sarcomere length and the tenderness properties of muscles still attached to the carcass. Force begins to develop at the beginning of the onset phase of rigor.[33] This force may be developing due to a rise in cytoplasmic calcium as

the ATP nears depletion or because of the activation of contraction due to binding of myosin heads to actin.[34]

4.3.3 Unusual Patterns of Postmortem Metabolism

4.3.3.1 Thaw Rigor

The term "thaw rigor" is somewhat of a misnomer. The name refers to the shortening that occurs when muscle is rapidly frozen prerigor and then subsequently thawed. Muscle that has been treated in this way shortens markedly (as much as 70–80%) and loses large amounts of liquid (more than 25% of the initial weight) as drip.[35] The mechanism of this phenomenon is believed to be the disruption of the sarcoplasmic reticulum due to ice crystal formation followed by the release of calcium upon thawing.[36] The calcium then activates contraction since there is ATP remaining in the region of the myofibrils. The degree of shortening depends largely on the size of the muscle piece frozen and thawed. As the surface regions thaw, the inner parts provide a rigid scaffold to prevent shortening. The ATP is used up rapidly after thawing, so the outer portions will go into rigor before the core regions are thawed. Thus the degree of shortening may be minimal on a large muscle piece that has been frozen and thawed prerigor.

4.3.3.2 Cold Shortening

The usual dependence of postmortem metabolism rate on temperature does not hold in certain cases. The muscles that rely primarily on oxidative metabolism may undergo a slow but significant shortening if excised and held at temperatures below 10°C. Locker and Hagyard[32] termed this phenomenon "cold shortening." The muscle length can decline as much as 50% in unrestrained muscle.[37] Cold shortening is a slow process; the time course may be minutes to an hour and depends on the cooling rate. The shortening occurs before there is any reduction in muscle ATP levels.[38] This shortening can also occur on the carcass, particularly with muscles not placed under stretch when suspended from the rail.[37] However, the force is weak; Bendall[6] estimates that it is only about 2–3% of the maximum produced in a muscle tetanus. Cold shortening is believed to be due to a gradual rise in the cytosolic calcium level by release from either mitochondria or the sarcoplasmic reticulum (the calcium pump operates more slowly at low temperature). The slightly elevated calcium causes a weak contractile response and the muscle shortens. The weak activation of the myofibrillar ATPase activity also increases the glycolysis rate compared to that at 15°C.[6] Cold shortening is usually only a significant problem in beef and lamb. The rapid cooling that occurs with high efficiency chillers exacerbates the problem. Since cold shortening leads to an increase in meat toughness,[37] improvements in processing efficiency and food safety are offset by deleterious effects on meat quality.

4.3.3.3 Pale Soft Exudative Condition

An unusual postmortem phenomenon (first described in pigs by Ludvigsen[39]) is one in which the muscle becomes pale in color, develops a soft texture, and exudes large amounts of liquid (Figure 4.11). The postmortem metabolic rate is vastly increased, with ATP depletion, completion of rigor mortis, and pH values as low as 5.3 occurring within 10–15 min after death.[40] The low pH that develops while the muscle temperature is still high leads to a denaturation of some of the muscle proteins, notably myosin. This reduces the water-holding activity of the muscle and results in excess drip loss. Currently, the mechanisms controlling water-holding capacity are not fully understood, but the structural organization of the muscle does influence the water distribution. More drip loss appears with short sarcomeres, and a higher proportion of water is held in the I-band compared with A-band of myofibrils as determined by nuclear magnetic resonance (NMR).[26,41–43] Degradation of some proteins such as the intermediate filament protein desmin[44,45,25,46–48] and costameric proteins vinculin and talin[48,49] improve water-holding capacity by disturbing the formation of drip channel between the myofibril and the sarcolemma.[26,50] However, the degradation of the protein integrin increases the water loss by the formation of drip channels in pork.[48,51]

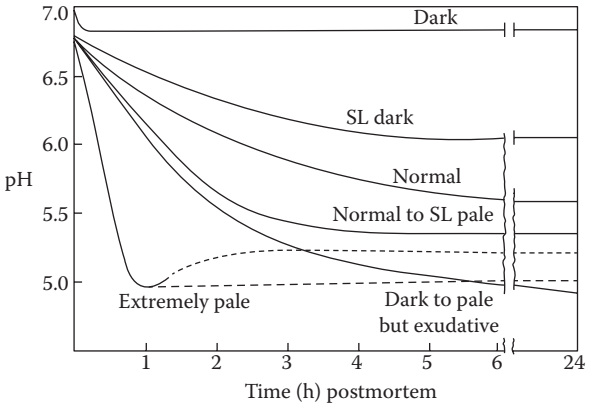

FIGURE 4.11 Variation in postmortem pH decline in pigs. The pH decline pattern is closely related to the resulting muscle color. (Modified from Briskey, E.J. 1964. *Adv. Food Res.* 13:89–168. With permission.)

The rapid postmortem glycolysis is often caused by a mutation in the ryanodine receptor (the calcium release channel, RyR) at the interface between the sarcoplasmic reticulum and T tubules.[52] The mutation of the RyRs arginine 615 to a cysteine results in the protein-leaking calcium even when there is no nerve signal for contraction. The elevated cytosolic calcium postmortem causes activation of the myofibrillar ATPase and the resulting acceleration of glycolysis. Attempts to remove animals carrying the mutation are in progress using genetic tests, but not all animals that develop the PSE condition can be identified by this procedure. It may be that there are other mutations in the RyR that cannot be picked up by the current test. Such is the case with RyR mutations in humans where over 30 different mutation sites have now been described.[53,54]

Attempts to change animal handling and postmortem processing procedures to reduce the incidence and severity of PSE development have been partially successful. The incidence of the PSE condition in the United States was approximately 15% for many years, but this has dropped to below 5% in more recent surveys. Gentle handling and allowing pigs a rest period before slaughter reduces the incidence of PSE.[55] Rapid cooling postmortem, such as briefly immersing a carcass in liquid nitrogen,[56] markedly reduces the PSE problem; however, this procedure has not been adopted commercially. PSE has been prevented by early postmortem injection of sodium bicarbonate.[57] The bicarbonate appears to slow the rate of pH decline as well as elevate the ultimate muscle pH.

It is now clear that a condition related to PSE also occurs in chicken and turkey muscle.[54,58,59] The biochemical characteristics of PSE turkey are strikingly similar to those of PSE pork. With some turkeys the ATP and postmortem pH decline are accelerated, and the resulting muscle has a reduced cooked yield.[60] There is also a precipitation of phosphorylase and a reduction in the myosin solubility in muscles that have undergone this rapid postmortem glycolysis.[60] The incidence is greater as the result of stress from hot weather, transportation, and struggling of birds.[61–63] Due to the obvious genetic differences between mammalian and avian species, Smith and Northcutt[64] suggested that the terms "pale chicken muscle" or "pale poultry muscle syndrome" as replacements for the use of PSE to describe pale poultry meat. No point mutation has been found in poultry RyR, but recent reports showed that alternative splicing in turkey RyR could lead to PSE development.[54,65,66]

4.3.3.4 Dark, Firm, Dry Condition

The DFD condition in pigs results from the same ryanodine receptor mutation that causes PSE. In this case, the glycogen has been largely depleted before death and lactic acid therefore does not accumulate in the muscle. The time to rigor mortis completion is very short and the resulting ultimate pH is high (it may be >6.5). The meat is dark in color and has a firm texture. The surface is dry and sticky to the touch. Such meat has excellent properties for use in processed meat products because of its high water-binding activity.

It's unusually dark color may deter purchase at the retail meat case. However, since it only occurs in animals that may alternatively become PSE, conditions that reduce its incidence are usually sought.

4.3.3.5 Dark Cutter

The dark cutter condition occurs in beef muscle having a high ultimate pH (see Tarrant[67] for review). Like the DFD condition in pigs, dark cutters result from the antemortem depletion of glycogen. However, this depletion is not due to a genetic condition but rather appears to result from a response to stress.[68] The incidence of the dark cutting is often high in bulls (as much as 15%) since they tend to fight. Dark cutting can be reduced by avoiding mixing unfamiliar animals prior to slaughter. Meat from dark cutters may cause difficulty in retailing, but it is a superior product for use in processing because of its high pH and resulting improved water-holding ability.

4.3.3.6 RN Phenotype in Pigs

A genetic condition in some pigs results in a different form of postmortem metabolism.[69] The RN condition is common in the Hampshire breed of pigs. The "RN" notation comes from the French "Rendement Napole" meaning "Yield Napole," a method for evaluating ham-processing yield. Certain pigs have a lower ultimate pH (typically about 5.3–5.4 versus the normal found in pigs of 5.5–5.6 in the Longissimus dorsi), and this results in a slightly paler fresh meat color and less water binding after processing. It is characterized by a significant increase (~70%) in the glycogen levels in the muscle of the live animal. The rate of postmortem pH decline is normal. Meat from pigs carrying the RN allele often is more tender than that from noncarriers.[70] Animals with this condition can be determined using estimates of the "glycolytic potential" (GP).[71] The GP equals 2 ([glycogen glucose units] + [glucose] + [glucose-6-phosphate]) + [lactate]. Since the glycolytic potential is the sum of glycogen and its major postmortem breakdown products, it should give similar results whether determined on biopsy samples or from muscle at any time point postmortem.

The first RN gene description localized the mutation to the protein kinase AMP-activated γ3 subunit gene (PRKAG3) where separate Arg200 → Gln and Thr30 → Asn changes were found, the former being identified as the causative alteration.[72] Subsequent work identified additional mutations in the same gene (Val199 → Ile and Gly52 → Ser).[73,74] In contrast to the RN Gln200 mutation, the Ile199 mutation was associated with low glycogen content, low lean meat percentage, and high ultimate pH.[73,74] It appears that the higher glycogen content in RN pigs is not due to a defect in glycogen degradation, but instead is caused by a higher glucose uptake.[74]

4.4 Summary

Muscle tissue is complex structurally and is nonhomogeneous in its protein composition and metabolic emphasis. The rate and extent of metabolic changes postmortem have important effects on the color of meat, its texture, and its usefulness for inclusion in processed meat products. In addition, there are profound differences in the rates of postmortem metabolism between muscles from different species. For example, electrical stimulation may be beneficial to speed postmortem metabolism in beef and therefore prevent cold shortening, but acceleration of metabolism in pigs may lead to greater incidence of pale, soft, exudative meat. Thus an understanding of the biochemical and physical processes that muscle undergoes postmortem is necessary for optimum design of slaughter and meat-processing procedures and the use of this tissue for food.

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Part III

Quality and Other Attributes

5

Meat Color[*]

Lorenzo Castigliego, Andrea Armani, and Alessandra Guidi

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5.1 Introduction

The organoleptic attribute of meat that more directly influences the consumer's choice is certainly the visual appearance. This is because it represents the first characteristic impacting on the end user and acts as a strong selecting filter, being perceived prior to other qualities, such as odors, flavors, or texture. In particular, other than shape, bones, fat, and lean tissues distribution, together with their chromatic characteristics are normally associated by the consumer to wholesomeness or freshness and color alterations are commonly considered as indicators of poor quality or hygienic problems. The relapses on the food industry economy strongly contributed to the carrying out of several studies to understand in detail what influences color in different kinds of meat and in different conditions of production, processing, and storing. The understandings achieved have led to improvements in the meat chain or to technological countermeasures aimed to avoid or limit problems associated to discoloration.

5.2 Color Measurement

The color, as it is perceived by the human eye, represents a sensorial experience, and for this reason it is always subjective, given that it is influenced by either physiological characteristics or by the psychological

[*] Part of the information in this chapter has been modified from Guidi A., Castigliego L. 2010. Poultry meat color. In: Guerrero-Legarreta I., Hui YH, editors. *Handbook of Poultry Science and Technology.* Vol. 2. Hoboken, NJ: John Wiley & Sons, Inc. pp. 359–88. Used with permission.

stance of the observer. In addition, the human visual system works more as a comparator than as an absolute sensor, so that even the background may substantially influence the overall perception of color. In fact, in order to investigate chromatic changes, to determine its origin and to trace its evolution over time, color measurement is needed.

Essentially, color depends not only on the measured material, but also on the light source and the detector, be it human or instrumental, making different measurement responses as a consequence of different experimental conditions. This implies that any type of color evaluation must be done according to standardized conditions, preferably using specific instruments, which are now in widespread use for research or quality control purposes. In general, the most common procedures used for color measurements are visual assessment, spectrophotometry, and reflectance colorimetry.

The first trials for color assessment were made using a panel of people who had to describe the object of the observation using predefined terms, each associated with a different shade of an established color. The responses to this test were too imprecise and varied. The comparative method was then introduced, by which sets of color range standards were developed, ordered on a numbered scale, and applied in a scoring system to carry out color matches for meat. Panel assessment for meat color is still common and is still based on reference color standards and specific rules. Recommendations by meat science associations are used primarily to limit the influence of the environment or characteristics of the meat other than color (e.g., fat, texture) (AMSA 1991). However, sensory panel assessment has some intrinsic limits and does not allow an objective response, which is instead based on instrumental measurements.

The first attempts to overcome subjectivity were carried out using spectrophotometers to measure the heme pigments of meat after chemical extraction. This method has often been used to correlate the quantity and form of myoglobin in meat (which is among the main factors responsible for meat color, as discussed later) with the potential for meat color or color defects.

Most research and quality control procedures contemplate the use of reflectance colorimeters, which express meat color in terms of color differences from a designated color standard. Yet despite the fact that this type of instrument ensures high levels of accuracy and reproducibility, it is designed for opaque surfaces, whereas meat is a translucent material and may scatter much of the light, reducing the reproducibility of the measurements and the comparison of color values (Uijttenboogaart 1991). The most commonly used colorimetric scale nowadays is the CIE L*, a*, b* (International Commission on Illumination 1986), even though other color scales can be used, such as the Hunter L, a, b, and YXZ space. The CIELAB color scale is organized in cube form, a sort of tridimensional space in which the color can be collocated and relies on three color parameters, expressed as L*, a*, and b* (Figure 5.1). L*

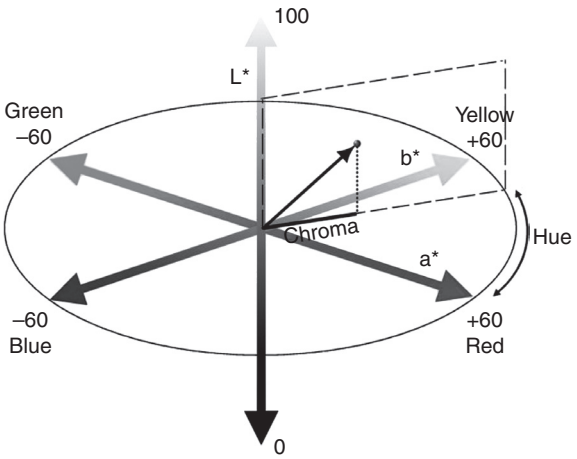

FIGURE 5.1 L*a*b* color space. The color of a surface is associated with a point in the 3D space with precise coordinates, related to each dimension (black (0) to white (100); green (−60) to red (+60); blue (−60) to yellow (+60)). Hue angle is the arctangent of b*/a* and chroma (intensity) is the distance between the origin of the axes and the orthogonal projection of the point on the circle surface $(a^{*2} + b^{*2})^{-2}$.

expresses the lightness, with 0 associated with black (complete absorption of light) and 100 with white (complete reflection). a^* indicates the level of redness (or greenness), since the two colors are complementary, ranging from −60 (pure green) to 60 (pure red). Finally, b* indicates yellowness (or blueness), also ranging from −60 (pure blue) and 60 (pure yellow). Hue angle is the arctangent of b*/a*, determined by rotation about the a* and b* axes and defines the color. The color intensity (chroma) is defined as $(a^{*2} + b^{*2})^{-2}$. If a comparative analysis is performed, delta values (ΔL*, Δa*, Δb*) indicate how much a sample and standard differ from one another. The total color difference (ΔE*) may also be calculated, and it takes into account all the L*, a*, and b* differences between samples and standards. The difference in chroma (ΔC*) and in hue angle (ΔH*) is described in a polar coordinate system.

Honikel (1998) specified a reference method for color measurements using the CIE scale, defining procedures for sample preparing, light source, geometry of illumination and viewing, observer angle, and all the parameters that have to be specified to ensure reproducibility of data. Despite instrumental measurement being more accurate and reliable than sensorial measurement, differences in sample presentation and measurement conditions make it difficult to compare absolute color values between laboratories. For instance, under practical industry conditions, meat sample size variation and background color (i.e., belt surfaces, packaging material, etc.) would be factors affecting the color difference or absolute color measurements (Bianchi and Fletcher 2002). This suggests that the choice of the sample must be accurate, since part of the incident light is diffused when interacting with a wet meat surface and partly penetrates, to a different extent inside the tissues, and is reflected internally. Because of the translucency of the meat, the sample to be measured must be thick enough to avoid the light reaching the background material and being reflected by it. Reflectance spectrophotometry also allows calculation of the proportion of the three major myoglobin pigments by means of measurements at the isosbestic point (i.e., the wavelength with the same extinction coefficient for all the pigments) and specific equations (Stewart and others 1965a; Van den Oord and Wesdorp 1971; Krzywicki 1979).

Values from instrumental color measurement have been shown to correlate with panel evaluation and consumer scores and were associated with the subjective perception of a number of meat defects, such as paleness, darkness, and discoloration. Some studies showed that pale, soft, exudative (PSE)-like or dark, firm, dry (DFD)-like meats can be associated with specific color measurement responses (Barbut 1993; Brewer and others 2001). Relationships were also found between color, and shelf life (Allen and others 1997), or composition (Qiao and others 2002), or genetic characters (Bertram and others 2000; Brewer and others 2004).

An interesting alternative for color measurement is computer vision based on an analysis of digital camera images. The advantages over other methods can be found in the possibility of processing the acquired digital images to remove the background fat and bone (Lu and others 2000); several measurement systems can be used (e.g., CIE, Hunter, XYZ), and the values can be measured on a given area. Only a single observation could be representative for the color evaluation, and the surface variation of the myoglobin redox state can be assessed (O'Sullivan and others 2003). Moreover, implementation of the data by statistical or neural network models can help predict sensory color scores (Lu and others 2000) since the camera takes measurements over the entire sample surface, resulting in a more representative outcome than that obtained by the point-to-point measuring of a colorimeter (O'Sullivan and others 2003).

5.3 The Basis of the Color: Meat Pigments and Their Chemistry

Many factors determine or influence the color of meat or, more properly, all its shades, making its final manifestation the effect of an intricate ensemble of causes. Some of them have been clearly identified, but a certain grade of uncertainty remains for others, due to the not always concordant results of the wide array of experimentation. This truth testifies to the great complexity and richness of the topic. Skipping for a moment argument as to its bases, it can be asserted that the color of meat depends primarily on

- The type and quantity of pigments present in the muscle.
- The types of fibers composing the muscle and their spatial relationships, which determine the scattering grade of light and thus its deepness of penetration.

- The intramuscular fats and surface dehydration, which confer different degrees of glossiness and thus affect light scattering and reflection.

The pigment most responsible for the color of meat is myoglobin, although there is also a small amount of hemoglobin, which is very similar, but is present only in small amounts in well-slaughtered animals, and of cytochromes and ribonucleases, which play a minor role because of their low concentrations. Myoglobin is a single-chain globular protein of about 16,700 kDa, whose role is to store oxygen (O_2) in the muscle once it is released by hemoglobin in the capillaries and spreads inside the myocytes before entering the oxidative phosphorylation cycle to produce new energy. Myoglobin consists of a peptide portion (apoprotein) and an O_2-binding prosthetic group, called heme, containing an iron atom in its core and placed in a hydrophobic pocket formed by the apoprotein (Figure 5.2). The iron is coordinated by four noncovalent bonds to pyrrole nitrogen of heme and by another bond to the proximal histidine 93 of the protein. A sixth coordination bond may potentially be formed by a number of small molecules, among them O_2, which is capable of penetrating the pocket and interacting with the iron itself. Iron's property to attract electron pair-donating ligands, due to its electron deficiency, leads to the formation of bonds with such molecules, whose nature depends on the ease with which electrons are donated. The intact conformation of the pocket containing heme is important to guarantee the stabilization of the iron's oxidation state, making the O_2 binding to the iron reversible (Morrison and Boyd 1981) and allowing access to only a limited number of molecules, such as cyanide, nitric oxide (NO), and carbon monoxide (CO) (Lycometros and Brown 1973). A distal histidine (His 64) present in the pocket controls the spatial relations between heme and bound compounds, influencing the chemical dynamics related to color. The heme core can also absorb electromagnetic radiation in the visible range, yet the absorbance spectrum may vary according to the oxidation state of the iron and the type of bound molecule (Giddings and Markakis 1972), conferring on the meat a great range of possible tonalities, from dark brown to bright red; some less common colors, such as green, can also appear, as in the case of bacterial contamination.

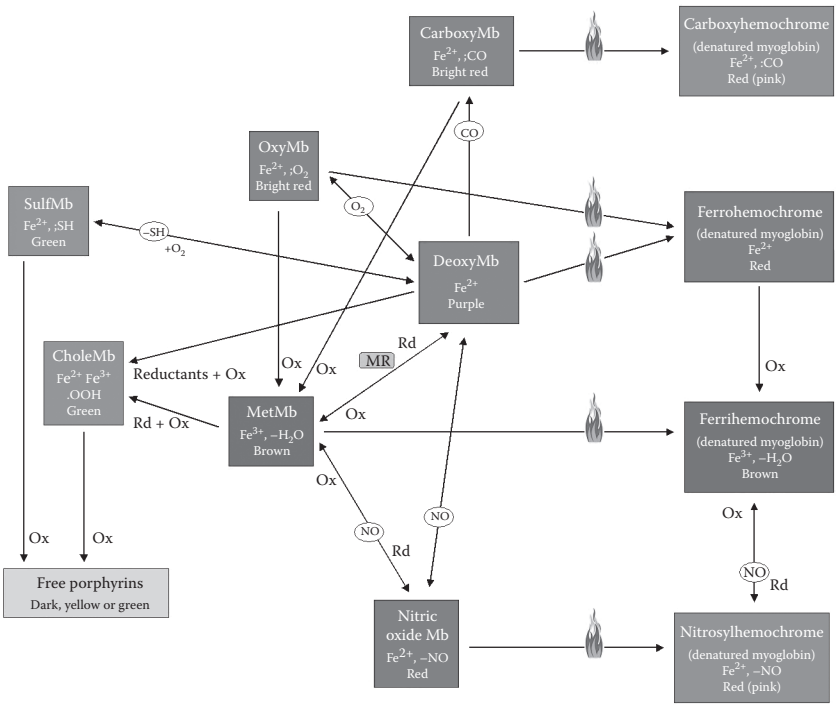

FIGURE 5.2 Major pathways of interconversion between different forms of myoglobin. MR, metmyoglobin reductase; Ox, oxidation; Rd, reduction.

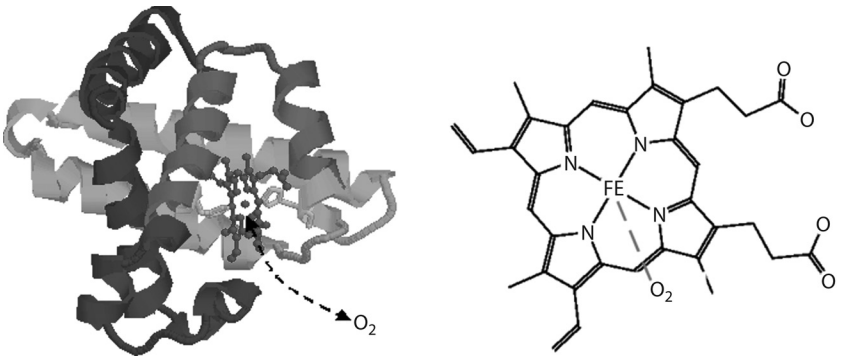

FIGURE 5.3 Schematic representation of myoglobin and heme, which is located in the pocket formed by the folding of the apoprotein.

There are three main forms of myoglobin in muscles: deoxymyoglobin (Mb^{2+}), oxymyoglobin (OMb^{2+}), and metmyoglobin (MMb^{3+}) (Stewart and others 1965a) (Figure 5.3), each conferring a different color to the meat. Under very low O_2 tension, iron in the form 2^+ (Fe^{2+}) tends to interact ionically with H_2O when no other electron donors are present in the pocket of the protein (Mb^{2+}), conferring a dark-red purple color to the tissue. On the other hand, at higher tension, the molecular O_2 tends to occupy the sixth coordination site and binds the iron, which maintains the lower oxidation state (Fe^{2+}): $Mb^{2+} + O_2 \Leftrightarrow OMb^{2+}$. This complex determines the bright red color that is typical of fresh meat. The two forms are rapidly convertible, even if the reaction is influenced by the temperature, O_2 partial pressure, pH, and possible competitors for binding the iron. However, in meat, the conversion from OMb^{2+} to Mb^{2+} may undergo more complex dynamics, characterized by a two-step reaction: $OMb^{2+} \rightarrow MMb^{3+} \rightarrow Mb^{2+}$.

The oxygenation of Mb^{2+} can frequently be observed when some parts of meat, appearing dark after being in prolonged contact with a gas-impermeable packaging film, which causes a temporary reduction in the local O_2 concentration, are exposed to the atmosphere (blooming). If the iron is oxidized further (Fe^{3+}), it loses its capability to bind molecular O_2, but can form a different type of complex with other molecules. An important example is the formation of MMb^{3+}, where iron interacts with a molecule of water, resulting in a covalent bound between Fe and a hydroxyl group. This determines a shift in the meat's color toward brown and its extent is related to the proportion of MMb^{3+} and to the amount of the meat surface with a high percentage of MMb^{3+}. Generally, despite the substantial harmlessness to health, the browning of meat produces a resolute rejection reaction in the consumer. The formation of MMb^{3+} is often characterized by an autoxidation process that causes the formation of superoxide anion (O_2^-) according to the reaction: $OMb^{2+} + H_2O + H^+ \Leftrightarrow MMb^{3+} + HO_2$. Superoxide anion can, in turn, be converted by spontaneous dismutation into hydrogen peroxide (H_2O_2), this also being a potent oxidant of OMb^{2+} (Tajima and Shikama 1987). This process was demonstrated to be dependent on the O_2 partial pressure, with a maximum around 1–1.4 mmHg (George and Stratmann 1952; Gill and McGinnis 1995), and it is favored by both acidic and basic pH (Shikama and Sugawara 1978). In particular, in the meat context, the range of slightly acidic pH is of more interest, since postmortem muscle pH is supposed to decrease. OMb^{2+} autoxidation to MMb^{3+} can also be enhanced by temperature (Brown and Mebine 1969), by an increase in free radicals in postmortem muscles for microbial growth or during meat processing (Giroux and others 2001), and by ions and microbial metabolite concentration (Renerre 1990; Osborn and others 2003). A high salt concentration decreases MMb^{3+} reductase activity (Stewart and others 1965b) and increases protein denaturation when associated to higher temperatures (Trout 1989), which facilitate pigment oxidation. In living muscle tissues, the enzymes MMb^{3+} reductase, in the presence of the cofactor NADH and the coenzyme cytochrome b4, bring the Fe^{3+} back to the Fe^{2+} of normal myoglobin (Livingston and others 1985). When tissues die and convert into meat, the conditions are not favorable for the reduction of MMb^{3+} to Mb^{2+} due to progressive depletion of the reducing enzyme systems and cofactors (such as NADH). As a consequence, MMb^{3+} formation becomes irreversible.

Undesirable color change from red to brown has also been observed in bone marrow, which, since it is rich in hemoglobin, undergoes oxidation–reduction reactions similar to those described for myoglobin. Lyon and others (1976) demonstrated that meat and bone darkening of thigh pieces was related to pigment migration from the femur to tissue.

Although many compounds can bind to iron other than O_2, only two molecules have some importance for meat: CO and NO, which, as well as O_2, can bind only Fe^{2+} but not Fe^{3+}, since the latter binds electrons of the donor molecules more tightly because of its higher charge, and is not able to donate back other electrons to form stabilizing bonds (Livingston and Brown 1982). CO has much more affinity for Mb^{2+} than O^2, so it can displace the latter and form a relatively stable compound called carboxymyoglobin ($COMb^{2+}$), which confers a bright red color to meat. Its importance derives from an increasing interest in packaging with a low CO level (Sørheim and others 1999; Hunt and others 2004). Nitric oxide myoglobin ($NOMb^{2+}$), which represents the pigment intentionally formed by cured-meat processing, also confers a red color to meat. $NOMb^{2+}$ is formed by the degradation of nitrates into nitrites and further into NO and is more stable than OMb^{2+}, but not enough to avoid the displacement of NO by O_2, which is normally far more abundant. In curing technology, meat and meat products can be cooked to prevent the loss of NO and form a stable pigment, the nitrosylhemochrome, which is responsible for the final pink color (Forrest and others 1975) (Figure 5.3). This pigment is desirable in cooked red meat but undesirable in poultry meat and meat products. Sharper chromatic alterations can occur in the flesh of slaughtered animals after denaturation of the muscle proteins and reduction of the heme's core, as a consequence of their exposure to hydrogen, sulfur, and O_2 or to H_2O_2 and ascorbic acid, with the formation of sulfmyoglobin (reversible) and cholemyoglobin (irreversible), respectively, which determine the appearance of a green color. Such events can be caused by the growth of microorganisms, favored by tissue lesions *in vivo*. Further oxidation of cholemyoglobin may cause heme opening, forming the verdoheme (green in color) or the loss of iron and the formation of a chain of porphyrin rings (Lawrie 1966; DeMan 1999). Any chemical process leading to the denaturation or destabilization of proteins, including myoglobins, may enhance the formation of pigment forms which can determine color alterations in the muscle. Besides the ability of the various myoglobin forms to catalyze lipid oxidation in an acidic environment, several studies have highlighted how transition elements, among them iron, can catalyze the decomposition of peroxides by generating free radicals, which in turn oxidize unsaturated fatty acids (Nawar 1996; Mancuso and others 1999). Lipid oxidation, also favored by low pH, may be correlated to myoglobin oxidation, which results in the formation of MMb^{3+} (Lynch and Faustman 2000). Thus, in an oxidizing and acidic environment, meats with high myoglobin and iron contents and rich in unsaturated fatty acid may show a tendency toward browning (Guidi and others 2006).

5.4 Factors Affecting Meat Color

The great differences in color shades, intensity, and stability of meats of different origins is the complex synergism of many pre- or postharvesting factors.

Some of the preharvesting factors are intrinsically associated with the animal, such as species, breed (strain), age, sex, muscle type, whereas some others depend on extrinsic conditions, such as diet, housing system, environmental conditions, stress associated to transport, or to any other preslaughter and slaughtering conditions. Postharvesting conditions are referred either to the whole carcass (electrical stimulation, chilling) or to cuts (storing, packaging, light exposure).

Before considering them in detail, a short digression must be made on the biochemical events that take place in the muscle after slaughtering, which are influenced by many of the factors mentioned above and may determine strong alterations in the organoleptic characteristics of the meat.

Once the lactic acid is formed during the postmortem residual activity, it cannot be removed from tissues, due to the termination of circulation; it contributes to the process of pH decrease until its value is as low as to inhibit enzymatic activities or the glycogen reservoir is terminated. At the end of the process, pH begins to increase slightly again until the meat tenderization has been concluded. The final pH can be very different, again according to the animal species and type of muscle. Red muscles will be characterized by a higher pH, due to the presence of a less efficient glycolytic apparatus with respect to muscle

rich in white fibers, whose role in supporting brief but intense contraction needs a larger amount of glycolytic enzymes.

Ultimate pH and the rate of its decrease, together with other factors, such as temperature, influence chemical reactions and determine some of the structural characteristics of the muscles, which, in turn, strongly affect the color of the meat. In general, a paler appearance than normal is associated with lower pH and a darker appearance with higher pH. It has clearly been demonstrated that excessively fast lowering of pH, associated with inadequate temperatures, determines the premature precipitation of muscle proteins, which tend to aggregate, causing water loss and the detachment of myofibrils from the cell membranes. The muscle's open structure thus formed indicates greater surface reflectance and the pH promotes the oxidation of OMb^{2+} and Mb^{2+} to MMb^{3+} (McKee and Sams 1998; Owens and others 2000).

These conditions together determine drip loss, soft consistency, and pale color, which characterize PSE meats, commonly found not only in pork, but also in poultry, especially in turkey. PSE meats are rejected by consumers and represent a growing concern in the industry. It is well-known that PSE conditions are induced by acute preslaughtering stress, which causes an increase in tissue metabolism and a rapid consumption of glycogen, with a prompt decrease in pH associated with the high muscle temperature. When the glycogen reservoir has been depleted before slaughtering, as happens in very active animals or in animals stressed over a long period, the ultimate pH of meat is usually higher, as observed in DFD meat. In beef, this kind of defect is also referred as dark cutting beef (DCB). High postmortem pH determines a higher solubility of proteins, which bind a greater quantity of water. This condition determines a higher compactness of the fibers, resulting in a more tightly packed assembly, and less free water to reflect light. The closed structure of the muscle implies a lower diffusion of O_2 and favors the maintenance of myoglobin in its deoxy form. Moreover, it implies a lower scattering capability of the muscle, so that light is absorbed rather than reflected (Warriss 2000).

5.4.1 Preharvest Factors

The following sections represent the principal preharvest factors affecting the color of the meat.

5.4.1.1 Intrinsic Factors

- *Animal species.* On an average, myoglobin content is highest in beef and lower in poultry, while ovine and pork have an intermediate content.
- *Strain.* Genetic selection implies differences in muscle growth rate, size, and biochemical characteristics in all the common species utilized for meat production.

In swine, the genetic characteristics associated to different strains have been shown to affect the color of the meat. This has been highlighted, for instance, by studies on loin chops from different sire lines, which show differences in lightness, pinkness, and redness (Brewer and others 2004).

The genetic influence on the final characteristics of the meat is particularly evident when taking into consideration the cases related to variants of two genes in swine: the halothane gene (HAL), or stress gene, which is associated with the Porcine Stress Syndrome (PSS), a genetic abnormality characterized by the animal inability to adapt to stressors encountered during its lifetime, and the Rendement Napole gene (RN) (Mabry and others 1998).

HAL is a mutation that determines a number of alterations in the carcass or muscle sizes and a higher incidence of PSE meat, as a consequence of an increased metabolism (Zhang and others 1992). Pigs affected by PSS have a homozygous single point mutation in the ryanodine receptor-1 gene (RYR1), which makes them more susceptible to halothane anesthesia and are classified as halothane sensitive (HS) (Allison and others 2005). It is well-known that many antemortem stress factors, including environmental temperatures, preslaughter-handling practices or stunning methods, may induce PSS and PSE meat. For this reason, most swine genetics companies eliminated this polymorphism from their herds.

RN is a swine gene commonly called the "acid meat gene" due to the low ultimate meat pH it causes. Unlike HAL, RN appears completely dominant. Its negative effects depend on a dramatic increase in glycogen levels in the muscles of pigs, which will determine a higher production of lactic acid after

slaughtering and thus a lower ultimate pH of the muscle. This low pH value causes a breakdown in proteins, which results in pale muscle color and poor water-holding capacity in the meat. This condition is very similar in characteristics to the PSE.

PSE meat is also observed with increasing frequency in turkey- and broiler-processing plants. Besides PSE, some authors described a significant decrease in color intensity in high-performance strains compared to that of less selected genotypes, both in broilers (Le Bihan-Duval and others 1999; Bianchi and others 2006) and in turkeys (Santé and others 1991).

- *Age*. Generally, the color tends to intensify with aging in relation to a different texture and a higher concentration of pigments that shift the meat color toward darker and redder tonalities, both in mammals and in birds (Froning and Hartung 1967; Latorre and others 2004).
- *Sex*. Females and gelded males have paler meat than whole males. For instance, studies on the comparison between heifer and steer carcasses showed different L*, a*, and b* values in muscle (Wulf and others 1997; Page and others 2001).

Regarding DFD meat in cattle, a number of studies showed that sex has the greatest influence in the defect onset and that heifers showed a higher risk than steers or spayed heifers, probably because of a more excitable temperament, sometimes influenced by estrogen secretion (Voisinet and others 1997a, b; Scanga and others 1998).

- *Muscle type: Anatomical district and functional activity of the muscle*. Within the same species, different muscles may present sharp variations. Muscles responsible for sustained activity, rich in red oxidative fibers, such as those belonging to the leg in poultry, have a higher myoglobin content than that of muscles responsible for short-power movement, such as the pectoralis of nonflying birds. Red muscles are characterized by a higher pH, due to the presence of a less efficient glycolytic apparatus with respect to muscle rich in white fibers, whose role in supporting brief but intense contraction needs a larger amount of glycolytic enzymes. Furthermore, oxidative fibers have a lower concentration of glycogen, which determines a higher ultimate pH. These differences cause color stability variations from muscle to muscle, from cut to cut, and also within a cut. For example, *Longissimus dorsi* as the hindquarter musculature tends to develop DFD more than forequarters muscles (Warriss 2000). In beef, some cuts, such as rumps and tenderloins, are more susceptible to browning than others, such as striploins (Food Science Australia 2000).

The position is also important in relation to muscle cooling efficiency, as observed by Rosenvold and Andersen (2003), who evaluated the effect of preslaughter stress in pigs on different muscles. *Longissimus dorsi* was the muscle least involved in terms of modification of L*, a*, and b* values with respect to *biceps femoris* and *semimembranosus*, due to its superficial position and more efficient cooling.

5.4.1.2 Long-Term Extrinsic Factors

- *Diet*. Diet is another parameter that influences the color of the meat. It is well-known that consumption of supranutritional levels of vitamins and other antioxidants by cattle improves meat color stability and oxidative stability. The contribution of α-tocopherol to the oxidative stability of skeletal muscle is largely influenced by diet (Chan and Decker 1994), and it acts as MMb^{3+} formation and lipid oxidation delayer proportionally to its content in feed (Garber and others 1996). Muramoto and others (2003) observed that in cattle dietary β-carotene supplementation lengthened the color life of *semimembranosus* and *longissimus dorsi*. In contrast to the successful results obtained in beef, the results obtained by supplementing diet with vitamins (i.e., vitamin E) have been indecisive in pork, and most studies do not confirm any significant effect on the color stability of the meat (Rosenvold and Anderson 2003).

The use of natural antioxidant also has been demonstrated to be effective in preserving meat color, and many different kinds of them have been successfully employed. When rosemary has been

supplemented in the diet, it was reported to be more effective than vitamin E in preserving oxidative and color stability of beef, in both aerobic and modified atmosphere environments (McBride and others 2007).

Also, finishing feeding may exert some effect on meat color, such as the use of feed low in digestible carbohydrates, which determine a darker and less intense color of certain muscles, such as *Longissimus thoracis*, in pigs (Rosenvold and others 2001; Tikk and others 2006).

The glycolytic potential of muscles, defined as the capacity for anaerobic metabolism by accounting for the various substrates found in muscle that can be converted to lactic acid, can be influenced by the lack of an adequate feed intake. In particular, longer fasting has been shown to produce marked effects, increasing the ultimate pH with a darkening in meat color, and decreasing the risk of PSE in pork meat (Eikelenboom and others 1991; Guàrdia and others 2004). However, too a long fasting can increase the incidence of DFD meat in pigs (Partanen and Siljander-Rasi 2007).

- *Housing system.* It may affect color through changes in physical activity of the animal, which could influence muscle fiber type and metabolism. For instance, Vestergaard and others (2000) reported both a darker muscle color and increased pigmentation for loose-housed bulls fed a roughage-based diet compared with bulls fed ad libitum concentrates in a tie stall. Loose housing and roughage-based diets increased the amount of slow-contracting fibers, vascularization, and muscle oxidative metabolic potential. Still, the effects of preharvest environment, particularly housing and pen space, have been shown to have an effect on pork color: Gentry and others (2004) reported that pigs born and reared outdoors had redder loins than pigs born and reared indoors. The mechanism by which rearing environment influenced muscle color was unknown but was attributed to environmental components such as space, soil, and vegetation.

Few records regarding the effects of the housing system on the broiler meat color exist at the moment. Sekeroglu and others (2009) compared two housing systems (traditional vs. free range) in broiler chickens and reported a decrease in redness and an increase in yellowness of breast meat color in free-range-reared birds.

5.4.1.3 Preslaughter and Slaughtering Conditions

Stress associated to the period close to slaughtering is very important in determining the final pH of meat. In fact, the muscle glycolytic potential can be influenced by several factors such as ambient temperature, humidity, stocking densities, transport or lairage duration, and handling practices, other than fasting (Partanen and Siljander-Rasi 2007).

- *Ambient temperature.* In cattle, the occurrence of DCB has also been associated with climatic conditions. Grandin (1992) and Smith and others (1993) reported a higher incidence during very cold weather, even though very hot temperatures have been shown to produce similar effects (Scanga and others 1998). In pigs, high temperature immediately before slaughtering may induce a decline in muscle color intensity (Sayre and others 1963). Many authors agree in considering the stress-related elevation of early postmortem muscle temperature as a key factor influencing color and color stability more than pH (Mancini and others 2005).

Antemortem heat stress has been shown to also influence turkey meat, especially with regard to breast muscles (Babji and others 1982; McKee and Sams 1997).

- *Transportation and densities.* Transportation conditions are critical for stress induction in the hours preceding slaughtering. In cattle, it has been shown that transport is a fundamental element responsible for DFD onset. Actually, even though it is difficult to correlate the incidence rate with the distance or the duration of the trip, the loading and unloading seems to be the major critical point (Van Logtestijn and Romme 1981).

Stress induced by transportation is very important in pigs. Guàrdia and others (2004) showed that the risk of PSE meat increases with stocking density during transport for transits longer than 3 h, while the opposite occurs for shorter transits. Still, Lynch and others (1998) found that also the kind of vehicle used for transportation (modern truck vs. a traditional trailer) can influence the final quality, and meat from carcasses of pigs transported in the trailer result to be darker than the other.

In the poultry industry, commercial problems are associated mostly with turkeys than with broiler chickens. Kannan and others (1997) observed that also the crating process increases the lightness of chicken breasts. Transportation may also affect this parameter, which has been reported to be either increased (Cashman and others 1989) or decreased (Debut and others 2003; Owens and Sams 2000). Finally, negative correlations between the redness of chicken breast fillets and the length of transportation have also been observed in chicken breast fillet (Bianchi and others 2006).

- *Lairage and handling practices.* One good example of lairage influence on meat color is given by the study of Mojto and others (1998), who showed that meat from bulls, which are physically more active in the loose housing at the slaughterhouse during the night, has higher pH values than meat from steers, as a consequence of depletion of glycogen reserves. This determines a higher occurrence of DFD cases. Physical exertion associated with mixing different animals in the same pen before slaughter is the main cause of DFD in cattles, and the prolongation of the time during which mixed cattle can interact increases the prevalence of the problem. Also animals sold through live auction market may show higher level of dark cutting, presumably for the same mixing stress causing responses, such as elevation of heart rate and body temperature and increased circulating corticosteroid level (treated in Warriss 2000).

In pigs, bruising and PSE meat, besides other problems, such as dead animals or broken bones, can be associated with improper handling before slaughter (Schultz Kaster 1998; Belk and others 2002). Furthermore, DFD muscle and blood splash are positively correlated with the number of animals in pens where pigs are placed before harvesting (Barton Gade and Christensen 1999). Other than the stocking density, lairage time can also increase the risk of DFD meats (Guàrdia and others 2005). Color can be also negatively affected by forcing pigs through the handling (Van der Wal and others 1999; Hambrecht and others 2004).

- *Stunning.* The commonest stunning systems in pigs are based on electricity or carbon dioxide (CO_2) utilization. Due to the fast falling of the muscle pH after the powerful activation of glycolysis in the muscles of electrically stunned pigs, the CO_2 method is sometimes preferred due to its capacity to improve meat quality by reducing blood splashing and PSE meat incidence (Barton Gade and others 1992; Raj and others 1997; Faucitano and others 1998). Kang and Sams (1999) showed that gas stunning can reduce carcass damages in poultry. Many authors agree with positive effect of CO_2 stunning system on pig meat quality in terms of higher 24 h muscle pH and more desirable muscle color (a* and b* values). On the other hand, considering the high number of variables influencing the final color, it has also been shown that CO_2 can reduce PSE meat incidence in pigs carrying the stress gene, but may increase PSE cases in animals that are free of this gene (Grandin 1998). Loins from pigs stunned with CO_2 have been shown to be darker and less yellow than those from pigs electrically stunned (Velarde and others 2001). However, the use of potentially different gas mixes or different types of electrical stunning may make difficult an efficient comparison of their effects on meat color.

In poultry, rigor mortis development, which is known to affect ultimate pH and thus meat color, was reported to be accelerated with CO_2 stunning by some authors (Raj and others 1997), whereas others observed no differences with electrical stunning (Kang and Sams 1999). CO_2 stunning has been reported to cause a loss of redness in turkey breast and thigh meat (Raj and others 1990), whereas gas argon killing has been reported to decrease darkness in broiler muscle with respect to electrical or CO_2 stunning (Fleming and others 1991a). Savenije and others (2002) reported that in normally processed carcasses from CO_2/argon-stunned chickens had higher L* values and lower a* values than those from electrically

stunned chickens, whereas those from chickens stunned with a $CO_2/O_2/N_2$ gas mixture had higher b^* values. Northcutt and others (1998) observed no significant differences in turkey breasts following 24 h after electrical or CO_2 stunning, nor did Poole and Fletcher (1998), who compared the effect of electrical stun and CO_2/argon killing in chicken breasts.

5.4.2 Postharvest Factors

As already underlined, there are many biochemical and structural events that take place in the first 24 h period after the animal is slaughtered and the muscle is converted into meat. This period greatly impacts meat tenderness and muscle color and is species-specific, for instance, in how the initial cooling process results in positive or negative consequences in meat quality.

- *Chilling.* At present, there are three types of chilling systems commonly used in commercial practices, including conventional, spray, and rapid chilling systems. Less common forms of chilling are cryogenic chilling, which utilizes liquid nitrogen and brine chilling. The most used are the rapid systems, included (but not limited to) "rapid," "ultrarapid," "blast," "very fast," and "extreme," with no consistent definition used by authors in defining these systems. Spray chilling lowers the temperature of the carcasses more quickly than does conventional chilling, but not as quickly as blast chilling, and is based on intermittent or continuous spraying the carcasses with water during the first few hours of the chilling phase followed by a drying period prior to loadout. Rapid chilling regimes produced darker meat color in beef, as reported by Aalhus and others (2002).

On the contrary, Greer and Jones (1997) noted that the spray chilling method greatly influences fat color, that acquires higher L* values in most locations, more than lean muscle.

Spray and rapid chilling systems are often used for pork carcasses chilling (Huff-Lonergan and Page 2001) and the use of different accelerated chilling systems is also an effective method to prevent or reduce the incidence of PSE in pork (Milligan and others 1998; Springer and others 2003; Savell and others 2005). In fact, fastly lowering the temperature postmortem can decrease the rate of the biochemical reactions, lowering the rate of pH decline with positive effect on PSE, primarily affected by pH falling (Offer 1991; Lawrie 1998). However, Tomovic and others (2009) reported that different chilling rates of pork carcasses did not produce significant differences in color.

Chilling conditions can also have a great impact on the development of PSE in turkey meat where large body size can produce an inadequate chilling procedure: the rapid decline in muscle pH at high carcass temperatures leads to the poor meat quality traits as shown by several studies reporting that inadequate chilling results in meat with lower pH and lighter color (i.e., Alvarado and Sams 2002; Molette and others 2006).

Chilling method seems not to affect the color of raw broiler breast fillets (Fleming and others 1991b; Huezo and others 2007).

- *Electrical stimulation (ES).* Electrical stimulation involves transmitting an electrical current through the carcass of freshly slaughtered animals. This electrical current accelerates postmortem glycolysis and causes pH decline by a rapid depletion of the muscle glycogen (Eikelenboom and others 1985). ES has received considerable attention for improving the meat quality, including not only tenderness and palatability attributes, but also the color in beef (Mckenna and others 2003), lamb (Polidori and others 1999), goat (Cetin and Topcu 2009), chicken (Birkhold and Sams 1993), deer (Wiklund and others 2002), and pork (Taylor and Martoccia 1995) meat.

At present, two kinds of ES exist: a low-voltage ES (voltage <100 V) (LVES) and high-voltage ES (voltage >100 V) (HVES). A recent study by Nazli and others (2010) showed that HVES applied to beef carcasses determined a more rapid pH decline and a better meat color compared to nonstimulated carcasses. Other reports on the influence of ES on meat color describe an increase of both lightness and yellowness in the *longissimus thoracis* and of yellowness and redness in the *gluteus* as a result of

high-voltage electrical stimulation in lambs (King and others 2004). Also, leans from electrically stimulated lamb carcasses have been described to have a brighter red color (Kerth and others 1999) or to be more "youthful" colored (Riley and others 1981) than lean from nonstimulated carcasses.

5.4.3 Storing

When the meat is stored, myoglobin may be present in its three main forms (Mb^{2+}, OMb^{2+}, and MMb^{3+}) in different proportions, according to the type of meat or meat product and the processing and storing history. As time elapses, the proportions of these myoglobin chemical states continuously change, being influenced by many intrinsic parameters, which have been described before. As a result, the oxidized form MMb^{3+} accumulates and contributes to meat browning.

Conditioned meat has been shown to reach a higher surface brightness after exposure to air with respect to unaged meat and gets greater a* and saturation values, resulting in a more attractive appearance. However, the decrease in enzyme activity during aging determines a faster accumulation of MMb^{3+} during the display life (MacDougall 1972).

Unprocessed or slightly processed meat is normally kept at refrigerated temperatures or frozen for a long-time storing. Refrigeration (temperature above –1.5°C, the freezing point of the meat) can be associated to different kinds of packaging, with the aim of both improving display conditions and inhibiting the growth of spoilage microorganisms, whose activity is an important factor in pigment changes as it reduces the O_2 tension in the surface tissue (Walker 1980). Experiments on pork meat (Jeremiah and Gibson 1997) and in beef muscles with good color stability (Gill and McGinnis 1995) showed that the lower is the storing temperature in aerobic condition, the better is the acceptability of color. Of course, low temperatures slow down all the chemical reactions that bring to browning or to discoloration due to microorganism metabolism and facilitates O_2 to penetrate the meat more easily.

Freezing for longer periods may negatively affect the reducing activity of the muscle (Stewart and others 1965b) and bring to a higher oxidation grade of unsaturated fatty acids, promoting the browning process. Moreover, the formation of crystals changes the optical properties of the meat, which results in a lower reflectance capacity. This contributes to confer a darker appearance with respect to the fresh meat, together with a higher tendency of MMb^{3+} to form. Moreover, crystals damage muscle fibers, allowing cellular component that are compartmentalized, in normal conditions, to mix together. The spreading of salts contributes to increase the ionic strength, and in the presence of O_2 the formation of radicals is facilitated. As a consequence, tissue degradation and oxidation is accelerated and discoloration promoted (Young and West 2001). Also the rate of freezing may influence the color of meat. At very low rates, there is a marked development of darkness, while at very fast rates an unnatural paleness is developed (Guenther and Henrickson 1962). Exposure of the meat to air prior freezing may help acquire an appearance similar to that of fresh meat (Tuma 1971). However, frozen meat can bloom in presence of O_2, but, even when thawed, it remains less reflective than raw meat and this determines a less attractive color (Jeremiah 1981).

5.4.4 Packaging

Packaging has been shown to influence meat color. This can be ascribed to the different degree of permeability to the gases and moisture in the packaging film and the type of gas mix introduced in modified-atmosphere packaging (MAP). The function of the various types of packaging is not only to extend the shelf life of the products, protecting it from microorganisms and dirt, but also to maintain a good appearance, controlling transpiration, and preserving or enhancing the most desirable shades of meat color. In MAP, it is important to find the best blend of gases to optimize color, stability, and shelf life of the meat, limiting microbial growth and lipid oxidation. In general, the chemical properties of the various molecules that interact with meat constituents may induce different chemical reactions and produce different effects on the macroscopical appearance of the meat during its shelf life. Myoglobin, lipids, and metals are primarily involved in this type of reaction.

High-O_2 atmospheres promote pigment oxygenation, increasing the redness of the meat, but enhanced lipid oxidation and different results on color stability have been reported. Some authors observed a

decrease in color deterioration and stabilization in redness during storage in beef and pork meat (Gill 1996; Jayasingh and others 2002). Different levels of decreased a* values at different times after packaging have been reported for ground turkey and chicken meat, probably as a consequence of myoglobin oxidation and the limited ability of poultry meat to form OMb^{2+} compared to beef and pork, due to high MMb^{3+}-reducing activity and high-O_2 consumption. A number of studies reported that high-O_2 atmospheres increase the likelihood of premature browning, especially in beef (Seyfert and others 2004; Suman and others 2005; Grobbel and others 2008), but discoloration or localized color alterations have been reported also in turkey thighs (Guidi and others 2006). In addition, the high-O_2 concentration increases the oxidation of lipids and leads to a shift from red to yellow (Saucier and others 2000; Dhananjayan and others 2006; Keokamnerd and others 2008).

With the increased use of high-O_2 MAP, several cases of bone darkening occurred during the retail life, involving beef and pork cuts, and affecting the choice of the consumers. Bone marrow discoloration is not related with the lipid portion, but it is primarily associated with the hemoglobin's redox state. The use of low- O_2, CO-enriched atmospheres, or reducing agents have been shown to be effective to improve color stability of the marrow (Mancini and others 2004, 2005).

In contrast, ultralow-O_2 atmospheres (usually N_2 as an inert filler, plus CO_2 at various concentrations) minimize the lipid oxidation and growth of aerobic microorganisms, and their use has been shown to be associated with the greatest color stability during shelf life in both turkey (Santé and others 1994; Dhananjayan and others 2006) and chicken meat (Saucier and others 2000). This could be due to the limited ability of the poultry meat to bloom as compared with beef and pork (Dhananjayan and others 2006). Generally, the prevalence of Mb^{2+} in low-O_2 atmospheres confers a darker color to beef and pork meat. To overcome this drawback, low concentrations of CO can be added to the modified atmosphere, since it forms $COMb^{2+}$ red-stable complexes on the meat surface and improves color stability in ground beef and steaks (Hunt and others 2004; John and others 2004, 2005) and pork (Krause and others 2003), but few data exist on poultry meat.

An alternative to N_2 plus CO_2 MAP, vacuum packaging is commonly used to exclude O_2. The drawback is represented by the fact that vacuum-packaged fresh meat turns to a dark, purplish color because the lack of O_2 and the reducing enzymes promote the conversion of the bright red pigment to Mb^{2+}. Nevertheless, once the meat is unpacked and it is exposed again to the atmosphere, the color converts to red in a short time for the rapid formation of OMb^{2+}. A too high-residual content of O_2 in the packaging, the presence of defects in the packaging itself (holes, poor seals), or the use of a film with a wrong grade of impermeability may determine a browning occurrence. This is due to the fact that a O_2 partial pressure of about 1 mmHg is sufficient to trigger a fast rate of myoglobin autoxidation with formation of MMb^{3+} (George and Stratmann 1952). However, the vacuum packaging does not represent the best choice for all kind of meat. For example, DFD meat in this condition may rapidly develop green discoloration due to the growing of spoilage microorganism-producing H_2S, which binds myoglobin and forms sulfmyoglobin (Nicol and others 1970).

Finally, the action of light on the meat pigment may be deleterious. Some works on myoglobin photooxidation provided evidences that ultraviolet lights enhance discoloration (Ramsbottom and others 1951; Bertelsen and Skibsted 1987). Therefore, meat shelf life can be improved by using packaging films able to reduce the amount of UV light that comes in contact with the meat.

5.4.5 Meat-Display Lighting

The color of meat at the retail level is strongly influenced by lighting, even though different experimental results leading to different opinions on this issue have been published. An adequate lighting system provides not only the right quantity of illuminance, but also the right type of light. In fact, the visible electromagnetic radiation that is reflected by the meat and that constitutes the color as it is perceived by the consumer is part of the total radiation that hits the product. Too weak lights will not be successful in exalting the brightness of the meat, while the prevalence of the blue or yellow component of the spectrum will diminish the possibility to appreciate the intrinsic redness of the meat, which represents one of its most appealing characteristics. Light sources are generally represented by incandescent, fluorescent, or metal-halide lamps. When the light source is introduced inside the display case, fluorescent lamps are

usually used due to their markedly lower heat production, resulting in a minor stress on the display's refrigeration unit (Barbut 2001). Studies on the effect of different kinds of illumination on the appearance of fresh beef, chicken (Barbut 2001), and pork (Calkins and others 1986; Barbut 2001) meat confirmed as incandescent lamps render the products more desired. The main reason can be ascribed to the fact that fluorescent and metal halide are not full-spectrum lights and lack in the red component. The differences in the color perception produce a higher effect in red beef, rather than in pork or chicken. However, the use of enhanced high spectrum fluorescent lights, even though more expensive, guarantees better results than the classical ones (Barbut 2005). Nevertheless, it must be underlined that the spectral composition of light illuminating meat products in retail displays does not depend exclusively on the fluorescent lamp model, but it is the result or combination of several factors, among which the contribute carried by the environment illumination (Sáenz and others 2005). Finally, the damaging action of light on the meat color should be prevented or reduced by using light source with a reduced emission in the UV (Djenane and others 2003).

5.4.6 Use of Antioxidants

A vast number of studies have shown the successful use of antioxidants not only to improve the flavor stability of the meat, but also to preserve its color, thanks to the fact that such substances inhibit or prevent the formation of peroxides and quench radical reactions, usually involving the lipid component. Lipid oxidation products interact directly with the protein portion of myoglobin and make the heme iron more susceptible to oxidation (Alderton and others 2003), thus accelerating the browning processes.

Antioxidants, other than included in the diet of livestock, can be incorporated into finished products or infused into packaging materials (Formanek and others 2001; Moore and others 2003). Among the most commonly used antioxidants, there are ascorbate (or ascorbic acid), citrate (or citric acid), and tocopherols or extracts of herbs and spices, whose effect is due primarily to phenolic OH groups (Cai and others 2005). The addition of reducing agents to meat products help maintain myoglobin in the reduced state, thus preventing MMb^{3+} formation and premature browning. Sodium ascorbate and sodium erythorbate added to ground beef have been shown to be effective in maintaining the red color and myoglobin in the reduced state in cooked patties (Sepe and others 2005). Wheeler and others (1996) observed a minor surface discoloration and increased redness in steak, after injecting beef cuts with a sodium ascorbate solution. The addition of ascorbic acid, tocopherol, or sesamol have been shown to be effective to improve the preservation of redness in ground beef during storage also prior to irradiation (Ismail and others 2009). Some polyphosphates have been shown to decrease TBARS (which is an index proportional to the oxidation grade of the substrate) and MMb^{3+} content, either alone or in combination with other antioxidants (ascorbate and tocopherol), thus improving color, as observed in ground beef (Lee and others 1998) and buffalo meat (Sahoo and Anjaneyulu 2000).

Natural antioxidants have also been successfully used. For instance, rosemary added to ground beef helps maintain redness both in fresh (Balentine and others 2006), or frozen meat (Akarpat and others 2008), to inhibit MMb^{3+} formation and lipid oxidation (Sanchez Escalante and others 2001; Djenane and others 2003), exerting its protecting action also in high-O_2 content packaging beef meat as well as ascorbate (Lund and others 2007). Other effective natural antioxidants are represented by oregano extracts. As well as rosemary, oregano combined with ascorbic acid can reduce TBARS values, inhibits MMb^{3+} formation in ground beef (Sánchez Escalante and others 2003), and prevents bone discoloration in high-O_2 MAP.

Besides vitamins or molecules with antioxidant activity present in some herbs, chelating agents bind metal ions avoiding their involvement in oxidation reactions (Fennema 1996).

5.5 Irradiation

The use of ionizing radiation is a common practice in some countries for meat processing and prevention of foodborne diseases, and it is regarded as one of the most effective methods to eliminate pathogens in meat (Gants 1996). The energy of the incident radiation on the meat may be absorbed by some electrons,

which can leave their orbital and transfer part of this energy to other electrons, in a sort of cascade reaction, until there is enough energy for the orbital leaving (the Compton Effect). This event may cause the scission of molecules; water is highly subject to radiolysis, which brings about the formation of free radicals, such as hydrated electrons, hydrogen radicals, and hydroxyl radicals (Thakur and Singh 1994). Radicals attack lipids and proteins and generate abnormal color and off-odors (Nanke and others 1998; Ahn and others 2001).

Irradiation can induce a number of different effects on myoglobin and on the molecules present in meat, whose alterations are responsible for the color change that occurs after treatment. Different final effects on color are related to the myoglobin concentration and its state before irradiation and also to substrate conditions, such as the pH and Eh values of the meat or to temperature. However, among the most important factors that influence the meat color after irradiation we have to mention the animal species and the atmospheric composition of the packaging. In particular, pork and beef may undergo different changes resulting in developing, other than bright red, brown, or green color even though beef seems to be the most susceptible to changes (Kim and others 2002). Furthermore, instrumental hue measurement reveals different color change trends after storage in beef, pork, and turkey (Nanke and others 1999; Kim and others 2002). Keeping the meat in the presence of O_2 after irradiation may lead to synergic effects on color development during the shelf life (Luchsinger and others 1996; Ahn and others 1998a; Murano and others 1998). However, experimental results have not always been concordant and some differences in outcomes have been reported in literature, partially due to slightly different experimental conditions. For instance, pork has been shown to decrease redness in aerobic conditions and to increase brown or gray shades (Tappel 1956; Ohene-Adjei and others 2004), but other reports attested an increase of a* values (Grant and Patterson 1991; Chen and others 1999). The same effects were observed in vacuum-packaged steaks (Luchsinger and others 1997). On the other hand, vacuum-packaged beef have been reported to become brown (Murano and others 1998; Nanke and others 1998), with a decrease in a* and a growth in b^* values.

A number of studies performed on different products, from both turkey and chicken meat, showed that irradiation determines an increase in redness regardless of the type of packaging used (Nanke and others 1998, 1999; Kim and others 2002; Yan and others 2006a), thus testifying to a common trend in poultry, regardless of the muscle of origin or the type of processing (Du and Ahn 2002). Since the red color of meat is often associated with its freshness, irradiated turkey appears to be preferred by consumers (Lee and others 2003; Yan and others 2006b). Even though irradiation increases yellowness in all species (Brewer 2004) inducing the partial formation of the pigment $MMbFe^{3+}$, the increase in brownness caused by an MMb^{3+}-like pigment perceivable in other species could not be observed in turkey (Nanke and others 1999).

Still, irradiation can magnify the natural tendency to increase L* values with aging due to the natural and progressive decrease in reductants (Nam and Ahn 2003a); its effects on lightness and yellowness seem to be markedly lower than the effect on redness, but the occurrence, the magnitude of possible changes of these parameters, and their development according to display time are still controversial as to details.

CO is a major radiolytic gas arising from irradiated foodstuffs (Pratt and Kneeland 1972; Simic and others 1979). It has been suggested that the red or pink color of irradiated meat is due to the CO produced, which has a very strong affinity to heme pigments, forming $COMb^{2+}$, which is more stable than OMb^{2+} (Kim and others 2002; Nam and Ahn 2002).

Irradiation also increases the reducing power of meat, which facilitates carbon monoxide–myoglobin complex formation, which is dose-dependent (Nam and others 2003). Nam and Ahn (2003b) found an increased redness of vacuum-packaged turkey breast by irradiation that was stable even after 10 days of refrigerated storage. However, in some cases, as reported before, the redness of aerobically packaged meat decreased significantly. This finding indicated that exposing irradiated meat to aerobic conditions was effective in reducing CO-heme pigment complex formation, probably due to the presence of O_2, which accelerates the dissociation of $COMb^{2+}$ (Grant and Patterson 1991). Nevertheless, the very importance of $COMb^{2+}$ complexes and their evolution during storage must be considered even in relation to the evolution of redness values in time, which still seems to be controversial, and $COMb^{2+}$ alone probably cannot explain all the irradiated meat color.

Irradiation has also been performed in a nitrogen atmosphere. Some studies have shown the formation of a pink color after treatment on chickens with a reflectance similar to that associated with OMb^{2+} (Satterlee and others 1972). The storage stability of the pigment formed and the irradiation atmosphere without O_2 caused others to ascribe the color to $COMb^{2+}$ or $NOMb^{2+}$ (Millar and others 1995).

Finally, the addition of antioxidants to the meat prior irradiation may improve color stability during display life (Giroux and others 2001). Supplementing diet with antioxidant has also been shown to improve the color of the meat in some cases (Ahn and others 1998b; Houben and others 2000), but results of other studies have not confirmed its benefits on color (Nam and others 2002).

5.6 Cooking

Heat has a strong effect on meat color, provoking the development of marked brownish hues. The factors most involved in determining the color of cooked meat are the different forms of myoglobin, which tend to denature along with other proteins. Myoglobins begin to denature after 55°C and the process is accomplished primarily around 75–80°C (Varnam and Sutherland 1995; Hunt and others 1999). Cooking causes an increase in pH, which is likely to be the reason for the slower rate of protein denaturation as the temperature arises (Geileskey and others 1998). According to the oxidation state of iron and the complex formed by myoglobin with other molecules, sensitiveness to heat varies. Mb^{2+} is less sensitive than OMb^{2+} and MMb^{3+} (Van Laack and others 1996; Hunt and others 1999). Denaturation of OMb^{2+} and Mb^{2+} with cooking leads to the formation of ferrohemochrome (Fe^{2+}), a red pigment that is rapidly oxidized to ferrihemochrome (Fe^{3+}); MMb^{3+} is converted directly into Fe^{3+}, which results in higher amounts in cooked meat, conferring its typical brown color (Varnam and Sutherland 1995) (Figure 5.3). Yet the final shade of the cooked meat depends on the Fe^{3+}/Fe^{2+} ratio, which is determined by either the type of cooking process (intensity and duration) or the initial relative concentration of the three main forms of myoglobin, even though under certain conditions meat can be enriched with other forms of myoglobin, as, for example, $COMb^{2+}$ or $NOMb^{2+}$, which are more stable than OMb^{2+}.

Different types of muscles or animal species are associated with different structural and biochemical characteristics, which may cause a different color evolution during cooking and also a final hue, generally ranging from off-white to gray or brown. Differences may also be determined by breeding management, preslaughter stress, and postmortem processing. As with raw meat, this implies that many variables influence the color development of meat during cooking. One of the most important is pH. An explanation of the association of pH with color development after cooking may be sought in the fact that a lower pH facilitates denaturation of the myoglobins, thus determining faster browning, due to the formation of Fe^{3+}, especially if subjected to heat treatment. However, different animal species are affected differently by pH. Experiments performed adjusting pork, beef, and turkey meat to different pH values cooked to reach different internal temperatures showed a common tendency to preserve the appearance of higher redness and a lower amount of denatured myoglobin, but at higher temperatures, turkey meat showed greater persistence of this tendency, with the percentage of denatured myoglobin at 83°C shifting from 75% to 95%, depending on pH, against 100% in other species at all pH levels (Trout 1989). Still, experiments performed on chickens showed that the darkness of raw meat tends to persist after cooking at higher pH (Fletcher and others 2000). Nevertheless, even though some studies correlate the grade and direction of color variation after cooking with the content and state of myoglobin or pH level of raw meat, meat being a complex biochemical system, the final color of cooked meat, and raw meat as well, must be influenced by many other factors that are still unclear, and whose synergy must be taken into consideration. For example, high pH has been shown to lessen the effects on cooked meat color produced by other factors, such as fat content, freezing, and rate of thawing (Berry 1998).

Beyond the factors described above, others may be regarded as determinant, such as muscle–fiber arrangement (e.g., DFD vs. PSE) and the denaturation processes of other meat proteins, including enzymes, which could also be affected by pH and to which myoglobin denaturation is intricately linked (King and Whyte 2006). The authors of some studies, in which myoglobin of ground lamb meat has been

shown to denature more slowly at lower pH, suggested a possible explanation in the fact that other proteins have been denatured first, which otherwise would interact with myoglobin destabilizing it (Lytras and others 1999).

The tendency to maintain a red or pink color during cooking (pinkness or pinking) is determined by the opposite causes of the browning: conditions are maintained to keep myoglobin undenatured and in the reduced state, which is the most heat stable. A higher pH is less effective in promoting pigment denaturation and contributes to the persistence of a pink–red color (Trout 1989). This condition is typical of meats from animals that underwent a long-stress period before slaughtering or from old animals (especially bovines). Meats with higher content of pigments may display longer a pink–red color. When the persistence of the typical uncooked meat color is determined by denatured reduced hemochromes, the problem involves not only the internal part of the product, but also the surface.

Incomplete burning of the gas or contaminants in the gas may result in the production of nitrogen dioxide and NO, which bind to the meat pigment and give a pink color. In this case, grilled meat or meat roasted in gas ovens may develop the so-called "pink ring," a superficial layer of few millimeters, which is often associated to undercooking by the consumers (Cornforth and others 1998). The pink pigment is the same formed in cured meat, which is intentionally treated with nitrates and nitrites to yield a pleasant color, besides controlling the growth of certain microorganisms, such as *Clostridium botulinum*.

In poultry, pinkness represents one of the most recurring defects after cooking, which can be observed even when the internal temperature exceeds 70°C. This defect is associated primarily with commercial problems, because of rejection by consumers. Factors involved in pinking incidence are several and include the presence of specific pigments, genetics, feed and stress, processing methods, and incidental nitrate/nitrite contamination through diet, water supply, processing equipment, freezing, and storing (Holownia and others 2003a). Other factors related to the chemical and physical properties of the meat, such as the presence of reducing agents, state and reactivity of pigments, and pH, may be involved in pinking (Holownia and others 2003a). Other than undenatured OMb^{2+} or Mb^{2+} and reduced hemochromes, pink defects are related primarily to the presence of nitrosylhemochrome, carbomonoxyhemochrome, or cytochrome *c*. One part per million of sodium nitrite is enough for the pinking occurrence, but high pH and low oxidation–reduction potentials may affect the pigment activity, permitting its occurrence even at lower concentrations (Holownia and others 2003b).

Another defect of cooked poultry meat is darkening, in which the tissue around the bone is discolored, attaining a burgundy or black appearance. This defect was first associated with frozen poultry (Spencer and others 1961; Lyon and Lyon 1986). The dark discoloration is seemingly determined by the infiltration of bone marrow onto the surrounding meat as a consequence of leaks in the bone. Finally, red discoloration of bone in fully cooked product is a defect often found in poultry. Marrow was determined to be the most important component in inducing red discoloration of breast meat (Smith and Northcutt 2004). Discoloration varied among product types, as suggested by the different lightness and redness values, and it is affected by cooking methods and piece type, as shown in a study by Smith and Northcutt (2003). However, cooking at a high-temperature endpoint reduces red or bloody discoloration. Reduced discoloration with a low endpoint is also possible if the product is frozen before cooking (Smith and Northcutt 2004).

5.7 Fat Color

Fatty tissues are a natural occurring part of the meat carcass and the color of the fat in meat cuts contributes to influence the choice of the consumers. Lipid oxidation and discoloration (myoglobin oxidation) reveal that color deterioration is an indicator of flavor deterioration (Djenane and others 2003). Normally, the whiter, the better, since yellow may be perceived by the consumer as being from old or diseased animals. As it happens for other meat components, the color of the fat is influenced by the animal species or even breeds and by the diet. In fact, under free-grazing conditions, the amount of carotenoids in fatty tissues increase and confer them a more yellow color. On the contrary, grain-fed animals will maintain a whiter color of fat (Zhou and others 1993). Grain finishing for at least 4 weeks may be adopted to reduce the intensity of the yellow due to carotenoids to acceptable levels (Forrest 1981).

However, discoloration of the fat portion may be due to oxidation processes, which involve fatty acids decomposition and are enhanced in presence of oxidizing atmosphere, as observed in beef cuts packaged in modified atmosphere with a high percentage of O_2 (Ramos Sagarnaga 2006). Vacuum or high content CO_2 packaging used for a long-period storage in refrigerated conditions have however been shown not to avoid fat discoloration after their placing onto the retail market, with a too fast development of a grayish hue that represented the greater limiting factor for retail display (Bell and others 1996). The fat oxidation occurs also in meat products and can lead to unpleasant colors, besides off-odors and flavors. Good results in hindering the process of fat oxidation in various meat products, such as turkey sausages (Barbut and others 1985) or beef steaks (Stoick and others 1991), have been obtained with the addition of natural ingredients containing antioxidant, such as rosemary.

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6

Flavors and Flavor Generation of Meat Products

Tzou-Chi Huang and Chi-Tang Ho

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6.1 Introduction

Characteristic meat flavor is a product of the volatile and nonvolatile compounds. Volatile compounds identified in meat flavor include hydrocarbons, alcohols, carbonyls, carboxylic acids, esters, lactones, ethers, sulfur-containing compounds, as well as different classes of heterocyclic compounds, namely furans, pyridines, pyrazines, oxazoles, thiazoles, and thiophenes (Table 6.1).

TABLE 6.1

Classification of Volatile Compounds Found in Meat

	Number of Compounds				
Compounds	**Pork (Uncured)**	**Pork (Cured)**	**Mutton**	**Chicken**	**Beef**
Hydrocarbons	45	4	26	71	123
Aldehydes	35	29	41	73	66
Ketones	38	12	23	31	59
Alcohols	24	9	11	28	61
Phenols	9	1	3	4	3
Carboxylic acids	5	20	46	9	20
Esters	20	9	5	7	33
Lactones	2	—	14	2	33
Furans	29	5	6	13	40
Pyridines	5	—	16	10	10
Pyrazines	36	—	15	21	48
Other nitrogen compounds	24	3	8	33	37
Surfur compounds	31	31	12	33	126
Halogenated compounds	4	1	—	6	6
Miscellaneous	7	11	—	6	16
Total	314	135	226	347	681

Source: Modified from Shahidi F, Rubin LJ, D'Souza LA. 1986. *CRC Crit Rev Food Sci Nutri* 24:141–243.

6.2 Precursors for Meat Aroma Formation

Raw meat is a rich reservoir of nonvolatile precursors of cooked meat flavor. The characteristic meat flavor is formed during the cooking process, when the nonvolatile flavor precursors, including free sugars, free amino acids, inosine monophosphate (IMP), thiamin, and ascorbic acid, react through a series of complex reactions (Mottram and others 2003). Components formed either by the enzymatic hydrolysis or thermal breakdown of fats, proteins, and carbohydrates are considered as these major precursors for thermally generated meat aroma. Strecker aldehyde, fatty aldehydes methanethiol, and 1-pyrroline are the degradation products of free amino acids, free fatty acids, methionine, and proline, respectively. The amount and nature of precursors present in meat depends on several factors including feed (Mottram and others 2003), aging (Koutsidis and others 2003), and genetic variations (Lawrie 1991).

Model systems composed of D-glucose and L-cysteine have long been used to study the thermal generation of nitrogen- and sulfur-containing flavor compounds (Mulders 1973; Scanlan and others 1973). An early attempt to synthesize meat flavor involved the heating of cysteine, plus some additional amino acids or hydrolyzed protein, with pentose or hexose (Morton and others 1960). Reaction of an aqueous solution of cystine with thiamin, glutamate, and ascorbic acid produces a complex mixture of compounds with an overall flavor resembling that of roasted meat (Werkhoff and others 1991).

6.2.1 Inosine Monophosphate

It was well established that a higher glucose concentration would increase the formation of Maillard reaction products and thus the fried attributes. It could be expected that higher concentrations of IMP also would increase the fried/grilled flavors and odors (Mottram and Madruga 1994a). Mottram and Nobrega (2002) pointed out that the relative amounts of IMP, ribose, and ribose 5-phosphate in meat may well be a determining factor in meat flavor quality. The role of IMP as a precursor of meat flavor has been

examined by heating muscle with and without added IMP. A number of thiols and disulfides containing furan groups were isolated from the meat systems, with much larger amounts formed in the meat containing IMP. The amounts of these sulfur compounds were also higher in meat systems in which the pH had been reduced by the addition of acid (Mottram and Madruga 1994b). Effects of addition of inosine-5′-monophosphate, to cooked meats on formation of volatile flavor compounds, were studied. The addition of IMP to both pork and beef has been shown to increase "meaty" and "roasted" aromas when added at four times the natural concentration (Mottram and Madruga 1994a). IMP, when added to beef at 10 times the natural concentration, increased the amount of generated thiols and disulfides containing furan groups during heating (Farmer and others 1999). Concentrations of sulfur substituted furans, mainly those containing the 2-methyl-3-furyl moiety, were significantly increased by the presence of flavor precursors, particularly 5′-IMP.

IMP is a product from the breakdown of ATP, and inosine, hypoxanthine, and ribose (or ribose 5-phosphate) are formed in meat from IMP by nucleoside phosphorylase or ribose dehydrolase, following the cleavage of IMP by 5-nucleotidase or nonspecific phosphomonoesterases (Lee and Newbold 1963). The rate of nucleotide hydrolysis in beef is much slower than in lamb, this may be due to a species effect or may reflect the physiological age of the animals (Rhodes 1965).

6.2.2 Thiamin

Thiamin has been recognized as an important precursor for the formation of meat aroma compounds, 2-methyl-3-furanthiol and bis-(2-methyl-3-furyl)disulfide. Thiamin degradation generates a series of sulfur-containing meaty compounds; hydrogen sulfide is an important precursor which can react with furanones to give an intense meat flavor. The acid degradation of thiamin leads to the formation of 4-amino-5-(hydroxymethyl)-2-methylpyrimidine and 5-(2-hydroxyethyl)-4-methylthiazole. The latter is supposed to be a key substance for the generation of series processed the meat flavors. On the other hand, the formation of meaty flavor compounds is favored by the thermal degradation of thiamin in a slightly alkaline pH value (Dwividi and Arnold 1963). Some key intermediates, hydrogen sulfide and 3-mercapto-5-hydroxy-2-pentanone, were formed. Further degradation of the latter can generate additional intermediates, 3,5-dimercapto-2-pentanone, 3,5-dihydroxy-2-pentanone, 3-hydroxy-5-mercapto-2-pentanone, and by a keto–enol tautomerization of 3,5-dihydroxy-2-pentanone, 1,4-dihydroxy-3-pentanone, 1-mercapto-4-hydroxy-3-pentanone, 1-hydroxy-4-mercapto-3-pentanone, and 1,4-dimercapto-3-pentanone (Guentert and others 1990). The intermediates 3-mercapto-5-hydroxy-2-pentanone and 3,5-dimercapto-2-pentanone are important precursors for aroma compound generation.

6.2.3 Alliin and Deoxyalliin

Alliin and deoxyalliin are two important nonvolatile flavor precursors of garlic. The isolate from the interaction of alliin and glucose possessed a very good roasted meaty character; the isolate from the interaction of deoxyalliin and glucose possessed a slightly roasted meaty flavor with garlic character. Thiazoles, especially 2-acetylthiazole, were found to be the predominant volatile interaction products of alliin and glucose (Yu and others 1994), whereas pyrazines, especially 2,5-dimethyl-, methyl-, and trimethylpyrazine, were found to be the predominant volatile interaction products of deoxyalliin and glucose. Some thiazoles together with some pyrazines, thiophenes, ketones, furans, and cyclic sulfur-containing compounds were thought to contribute to the roasted meat-like flavor in the model systems.

6.2.4 Strecker Aldehyde

The amount of free amino acids has been shown to increase during aging of pork (Moya and others 2001) and beef (Mullen and others 2000). There were highly significant differences ($p < 0.01$) in the concentrations of all the free amino acids between goats, suggesting that genetic factors may be important. Koutsidis

and others (2008) and Aliani and Farmer (2005) reached similar conclusions for beef and chicken, respectively. The concentrations of free cysteine (range of values for individual animals 0.02–0.06 mg/100 g) and free methionine (1.3–2.1 mg/100 g) were lower than values recorded in the literature for most goat meat types, since the concentration of free methionine found in meat is typically 2–6 mg/100 g, while cysteine ranges from 0.03 to 2.1 mg/100 g. These increases were thought to reflect the activity of variety of endoproteases and exoproteases, though it could not be concluded whether they were of microbial or indigenous meat origin.

High temperature treatment of meat such as roasting or grilling may lead to the pyrolysis of peptides and amino acids. When foods are roasted at temperatures higher than 100°C–200°C, pyrolysis may participate in aroma formation taking place on the surface of the foods (Fuzimaki and others 1969). The pyrolytic products frequently include ammonia, carbon dioxide, amine, hydrocarbon, nitriles, and carbonyl compounds. The compounds formed when pyrolyzing a peptide depended on the sequence of amino acids. As suggested by Dwivedi, this could explain the formation of volatile compounds in meat not obtained on heating model systems of single amino acids (Dwivedi 1975). Degradation of glutathione releases hydrogen sulfide rapidly when heated (Ohloff and others 1985), but ammonia is released relatively slowly producing heterocyclic compounds containing nitrogen (Zhang and others 1988). Ammonia released from amino acids, peptides, and proteins may impart numerous reactions leading to the formation of meat aromas. Different amino acids liberate free ammonia at different rate. The effects of an oil medium on the release of free ammonia from each of the five amino acids (glycine, L-aspartic acid, L-asparagine, L-glutamic acid, and L-glutamine) and the formation of pentylpyridines were studied. Among the five amino acids, only asparagine and glutamine generated ammonia readily by deamidation of amide side chains at 180°C under oil conditions, even though both of them produced less ammonia than under aqueous conditions (Kim and Ho 1998).

Strecker degradation is one of the most important reactions associated with the generation of the precursors for thermal formation meat aroma and observed by Strecker (Strecker 1862). It involves the oxidative deamination and decarboxylation of an amino acid in the presence of a dicarbonyl compound. This leads to the formation of a Strecker aldehyde, containing one fewer carbon atom than the original amino acid, and an α-aminoketone (Mottram 1994). Ammonia may also be produced from amino acid by the Strecker degradation. A number of α-dicarbonyls or vinylogous carbonyls as well as reducing sugar (glucose) are able to degrade α-amino acids via Strecker degradation. Mechanism of the Strecker degradation of l-phenylalanine initiated by 2-oxopropanal was proposed by Strecker (Hofmann and others 2000).

Several Strecker aldehydes are well-known cooked beef components, for example, acetaldehyde (sharp, penetrating, fruity), 2-methylpropanal (penetrating, green), 3-methylbutanal (malty, green), 2-methylbutanal (ethereal, bitter, almond, green), and phenyl and may be derived from alanine, valine, leucine, isoleucine, and phenylalanine, respectively. The Strecker degradation of methionine is another source of the sulfur-containing intermediate, methional. Methanethiol and 2-propenal are derived from methional (Schönberg and Moubacher 1952).

Besides the thermal degradation of thiamin, the amino acid cysteine is a well-known precursor of meat flavor. Hydrogen sulfide, mercaptoacetaldehyde, and acetaldehyde were identified as common products when cysteine was boiled with various carbonyl compounds. Among them, acetaldehyde is known to be formed from the hydrolysis or Strecker degradation of cysteine (Fuzimaki and others 1969). Addition of cysteine to raw muscle favored the formation of thiophenes in a cooked meat system (Ho and others 1982). In addition to cysteine, sulfur containing amino acid cystine and peptide glutathione will liberate hydrogen sulfide, which is a major reactant for meaty aroma generation (Tai and Ho 1998). The amino group in the free cysteine molecule is accessible to a dicarbonyl compound, thus making Strecker degradation possible (Zheng and others 1997).

6.2.4.1 Methanethiol

Precursors for odor-active sulfur-containing compounds may also come from the breakdown of methionine. Methionine plays a major role in the formation of some odorous sulfur-containing compounds.

Demethiolase catalyzes the bioconversion of methionine to methanethiol (Yvon and Rijnen 2001). Cooking may also lead to the decomposition of sulfur-containing meat components, such as cysteine, methionine, glutathione, taurine, and thiamine, results in the formation of sulfur compounds, such as hydrogen sulfide and methanethiol. These small sulfur-containing compounds may react either with each other or with compounds such as formaldehyde or ammonia. Methanethiol is the direct precursor of numerous sulfur compounds with very low perception thresholds (Arfi and others 2003). Dimethyl trisulfide comes from the condensation of methanethiol (López Del Castillo-Lozano and others 2007), and methional or 3-(methylthio)-propanal ("potato" odor) is formed by the oxidative catabolism of methionine via Strecker degradation. This catabolism may be linked to the action of endogenous enzymes in muscle tissue (theoretically sterile), and also to the action of surface microbial flora followed by the migration of methional inside the ham (McSweeney and Sousa 2000).

6.2.5 1-Pyrroline

1-Pyrroline was identified as effective intermediates in generating the roast-smelling food odorant 2-acetyltetrahydropyridine and 2-acetyl-1-pyrroline (Hofmann and Schieberle 1998a). The oxidation of proline with periodate may lead to the formation of 1-pyrroline (Bragg and Hough 1958). Heating L-proline with various reducing sugars leads to the formation of 1-pyrroline as well (Tressel and others 1985). Strecker degradation of proline and/or arginine and/or ornithine may produce Strecker aldehyde, 4-aminobutanal, which is an equilibrium compound of 1-pyrroline (Kubota and others 1991). Biologically, in yeast (Munch and Schieberle 1998) and *Bacillus cereus* (Romancyzk and others 1995), 1-pyrroline has been characterized as an important intermediate for the formation of 2-acetyl-1-pyrroline. 1-Pyrroline is proposed to be derived enzymatically through decarboxylation of proline and reduction of glutamic acid. In addition to yeast and bacteria, 1-pyrroline and 2-acetyl-1-pyrroline were identified in taro (Wong and others 1998) volatiles, indicating that 1-pyrroline may be synthesized *in vivo* as well.

Flavor contribution and formation of the intense roast-smelling odorants 2-propionyl-1-pyrroline (PP) and 2-propionyltetrahydropyridine (PTHP) in Maillard-type reactions have been proposed (Hofmann and Schieberle 1998b). A formation pathway for both odorants is created from the same intermediate, 1-pyrroline, when reacted with either 2-oxobutanal (yielding PP) or 1-hydroxy-2-butanone (yielding PTHP).

6.2.6 Fatty Aldehydes

Three main mechanisms for the formation of volatile reactive carbonyls are discussed: (1) Strecker degradation of amino acids; (2) nonenzymatic oxidation of fatty acids; and (3) retro–aldol condensation. The identification of many heterocyclic compounds from reactions between aldehydes and products from Maillard reactions indicates that these compounds could be formed during the cooking of meat. Some of the aldehydes investigated must be considered as Strecker degradation products of amino acids mentioned above. However, other studies have shown that aldehydes from lipid oxidation such as pentanal, hexanal, 2-hexenal, 2-decanal, and 2,4-decadienal can enter these reactions as those of shorter chain length (Whitfield 1992).

The effect of cooking conditions on the development of thermally generated meat aroma depended on the quantity and quality of the precursors. It is well-known that aging treatment influences the level of flavor precursors in meat for further thermal processing. The lipolysis of triglycerides and phospholipids is responsible for the release of free fatty acids (Flores and others 1997), which can be more easily oxidized to highly reactive compounds such as aldehydes, ketones, and dicarbonyls produced throughout the ripening stage.

Lipolysis is governed by the activity of lipases and phospholipases and leads to the formation of free fatty acids. Both endogenous enzymes of fat cells and muscle fibers and enzymes of bacteria are involved in lipolysis. Contents of free fatty acid increase significantly during the curing process both in dry-cured sausages (Hernandez and others 1999) and hams (Coutron-Gambotti and Gandemer 1999). The amounts of free fatty acids sharply rise during dry-cured processing. For instance, in dry-cured hams, level of free

fatty acid rises from 1%–2% to 10%–12% of the total lipids in 10 months in adipose tissue. Whereas, free fatty acids account for 8%–20% of total lipids in muscle according to the technology used and the raw material (Gandemer 2002).

Degradation of lipid is one of the major causes of quality deterioration in raw and cooked meat products. Processes involved in meat product processing which include cutting, grinding, mixing, and cooking can enhance the degradation of polysaturated fatty acid hydroperoxides into secondary products. During cooking, thermal and oxidative degradation of depot triglyceride and tissue phospholipids occur simultaneously. In the absence of oxygen, lipids thermally degrade through dehydration, decarboxylation, hydrolysis, dehydrogenation, and carbon–carbon cleavage. Through hydrolysis, free fatty acids are released (Skibsted and others 1998). The procedure of direct heating the meat sample under acidic (pH 3 or lower) conditions enhances the degradation of the existing lipid hydroperoxides and generates additional degradation products.

Of the volatiles produced by lipid oxidation, aldehydes are the most significant flavor compounds. The accepted mechanism of lipid oxidation involves hydroperoxide formation, followed by the production of fragments containing various functional groups (Nawar 1969). Autoxidation is a free radical chain reaction and is initiated by the extraction of a hydrogen atom from the methylene carbon of an unsaturated fatty acid. For fatty acids with two or more double bonds in a nonconjugated system, the release of the methylene hydrogen is stabilized by delocalization of the free radical over five carbons. Reaction of oxygen with the free radical generates peroxy radicals, and the reaction is then propagated by the formation and decomposition of hydroperoxides. An aldehyde can be produced by scission of the lipid molecules on either side of the radical. The products formed by these scission reactions depend on the fatty acids present, the hydroperoxide isomers formed, and the stability of the decomposition products. Temperature, time of heating, and degree of autoxidation are variables which affect thermal oxidation (Ho and Chen 1994; Frankel 1982). Aldehydes are the major components identified in volatiles of cooked meat. Octanal, nonanal, and 2-undecenal are oxidation products of oleic acid, and hexanal, 2-nonenal, and 2,4-decadienal are major volatile oxidation products of linoleic acid (Bading 1970).

6.2.6.1 2,4-Decadienal

The autoxidation of linoleic acid creates 9- and 13-hydroperoxides. Cleavage of 13-hydroperoxide leads to hexanal and the breakdown of 9-hydroperoxide produces 2,4-decadienal (Ho and Carlin 1989). Subsequent retroaldolization of 2,4-decadienal will produce 2-octenal and hexanal (Josephson and Lindsay 1987). 2,4-Decadienal is known to be one of the most important flavor contributors to deep-fat-fried foods (Ho and others 1987).

The phospholipid fraction has been identified as the primary substrate in the development of oxidative deterioration in muscle foods (Ho and others 1987; Igene and others 1980). The significant decrease in phospholipid content in muscle during dry-cured ham processing indicates a phospholipid origin of free fatty acids (Coutron-Gambotti and others 1999). This conclusion is supported by the fact that the free fatty acid composition is closer to the fatty acid composition of phospholipids than to that of triglycerides whatever the type of ham (Gandemer and others 2000). Phospholipids are essential structural components of all cells and contain a much higher proportion of unsaturated fatty acids than the triglycerides. The phospholipid fraction had a high content of polyunsaturated fatty acids, linoleic (C18:2) acid, and arachidonic (C20:4) acid. Arachidonic acid constitutes greater than 50% of the polyunsaturated fatty acids in meat phospholipids. Autoxidation of arachidonic acid may have a role in the development of off-flavors in meat. Various aldehydes, ketones, aldehyde esters, hydrocarbons, and alcohols were identified. The major products included hexanal, methyl 5-oxopentanoate, pentane, methyl butanoate, and 2,4-decadienal, which could be important to off-odor development in oxidized food systems containing arachidonate (Artz and others 1993). Reactions involving phospholipids contribute considerably to the flavor and aroma of cooked meat. After 1 h at 132°C, ethanolamine-containing phospholipids rapidly lost most of their component aldehydes, but not their fatty acids, whereas choline-containing phospholipids lost their aldehydes much more slowly. In both cases, the presence of ribose or glycine had little effect on the loss of aldehydes. After heating at 132°C, there was a 90% loss of aldehydes in

the meat, which was similar for both the ethanolamine- and choline-containing phospholipids (Fogerty and others 1989).

6.2.6.2 Short-Chain Aldehydes

Short-chain aldehydes such as acetaldehyde, butanal, and hexanal are probably derived from the decomposition of 2,4-decadienal. Under aqueous conditions, 2,4-decadienal undergoes α,β-double-bond hydration and retro–aldol condensation to give hexanal and acetaldehyde (Huang and others 1987). Acetaldehyde is suggested to be involved in the formation of 3,5-dimethyl-1,2,4-trithiolane or 5,6-dihydro-2,4,6-trimethyl-4H-1,3,5-dithiazine. Benzaldehyde is considered a typical thermal degradation product of 2,4-decadienal. The effect of adding glycerophosphorylcholine (GPC) and glycerophosphorylethanolamine (GPE) to the decomposition of linoleic (18:2) acid was evaluated at 70°C to study the effects of phospholipids on off-flavor production during lipid oxidation in cooked meat products by monitoring the production of low-molecular-weight headspace volatiles. Hexanal was the most prominent volatile produced in all treatments. Volatile production rate in the 18:2 + GPE treatment was generally greater as compared to the 18:2 + GPC and 18:2 treatments (Dawson and others 1991).

Water-mediated retro–aldol degradation of α,β-unsaturated carbonyls appears to be significant as a means to thermally generate flavor active carbonyl. The reaction cascade proceeds rapidly from the conjugated carbonyl through its hydration and subsequent fragmentation (Josephson and Glinka 1995). Retro–aldol degradation of (*E,Z*)-2,6-nonadienal to (*Z*)-4-heptenal and acetaldehyde, and (*E,Z*)-2,4-decadienal to 2-octenal, hexanal, and acetaldehyde, have been demonstrated to be thermally driven at neutral pH.

6.2.7 Carbohydrate Degradation Products

Addition of certain carbohydrates to meat has been shown to increase the roasted aroma. The effect of aging on the level of several precursor carbohydrates in processed beef has been studied. It was found that the concentration of free ribose, glucose, and glucose 6-phosphate changed significantly during aging; ribose and glucose 6-phosphate increased from day 14 to day 21, while the concentration of glucose decreased from day 7 to day 14 and then increased to the highest concentration at day 21 (Koutsidis and others 2003). Glucose was present in the highest amount (4.0 μmol/g), followed by glucose 6-phosphate (2.6 μmol/g), mannose (0.3 μmol/g), and finally ribose (0.1 μmol/g) in pork loin (Bjergegaard and others 2007; Meinert and others 2007). The effectiveness of glucose, glucose 6-phosphate, ribose, and mannose as flavor precursors in pork were compared. Glucose generated the highest amounts of volatiles followed by glucose 6-phosphate (Meinert and others 2009).

Ribose is believed to be the most important precursor for a roasted aroma. The addition of ribose was found to increase the quantity of 2-furanmethanethiol in roasted chicken (Aliani and Farmer 2005). Ribose can be formed from the degradation (dephosphorylation) of ribose 5-phosphate. It has been shown that ribose 5-phosphate undergoes Maillard reactions significantly faster than similar carbohydrates and phosphorylated carbohydrates (Sandwick and others 2005). The reactive nature of ribose 5-phosphate was attributed to the formation of several minor peaks via Maillard reaction (Bjergegaard and others 2007). Ribose 5-phosphate may also undergo conversion to ribose and glucose 6-phosphate in the minced pork (Berg and others 2007).

During thermal treatment of carbohydrates, several α-dicarbonyls are formed (Hofmann and others 2000). The condensation of the carbonyl group of the reducing sugar with the amino compound gives a glycosylamine. Subsequently, this rearranges and dehydrates, via deoxyosone, to various sugar dehydration and degradation products such as furfural and furanone derivatives. When a ketose is involved instead of an aldose sugar, then a ketosylamine is formed that undergoes a Heyns rearrangement to form a 2-amino-2-deoxyaldose (Heyns product). At temperatures above 100°C, 1-amino-1-deoxy-2-ketoses undergo 2,3-enolization to give a 1-amino-2,3-enediol from which an amine is eliminated to form a methyl-2,3-dicarbonyl intermediate (Blank and Fay 1996). Yaylayan has recently proposed an alternative mechanism, for example, direct dehydrations from cyclic forms of the Amadori compounds to account for the many products observed in model systems and in foods (Yaylayan 1990). 1,2-Enolization of the Amadori product will result, after dehydration and deamination, in the formation of a 3-deoxyosone. The latter compound

can be formed by a similar pathway from the Heyns product. The 3-deoxyosone is readily converted to the corresponding furfural from a pentose, and 5-methylfurfural from a hexose. Furan derivatives are also formed by the further dehydration of the 1-methyl-2,3-dicarbonyl compounds, yielding 4-hydroxy-5-methyl-3(2H)-furanone or the 2,5-dimethyl homologue, from the pentose and hexose sugars, respectively.

Ribose is one of the main sugars in muscle. It is usually associated with phosphate and exists as ribose phosphate. In meat, it has been proposed that ribose phosphates from ribonucleotides is the principal precursor of furan (Zhang and Ho 1991). Dephosphorylation and dehydration of ribose phosphate form the important intermediate, 4-hydroxy-5-methyl-3(2H)-furanone (HMF), which readily reacts with hydrogen sulfide (Ouweland and Peer 1975). 4-Hydroxy-5-methyl-3(2H)-furanone and the structurally related 2,5-dimethyl-3(2H)-furanone (HMF) have been isolated from natural beef broth (Tonsbeek and others 1969). These compounds are considered to be involved in the formation of meaty flavors through their reaction with either hydrogen sulfide or sulfur-containing amino acids. A method is described for producing a pure, food-grade 2,5-dimethyl-4-hydroxy-2,3-dihydrofuran-3-one by heating a 6-deoxyhexose in the presence of an amino acid in an aqueous medium at pH 4–8. The hydroxyfuranone thus obtained is suitable for flavoring meat products (Anonymous 1989).

In addition to the ring-type furan derivatives, a number of hydroxycarbonyl and dicarbony fragmentation compounds can also be formed, for example, glyoxal, glycolaldehyde, acetaldehyde, glyceraldehyde, methylglyoxal, hydroxyacetone, dihydroxyacetone, diacetyl, acetoin, and hydroxydiacetyl. 2-Oxobutanal was shown to be formed in high yields (29 mol%) by reacting acetaldehyde and glycolaldehyde, two well-known degradation products of carbohydrates (Fay and others 1997). Recently, the reaction between 4-hydroxy-5-methyl-3(2H)-furanone and cysteine or hydrogen sulfide was reinvestigated. Formation of 2,3-pentanedione was proposed. The key steps are the acid hydrolysis of 4-hydroxy-5-methyl-3(2H)-furanone to yield 1-deoxypentatosone followed by the reduction and acid-catalyzed dehydration of the latter. Retroaldolization of the 1-deoxypentatosone, produced from the hydrolysis of 4-hydroxy-5-methyl-3(2H)-furanone, can yield methylglyoxal and, subsequently, hydroxyacetone by reduction. Aldol condensation of the latter with acetaldehyde, followed by dehydration, would yield 2,3-pentanedione. The formation of diacetyl from 4-hydroxy-5-methyl-3(2H)-furanone could follow similar pathways to those involved in the formation of 2,3-pentanedione. The fragmentation of the carbohydrate chains of the Amadori and Heyns products or the rearranged 1- and 3-deoxyketone proceeds by a series of retroaldolization reactions. These reactions can lead to a series of α-dicarbonyl compounds such as methylglyoxal, 2,3-butanedione, glyoxal, and hydroxycarbonyl compounds such as 1-hydroxy-2-propanone, 2-hydroxyethanal, 2,3-dihydroxypropanal, and 1,3-dihydroxy-2-propanone. The loss of one carbon to give diacetyl occurs via retro–aldol condensation with the elimination of formic acid. In the presence of cysteine, the formation of 3-mercaptobutan-2-one requires aldol condensation between acetaldehyde and methylglyoxal followed by elimination of formic acid by retro–aldol condensation. The resulting hydroxybutanone readily reacts with hydrogen sulfide to yield 3-mercaptobutan-2-one. In the formation of mercaptoalkanones, it has been proposed that α-dicarbonyls are reduced to hydroxyalkanones before substitution by hydrogen sulfide (Whitfield and Mottram 1999).

Mercaptoalkanones are known to form readily by the reaction of α-dicarbonyl compounds with hydrogen sulfide (Mottram 1998). The formation of α-dicarbonyls from 4-hydroxy-5-methyl-3(2H)-furanone is a key step in the formation of mercaptoalkanones, such as 3-mercaptopentan-2-one and 2-mercaptopentan-3-one. Three alkanediones, 2,3-pentanedione, 2,4-pentanedione, and 3,4-hexanedione were identified in the reaction mixtures containing cysteine. Three interesting meat aroma precursors, 3-mercaptobutan-2-one, 3-mercaptopentan-2-one, and 3-mercaptobutan-2-one, which had been reported previously in cysteine–ribose model systems, were detected in systems that contained hydrogen sulfide.

6.2.8 Ascorbic Acid

The reaction between L-ascorbic acid and L-cysteine leads mainly to the formation of alicyclic sulfur compounds with meaty aroma. Among them, thiophenes, thienothiophenes, thiophenones, thiazoles, and pyrazines are typical products of thermal generation (Yu and Zhang 2010a). Furans, such as furfural, 2-furanmethanol, benzofuran, 2,5-furandicarboxaldehyde, and 2-furfurylfuran, were mainly formed in acidic environments. These furans may provide the carbon skeleton for the meaty sulfur compounds

formed in food systems. L-ascorbic acid was also recognized as the origin of pyrazines. Formation of pyrazines from ascorbic acid and amino acids under dry-roasting conditions was reported (Adam and De Kimpe 2009). The reaction between L-ascorbic acid and L-threonine/L-serine led mainly to the formation of pyrazines. Many of these were alkylpyrazines, such as 2-methylpyrazine, 2,5-dimethylpyrazine,2-ethylpyrazine,2-ethyl-6-methylpyrazine,2-ethyl-5-methylpyrazine,3-ethyl-2,5-dimethylpyrazine, 2,3-diethyl-5-methylpyrazine, and 3,5-diethyl-2-methylpyrazine (Yu and Zhang 2010b). The thermal rearrangement and fragmentation of L-ascorbic acid in the presence of amino acids can produce many carbonyl compounds (Vernin and others 1998).

6.3 Pathways for the Formation of Some Meat Aroma Volatiles

Heterocyclic compounds have been identified as important volatile components in many foods. The odor strength and complexity of these compounds makes them desirable as flavoring ingredients. Heterocyclic compounds are primarily formed through nonenzymatic browning reactions. Temperature and aging were the most influential factors on the thermal generation of flavor compounds in meat products. In sensory analysis, a more intense roasted flavor was found at 250°C as compared with 150°C, and this is most likely due to the formation of Maillard-derived flavor compounds, which is accelerated by local dehydration of the meat surface at elevated temperatures (Meinert and others 2007).

6.3.1 Acetaldehyde-Derived Meat Aroma

Acetaldehyde is involved in many reactions, explaining the formation of meaty aromas: 1-(methylthio) ethanethiol (Brinkman and others 1972), 3,5-dimethyl-1,2,4-trithiolane (Kubota and others 1980), trithioacetaldehyde (Wilson and others 1974), thialdine (Kubota and others 1980), 2,4-dimethylthiazole (Katz 1981), 2,4-dimethyl-5-ethylthiazole (Pittet and Hruza 1974), 2,4,5-trimethyl-3-thiazoline (Mussinan and others 1976), 2,4,5-trimethyloxazole (Ohloff and Flament 1978), and 2,4,5-trimethyl-3-oxazoline (Self and others 1963).

3,5-Dimethyl-1,2,4-trithiolane was first identified by Chang and others (1968) in the volatiles of boiled beef and has been found in the volatiles of cooked chicken (Tang and others 1983). It is reported to have roasted and onion-like flavors (Forss 1983). In addition to 3,5-dimethyl-1,2,4-trithiolane, 3,5-diisobutyl-1,2,4-trithiolane were identified in the volatiles isolated from fried chicken flavors (Hartmann and others 1984). These trithiolanes possess roasted, roasted-nut, crisp bacon-like, and pork rind-like aromas.

3,5-Diisobutyl-1,2,4-trithiolane has been produced in a model system containing isovaleraldehyde, ammonia, and hydrogen sulfide (Shu and others 1985). Instead of acetaldehyde, isovaleraldehyde is involved in the reaction leading to the formation of 3,5-diisobutyl-1,2,4-trithiolane. Isovaleraldehyde arises via the Strecker degradation of leucine. Ammonia and hydrogen sulfide may arise via thermal degradation of amino acids and cysteine or cystine.

Several cyclic sulfur-containing compounds have been identified in meat flavor. Thialdines are sulfur and nitrogen-containing heterocyclic flavor components that are produced nonenzymatically during thermal processing (e.g., by cooking, frying, boiling, roasting, baking etc., of food stuffs). Their origin in food flavors lies in a series of complex reactions in which lipids and amino acids play a dominant role (Werkhoff and others 1992). Cyclic dithia compounds, especially those containing a keto group, are believed to contribute to meat flavor (Weyerstahl and Oldenburg 1998). A number of cyclic dithia compounds impart fatty, spicy, roasted, mushroom, or sulfury notes to overall meat flavor.

Possible formation mechanisms of thialdines have already been described (Boelens and others 1974). At high temperature approximating roasting conditions (i.e., Shigematsu condition), the products formed reflect the fact that amino acid pyrolysis (rather than Strecker degradation) is an important reaction (de Riike and others 1981). Cysteine is primarily transformed into six products, namely mercaptoacetaldehyde, acetaldehyde, cysteamine, ethane-1,2-dithiol, hydrogen sulfide, and ammonia (Tressel and others 1983). When cysteine was degraded at 180°C, a representative frying temperature, both 3,5-diisobutyl-1,2,4-trithiolane and 2,4,6-trimethylperhydro-1,3,5-dithiazine (thialdine) were identified as major products (Zhang and others 1988). A similar mechanism for the formation of thiadiazines and dithiazines was proposed (Kawai 1991).

Fatty aldehydes, hydrogen sulfide, and ammonia are often constituents of food flavor which are converted into mixtures of alkyl-substituted thialdines by heating. 2,4,6-Trimethylperhydro-1,3,5-dithiazine (thialdine), the main representative of this class of compounds, was described as early as 1847. The first identification of the trimethyl derivative in a food product was described in heated pork. Thialdine was isolated and identified from pressure-cooked beef, cooked beef flavor, roasted lamb fat, and cooked mutton (Kubota and others 1980). In the presence of ammonia, dithiazines and thiadiazines are formed. One dithiazine (i.e., thialdine) has been reported in heated beef aroma (Mottram 1998).

Model reactions using aldehydes and hydrogen sulfide lead to the formation of another group of interesting sulfur compounds. At atmospheric pressure, acetaldehyde and hydrogen sulfide give mainly cyclic trimers, for example, dioxathianes, oxadithianes, and trithiane (MacLeod and Seyyedain-Ardebili 1981). 2,4,6-Trimethyl-1,3,5-trithiane has been found in cooked beef aroma (Kubota and others 1980). 1,3,5-Trithiane has also been identified in model reactions of fatty aldehydes, hydrogen sulfide, thiol, and ammonia (Boelens and others 1974) and from furfural, hydrogen sulfide, ammonia reaction mixture as well (Shibamoto and Russell 1977). However, the reaction between acetaldehyde and excess hydrogen sulfide in a closed vessel gave rise via bis-(1-mercaptoethyl) sulfide as an intermediate to some compounds, namely 3,5-dimethyl-1,2,4-trithiolane and diethyldisulfide.

In 2,4,6-trimethyl-1,3,5-dioxathiane, 2,4,6-trimethyl-1,3,5-oxadithianes, 2,4,6-trimethyl-1,3,5-trithiane, 2,4,6-trimethyl-1,3,5-thialdine, 2,4,6-trimethyl-1,3,5-thiadiazine, and 3,5-dimethyl-1,2,4-trithiolane, acetaldehyde provides the carbon skeleton on the ring structure (Figure 6.1).

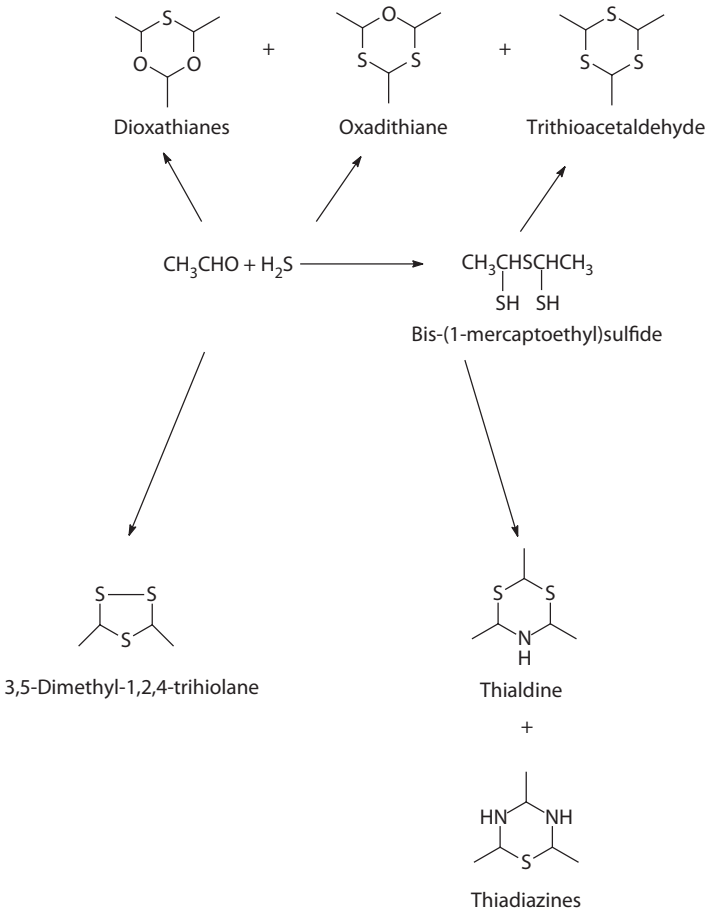

FIGURE 6.1 Formation mechanism for acetaldehyde-derived meat aroma.

6.3.2 Dicarbonyl-Derived Meat Aroma

An extensive and systematic study of similar reactions, but using α-dicarbonyls in addition to aldehydes, hydrogen sulfide, and ammonia have been conducted (Shu and others 1985). Various combinations were studied, the α-dicarbonyls being either 2,3-butanedione, 2,3-pentanedione, or methylglyoxal; the aldehydes used were acetaldehyde or propanal. All reaction mixtures gave relatively similar products, for example, thiazoles, thiazolines, oxazoles, oxazolines, isothiazoles, thialdine, dithiolenes, trithianes, and mercaptoalkenes.

Aldehydes formed by Strecker degradation of amino acids in the presence of α-diketones react with ammonium sulfide in the presence or in the absence of acetoin (3-hydroxy-2-butanone) giving a variety of oxygen, sulfur, and/or nitrogen-containing heterocyclic compounds (Vernin and others 1993). Sugars are the major suppliers of carbonyls and amino acids for nitrogen.

Thiazole and oxazoles may be formed from related mechanisms, involving the formation of an intermediate imine from the reaction of an aldehyde with ammonia. Reaction of the imine with a carbonyl produces an oxazoline which may undergo oxidation to the corresponding oxazole. If hydrogen sulfide is present, it may react with the dicarbonyl to give an α-mercaptoketone which then yields thiazoline and thiazole from reaction with the imine.

Oxazoles and oxazolines, which are oxygen and nitrogen-containing heterocyclics, have been identified in many kinds of heated foods and have significant sensory contribution (Maga 1981). They possess potent sensory quality at very low concentrations and can be described as green, sweet, and nutty aroma and have been identified in soy sauce (Numomura and others 1978) and cooked beef (Pittet and Hruza 1974). 2,4,5-Trimethyl-3-oxazoline was first found in the flavor of meat as early as 1968 (Chang and others 1968). 2,4,5-Trimethyl-3-oxazoline was reported later to be the major compound in boiled beef (Hirai and others 1973) and canned beef stew (Peterson and others 1975). 2,4,5-Trimethyl-3-oxazoline was isolated and characterized from a model reaction of ammonia, acetaldehyde, and 3-hydroxy-2-butanone.

The condensation between the amino group of 3-amino-2-butanone and the keto group of 3-hydroxy-2-butanone leads to the formation of a Schiff base. The Schiff base may rearrange to form 2-(1-hydroxy ethyl)-2,4,5-trimethyl-3-oxazoline and then 2,4,5-trimethyl-3-oxazoline. A similar 2,4,5-trimethyl-3-oxazoline formation mechanism has been proposed by Piloty and Baltes (1979). These researchers reported that oxazoles, pyrazines, pyrroles, and pyridines were formed in the heated model systems of amino acids and 2,3-butanedione (Figure 6.2).

Thiazoles are a class of compounds possessing a five-membered ring with sulfur and nitrogen in the 1 and 3 positions, respectively. A number of thiazole derivatives were found in cooked meat (Shibamoto 1980). 2-Acetyl-2-thiazoline has been found in beef broth (Tonsbeek and Copier 1971). Thiazoles are considered to be one of the main constituents which give a meaty flavor. Alkylthiazole formation in meat products arises from a Maillard-type reaction between amino acids and sugars or sugar-derived carbonyl compounds. An alternative formation pathway has been reported (Ho and Carlin 1989). Four alkylthiazoles, namely 4-methylthiazole, 4,5-dimethylthiazole, 4-methyl-5-ethylthiazole, and 4-methyl-5-vinylthiazole, identified in pork may be the thermal degradation products of thiamin.

Several thiazoles with C_4–C_8 *n*-alkyl substituents in the 2-position have been reported in roasted beef (Hartman and others 1983) and fried chicken (Tang and others 1983). Other alkylthiazoles with longer 2-alkyl substituents (C_{13}–C_{15}) were found in the volatiles of heated beef and chicken with the highest concentrations in beef heart muscle (Farmer and Mottram 1994). Aliphatic aldehydes from lipid oxidation are the likely sources of the long *n*-alkyl groups in these compounds. Recently, a large number of alkyl-3-thiazolines have been isolated from cooked beef (Elmore and Mottram 1997). Most of the thiazolines contained C_5–C_9 *n*-alkyl substituents in the 2-position. The most likely routes to the 3-thiazolines and thiazoles are from α-hydroxyketones or α-diones (α-dicarbonyls), hydrogen sulfide, ammonia, and aldehydes (Figure 6.2). Lipid oxidation provides long chain aldehydes.

Formation of 3-thiazolines from α-hydroxyketone involves the substitution of the hydroxyl group with a thiol group. This is followed by nucleophilic attack by the sulfur atom at the carbon atom of an imine intermediate formed by the reaction between ammonia and an aldehyde. Subsequent ring closure, with the elimination of a molecule of water, leads to the formation of the 3-thiazolines. Oxidation of the thiazoline results in the formation of the corresponding thiazole (Zhang and others 1992). A slightly

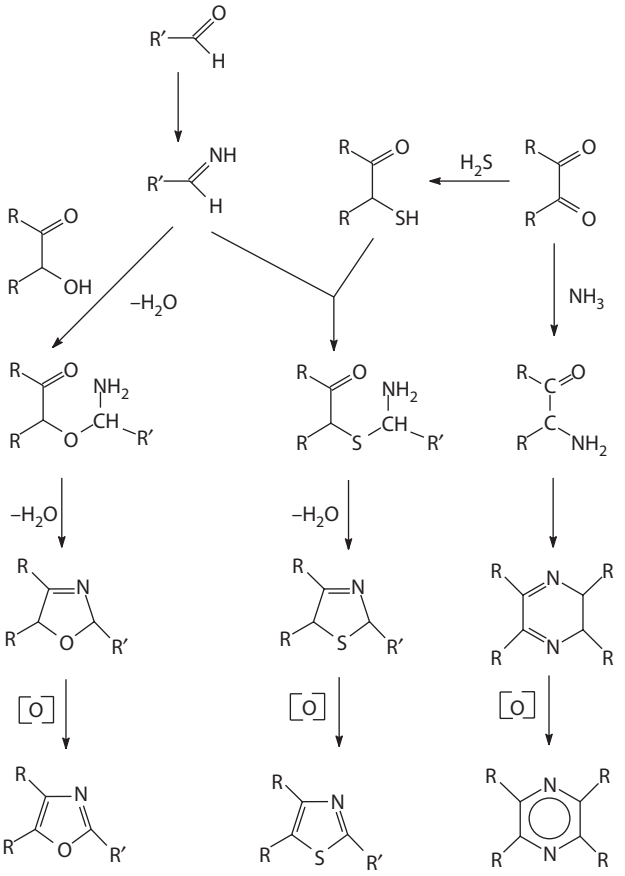

FIGURE 6.2 Formation of thiazoles, thiazolines, oxazoles, oxazolines, and pyrazines by the reaction of aldehydes with dicarbonyls, hydrogen sulfide, and ammonia.

different mechanism could occur with the alkane-α-diones. The initial step requires nucleophilic addition of a thiol group to one of the carbonyl groups. This intermediate could be reduced to a mercaptoketone, or it could react with an imine intermediate leading to a thiazole (Figure 6.2).

Pyrazines are very important constituents of meat aromas. Most of the pyrazines contain a methyl group as a substituent that demonstrates the outstanding role of diacetyl, methylglyoxal, and corresponding sugar decomposition products. Other important substitutes are ethyl-, propyl-, vinyl-, allyl-, and propenyl groups. In the simple Maillard reaction mixture, the alkylpyrazines were quantitatively major products when amino acid was the nitrogen source. Volatile aroma compounds from beef steaks, shallow fried without oil have been studied. Pleasant flavor qualities were much stronger for the 280°C extract than for the 300°C extract; this was mainly due to the combination of 2-ethyl-3,5-dimethylpyrazine and 2-propyl-3-methylpyrazine (Specht and Baltes 1994). At least 22 pyrazines were identified in the flavors of fried chicken and roasted chicken (Noleau and Toulemonde 1986). The alkylpyrazines accounted for almost 80% of the total headspace volatiles of well-done grilled pork (Mottram 1985).

Pyrazine formation was studied as a model for carbohydrate fragmentation in the Maillard reaction (Weenen and others 1994). Thus 1-^{13}C-glucose, 2-^{13}C-glucose, and 1-^{13}C-fructose were reacted with asparagine in 1,2-propanediol, and the volatile products isolated by steam distillation and extraction. The product mixture consisted mainly of dimethyl-, monomethyl, and to a lesser extent trimethylpyrazines. The ^{13}C-incorporation in the pyrazines obtained from all three labeled hexoses was in agreement with retroaldolization of the intermediate deoxyglucosones as the main cleavage mechanism. Both 1- and 3-deoxyglucosone appear to play an approximately equal and important role in the formation of the methylated pyrazines.

Alkylpyrazines can be formed from a reaction of the degraded nitrogenous substances, NH_3, RNH_2 from proteins, peptides, amino acids and phospholipids, and α-dicarbonyl compounds in food (Shibamoto 1990). Formation pathways for alkylpyrazines have been proposed by numerous researchers (Shibamoto and Bernhard 1977). An important route to alkylpyrazines is from α-aminoketones which are formed in Strecker degradation or from the reaction of α-dicarbonyls with ammonia. Condensation of two aminoketone molecules yields a dihydropyrazine which oxidizes to pyrazine (Figure 6.2).

6.3.3 Fatty Aldehyde-Derived Meat Aroma

6.3.3.1 Pyrazines

Long *n*-alkyl-substituted pyrazines, butyl-, and pentyl-pyrazines are found in meat (Ho and Chen 1994). Two pentyl-substituted and one butyl-substituted pyrazines are also observed in grilled pork. Propylpyrazine may be formed from the interaction product of lipids, proteins, and carbohydrates following a similar mechanism proposed by Chiu and others (1990). The combination of these alkylpyrazines may lead to the formation of the characteristic-fried meaty aroma of Chinese-fried pork bundle (Huang and others 1989).

Recent studies on the interaction between the Maillard reaction and lipid degradation leading to the formation of desirable flavor compounds of foods are also discussed (Huang and others 1995). The effect of long-chain aldehydes on the formation of long-chain alkyl-substituted pyrazines was investigated in model systems of acetol and ammonium acetate with the addition of pentanal or hexanal. When the systems were reacted at 100°C for 4 h, 2,5-dimethyl-3-pentylpyrazine and 2,6-dimethyl-3-pentylpyrazine were formed in the model system with added pentanal, and the corresponding hexylpyrazines were formed in the hexanal system. Formation pathways are proposed for some of these pyrazines (Chiu and others 1990). The formation of higher carbon number-substituted pyrazines is hypothesized to be due to the intervention of aldehydes. The aldehydes, originating from lipid degradation, could facilitate addition to the metastable dihydropyrazine compound, formed by the condensation of two molecules of aminoketones (Huang and others 1995). The latter is a product of the Strecker degradation of amino acids with α-dicarbonyl compounds. Figure 6.2 shows the mechanism for the formation of long-chain alkyl-substituted pyrazine.

6.3.3.2 Pyridines

2-Isobutyl-3,5-diisopropylpyridine was identified in fried chicken and has a roasted cocoa-like aroma. The formation of pyridines involves the reaction of aldehyde and ammonia at high temperature and is known as the Chichibabin condensation (Su and others 1985). As a matter of fact, the investigation of the condensation of aldehydes, ketones, α,β-unsaturated carbonyl compounds with ammonia to form substituted pyridines can be dated back to 1950 (Mosher 1950). In a continuation of the above studies, the same authors showed that, when glycine and propanal were heated at 180°C, 3,5-dimethyl-2-ethylpyridine, 3,5-dimethylpyridine were formed immediately (Suyama and Aachi 1980).

6.3.4 Cysteamine-Derived Thiazoles and Thiazolidine

An alternative pathway for thiazole formation derived from the condensation between cysteamine and aldehyde or 2,3-butanedione has been proposed. Cysteamine, which is a decarboxylated derivative of cysteine, produced a series of alkylthiazolidines (Sakaguchi and Shibamoto 1978). Aroma compounds, thiazolidine, and thiazine were reported by different research groups from the same model system, cysteamine and 2,3-butanedione. Thiazolidines generally possess a characteristic popcorn flavor (Yeo and Shibamoto 1991). 2-Acetyl-2-methylthiazolidine was first characterized by Umano and others (1995) from the head space of a heated D-glucose/L-cysteine model system. The yield of 2-acetyl-2-methylthiazolidine is <0.01% (GC peak area). They hypothesized that, the reaction between cysteamine, the decarboxylated cysteine, and 2,3-butanedione, a glucose degradation product may lead to the formation of 2-acetyl-2-methylthiazolidine. Under the experimental conditions described, a standard mixture composed of formaldehyde, acetaldehyde, propionaldehyde, butyaldehyde, valeraldehyde, and

hexanal was reacted with cysteamine as described. Thiazolidine, 2-methylthiazolidine, 2-ethylthiazolidine, 2-popylthiazolidine, 2-butylthiazolidine, and 2-pentylthiazolidine were formed (Huang and others 1998a).

A mechanism was proposed to elucidate the formation of a thiazole and thiazolidine, in aldehyde or 2,3-butanedione/cysteamine model systems (Figure 6.3). Buffering dramatically promotes thiazolidine formation from aldehyde or 2,3-butanedione and cysteamine. Phosphate tends to stabilize the carbocation

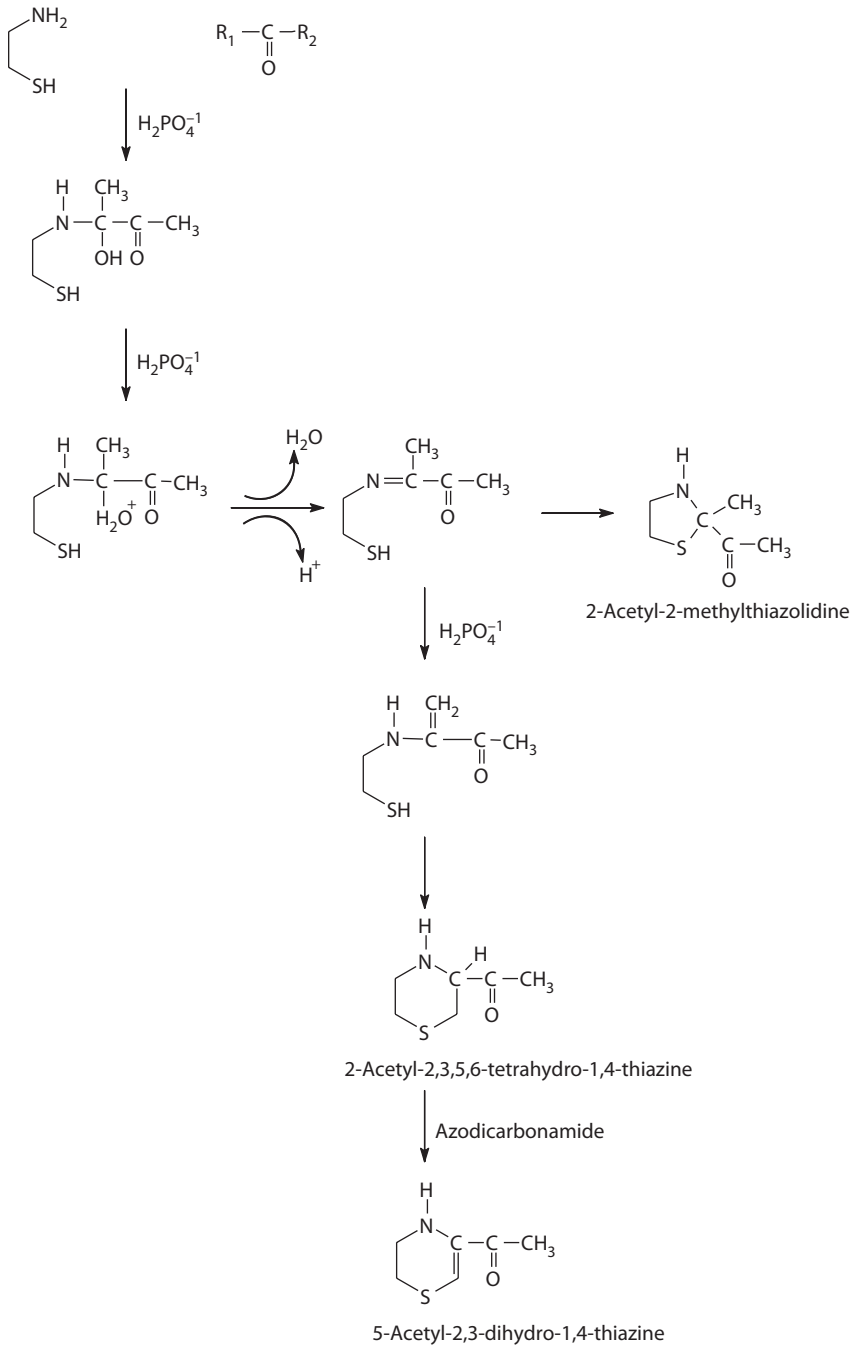

FIGURE 6.3 Formation of thiazoles, thiazolines, and thiazine by the reaction of aldehydes with cysteamine.

formed, and this may lead to completion of the cyclization by attacking the amino nitrogen on the activated carbon. Protic solvent, by removing the water molecule, further enhances thiazolidine formation. Redox reaction catalyzed by phosphate ions results in the conversion of thiazolidine to the corresponding thiazoline through hydride transfer (Huang and others 1998b).

6.3.5 1-Pyrroline-Derived 1,3,5-Dithiazine and Pyrroline

A formation pathway has been proposed for both odorants from the same intermediate, 1-pyrroline, when reacted with either 2-oxobutanal (yielding 2-acetyl-1-pyrroline) (Hofmann and Schieberle 1998c) or acetaldehyde and hydrogen sulfide (yielding pyrrolidino(1,2-e)-4H-2,4-dimethyl-1,3,5-dithiazine) (Kubota and others 1991). 2-Oxobutanal was shown to be formed in high yields (29 mol%) by reacting acetaldehyde and glycolaldehyde, two well-known degradation products of carbohydrates. Pyrrolidino(1,2-d)-4H-2,4-dimethyl-1,3,5-dithiazine possesses an extremely low odor threshold. 1-Pyrroline and compound pyrrolidino(1,2-d)-4H-2,4-dimethyl-1,3,5-dithiazine were produced at the same time during heating and were increased by longer heating. It is postulated that pyrrolidino(1,2-d)-4H-2,4-dimethyl-1,3,5-dithiazine was formed secondarily from 1-pyrroline with acetaldehyde and hydrogen sulfide in the shellfish during heating (Kubota and others 1991).

The reaction between 2-oxopropanal and proline was suggested earlier (Hodge and others 1972) as a key step in the formation of 2-acetyltetrahydropyridine. The reaction of 1-pyrroline with 2-oxopropanal was previously postulated to be a key step in the generation of 2-acetyl-1-pyrroline (Figure 6.4).

The production of volatile compounds in the reaction of the lipid peroxidation product (*E*)-4,5-epoxy(E)2-heptenal with lysine or bovine serum albumin was studied in order to characterize the alternative mechanisms for production of volatile pyrrole derivatives in foods (144). A mixture of the lipid and the amino acid or the protein was stirred overnight at 37°C and the volatiles formed were collected using a Tenax TA trap. Several aldehydes, ketones, alkylfurans, alkyl- and acyl-pyrroles, and alkylpyridines were identified by gas chromatography-mass spectrometry (GC–MS). Formation of pyrrole derivatives in these reactions suggested the existence of alternative mechanisms for production of these flavor-associated compounds, previously considered to be formed mainly via Maillard reactions. This new route does not need the presence of sugars, and might contribute significantly to volatile pyrrole production in foods under conditions unfavorable for classic Maillard reactions (Zamora and others 1994).

In addition to 2,4,6-trimethylperhydro-1,3,5-thiadiazine, other alkyl-substituted dithiazines, including 2-ethyl-4,6-dimethyl,4-ethyl-2,6-dimethyl, 2-propyl-4,6-dimethyl-, 2-butyl-4,6-dimethyl-, 2-pentyl-4,6-dimethyl-, and 4-pentyl-2,6-dimethyl-1,3,5-dithiazines, were also found in chicken and beef (Werkhoff and others 1992) flavor. These alkyl-substituted dithiazines were identified in the flavor of pork as well.

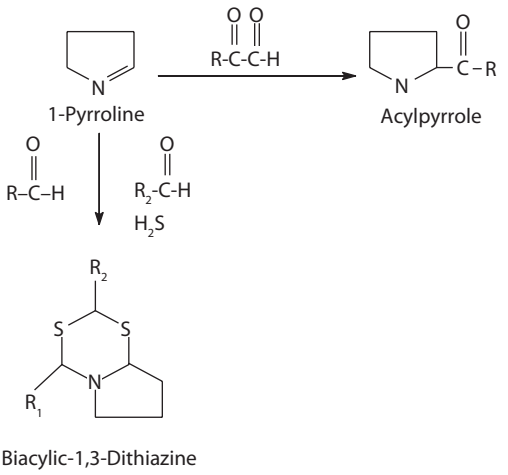

FIGURE 6.4 Formation of acylpyrrole and bicylic-1,3-dithizine by the reaction of aldehydes and 1-pyrroline.

The higher homolog of 3,5-dimethyl-1,2,4-trithiolane, 3,5-diisobutyl-1,2,4-trithiolane, was identified in the volatiles isolated from fried chicken flavor (Werkhoff and others 1992).

6.3.6 Furan-Derived Meat Aroma

Sulfur-containing furans and thiophenes and related disulfides are known to possess strong meat-like aromas and exceptionally low odor threshold values. Such compounds have been found in model Maillard reaction systems and in cooked meat where they are considered to contribute to the characteristic aroma (Mottram and Madruga 1994a). Possible precursors of these compounds in meat are pentose sugars and cysteine. One of the main sources of pentoses in meat is the ribonucleotide, inosine-5′-monophosphate (IMP), which accumulates in meat during postmortem glycolysis (Mottram and Madruga 1994a).

Furans or thiophenes substituted on position 3 by a thiol, sulfide, or disulfide group are considered as key compounds to meat flavor with savory and meaty aromas and very low threshold values (Buttery and others 1984). An essential structural requirement for meaty aroma is a five- or six-membered ring, which is more or less planar and substituted with an enol, thiol, and a methyl group adjacent to the thiol (Figure 6.5). Meat, such as beef, mutton, pork, or poultry, treated with a raw soy sauce, retains the flavor of soy sauce and the well-balanced enzyme activities (proteinase, collagenase, elastase) acquired during brewing. The treatment eliminates the powdery (roughened) surface and other disadvantages attributable to conventional-tenderizing agents. The raw soy sauce acts intensively on the connective tissue of the meat, and to a lesser extent on myofibrils, producing meat with good flavor and tenderness (Tsuji and Takahashi 1989).

2-Methyl-3-furanthiol has been identified as the most important flavor compound contributing to the meaty perception of chicken broth (Gasser 1990) and as a character impact compound in the aroma of cooked beef (Gasser and Grosch 1988).

Another compound structurally related to 2-methyl-3-furanthiol and identified as a primary odorant in chicken broth is 2-furfurylthiol (Gasser 1990). This compound possesses a threshold of 5 ppt and aroma qualities such as roasted and sulfury. Such compounds are thermally generated from the reaction of furfural and cysteine (Tressel and Silwar 1981).

Aroma concentrates from cooked and roasted beef, pork, and chicken were isolated and reported to contain aliphatic, cyclic, and heterocyclic *S*-containing components. Constituents identified included several iso- and anteiso-methyl-branched long-chain aliphatic aldehydes primarily found in beef aroma as well as a homologous series of alkyl-substituted cyclopentene-1-carbaldehydes in chicken flavor (Werkhoff and others 1993).

Reaction products formed from the aqueous mixture (1:1:1:1 molar ratio) of cysteine, xylose, thiamin, and ascorbic acid that was heated at 120°C for 5 h in a laboratory autoclave were isolated and characterized (Werkhoff and others 1991). Compounds were identified as: 6-methylbicyclo[4.3.0]-2,5-dithia-7-oxanonene-3, 2-(2-furyl)-1,3-dithiol, *cis*- and *trans*-2-(2-furyl)-4-acetyl-1,3-oxathiane, 4-(2-methyl-3-furylthio)-4-(2-furyl)-3-thiabutanal, 4,6-bis-(2-furyl)-3,5-dithiahexanal, and 4-(3-thienylthio)-4-(2-furyl)-3-thiabutanal (152).

Thiophene, thiophenone, thiirane. Thiophenes are responsible for the mild sulfurous odor of cooked meat (Shibamoto 1980). Four reactions were carried out to compare the sulfur-containing compounds formed via Maillard reaction/Strecker degradation of cysteine with furaneol and via the participation of hydrogen sulfide in the thermal degradation of furaneol. GC–MS analysis showed that certain sulfur-containing compounds, such as 2,5-dimethylthiophene, 2,5-dimethyl-4-hydroxy-3(2H)-thiophenone, and 3,5-dimethyl-1,2,4-trithiolane, were found in four reactions, while thiirane and 2-methylthiophene were only found in the Strecker degradation of cysteine and furaneol. Furthermore, this study showed that more sulfur-containing compounds were formed in the participation of hydrogen sulfide than in the Maillard reaction/Strecker degradation of glutathione and even cysteine, indicating that the availability of hydrogen sulfide in the reaction may be the limiting factor in the amount and the type of sulfur-containing compounds formed in the reactions. The amino group of the cysteine residue in glutathione is peptide bonded and cannot participate in Strecker degradation with a dicarbonyl compound. Therefore, the reaction mechanisms involved in the reaction between cysteine and furaneol would be different from those in the reaction between glutathione and furaneol. Thiophenes are responsible for the mild sulfurous odor of cooked

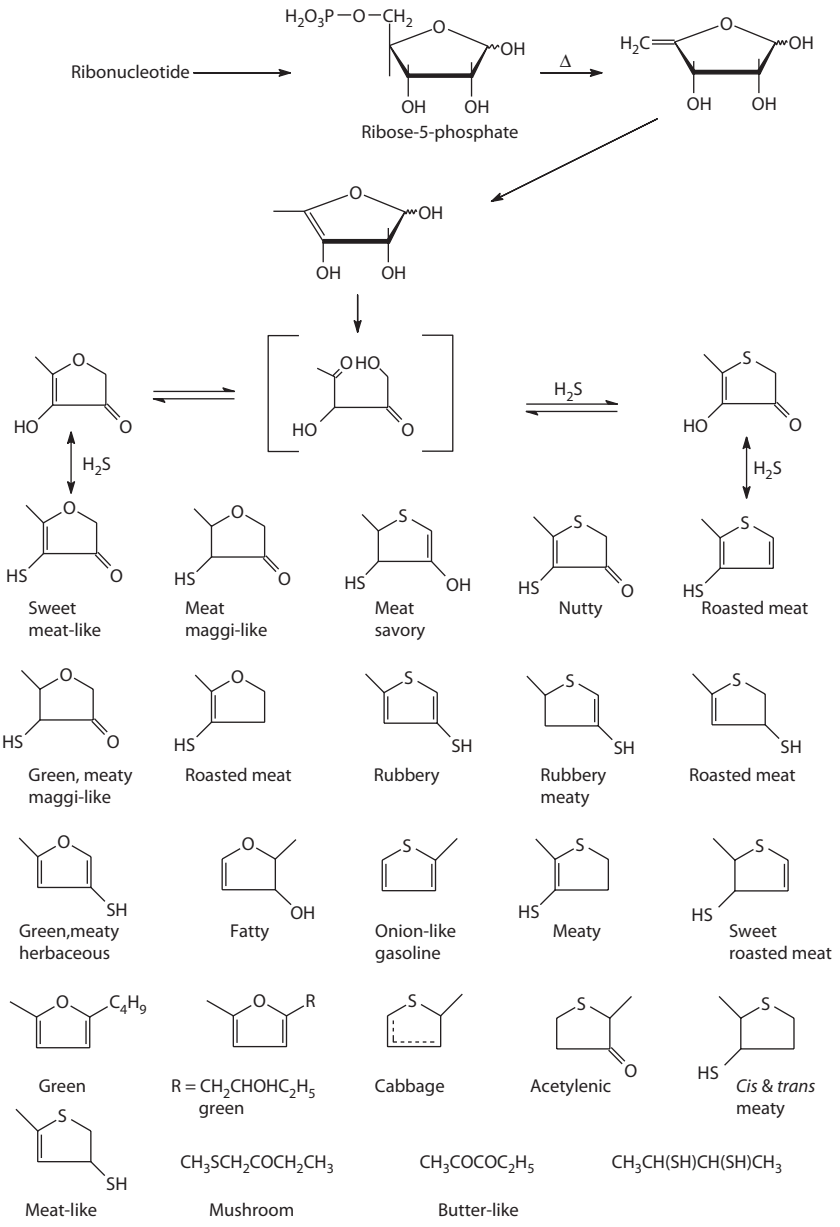

FIGURE 6.5 Formation from ribonucleotides of 4-hydroxy-5-methyl-3(2H)-furanone, and its reaction with hydrogen sulfide.

meat (Shibamoto 1980). Several thiophenes were identified in grilled pork. Alkyl-substituted dithiazines have been found in the flavors of beef and pork (Werkhoff and others 1992).

Three compounds, 2-methyl-3-furanthiol, 2-methyl-3-thiophenethiol, and 3-thiophenethiol, were recently found in the 4-hydroxy-5-methyl-3(2H)-furanone/cysteine reaction mixture (Whitfield and Mottram 1999). 2-Methyl-3-furanthiol and 2-methyl-3-thiophenethiol had both been found in cysteine–ribose model systems (Farmer and others 1989). They were also reported by van den Ouweland and Peer (1975) to be the major products of the reaction between mmf and hydrogen sulfide. These authors also reported that the di- and tetrahydro derivatives of 2-methyl-3-furanthiol and 2-methyl-3-thiophenethiol were also found. Pathway for the formation of 2-methyl-3-furanthiol involves the initial reduction of

HMF to give a hydroxyhydrofuranone, which undergoes acid-catalyzed dehydration to 2-methyl-3-hydroxyfuran. Ketonization of this, followed by the addition of hydrogen sulfide and the elimination of a molecule of water, gives the thiol 2-methyl-3-furanthiol. The substitution of the oxygen ring in 2-methyl-3-furanthiol by a sulfur atom via ring opening was suggested previously (Ouweland and Peer 1975). 2-Methyl-3-thiophenethiol was suggested to form via a route similar to that proposed above for the furanthiol. If the intermediate 2-methyl-3(2H)-furanone or 2-methyl-3(2H)-thiophenone were reduced to the corresponding hydroxy derivatives prior to substitution of the –OH by –SH, 4,5-dihydro-2-methyl-3-furanthiol and the corresponding thiophenethiol would result.

6.3.7 2,4-Decadienal-Derived Thiophene, Thiapyran, Pyridine, and Dithiazines

A large number of long-chain alkyl-substituted heterocyclic compounds, including thiophenes, pyridines, thiazoles, and other sulfur-containing compounds, were detected in the interaction systems. The occurrence of such compounds in cooked meat products has been reviewed (Whitfield 1992). These compounds are O-, N-, and S-heterocycles containing *n*-alkyl substituents (C_5–C_{15}). The alkyl groups are usually derived from aliphatic aldehydes, obtained from lipid oxidation. The reaction pathway for the formation of 2-pentylpyridine, 2-hexylthiophene, and 2-pentylthiapyran from 2,4-decadienal has been proposed (Figure 6.6). The reaction of 2,4-decadienal with ammonia is a likely route to 2-pentylpyridine. Related reactions between dienals and hydrogen sulfide may be responsible for the formation of 2-alkylthiophene with C_4–C_8 alkyl substituents which have been reported in pressure-cooked beef.

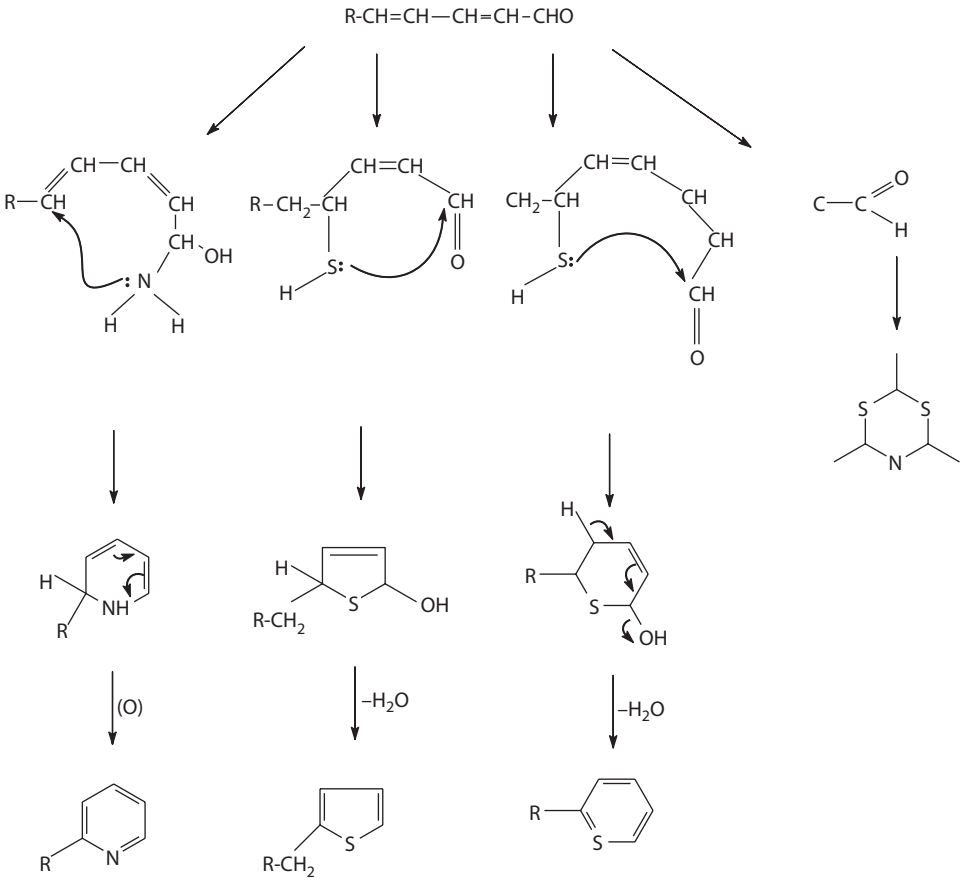

FIGURE 6.6 Formation of 2,4-decadienal-derived thiophene, thiapyran, pyridine, and dithiazines by the reaction of aldehydes.

2-Pentylpyridine is commonly found in the volatiles of cooked meat. A number of other alkylpyridines have been reported in lamb fat (Buttery and others 1977). Recently, the mechanism for the formation of 2-pentylpyridine in the reaction of 2,4-decadienal with either cysteine or glutathione (γ-glu-cys-gly) in an aqueous solution at high temperature (180°C) was studied (Zhang and Ho 1989). The release of free ammonia from amino acids, especially L-asparagine and L-glutamine, leads to the formation of pentylpyridines as reported by Kim and Ho (1998). When each of the amino acids was reacted with 2,4-decadienal under oil conditions, an increased amount of 2-pentylpyridine could be produced from asparagine and glutamine as compared to aqueous conditions. However, only a small amount of 3-pentylpyridine was formed in the asparagine and glutamine that produced a large amount of 2-pentylpyridine under oil conditions. When each of the five amino acids was reacted with 2,4-decadienal under oil conditions, an increased amount of 2-pentylpyridine could be produced from asparagine and glutamine compared to aqueous conditions. Specifically, larger than 10 times the amount of 2-pentylpyridine was found in the reaction of glutamine and 2,4-decadienal under oil conditions than under aqueous conditions, despite the fact that both asparagine and glutamine could liberate less ammonia in oil systems (Kim and Ho 1998).

2-Alkylthiophenes with C_4–C_8 alkyl substituents, which derived from the interaction between dienals and hydrogen sulfide, have been reported in pressure-cooked beef (Min and others 1979).

6.4 Factors Controlling Meat Aroma Formation

6.4.1 Effect of Cooking Conditions on Meat Aroma Formation

A wide range of temperature conditions exist during normal cooking of meat; the center of a rare steak may only reach 50°C, the center of roast meat may attain 70°C–80°C, while the outside of grilled or roast meat will be subjected to much higher temperatures and localized dehydration of the surface will occur. On the other hand, in stewing, the meat remains at a temperature of 100°C, in the presence of excess water, for several hours (Mottram 1998).

The large numbers of heterocyclic compounds reported in the aroma volatiles are associated with roasted, grilled, or pressure-cooked meat rather than boiled meat where the temperature does not exceed 100°C (Mottram 1985). In pressure-cooked pork liver at 163°C, quantitatively over 70% of the total amount of volatiles were furans and pyrazines (Mussinan and Walradt 1974), while in boiled pork, the volatiles were dominated by aliphatic aldehydes and alcohols with only very small quantities of heterocyclic compounds. The effect of cooking conditions on the formation of volatile heterocyclic compounds in pork has been compared (Mottram 1985). Well-done grilled pork contained 66 heterocyclic compounds including pyrazines, thiazoles, thiophenes, furans, and pyrroles. Pork cooked under less severe roasting or boiling conditions contained considerably fewer heterocyclic compounds. The alkylpyrazines accounted for almost 80% of the total headspace volatiles of well-done grilled pork (Mottram 1985).

It has been well accepted that alkylpyrazines are formed from a reaction of the degraded nitrogenous substances, ammonia, primary amine from proteins, peptides, amino acids and phospholipids, and α-dicarbonyl compounds in food (Shibamoto 1980). Like alkylpyrazines, alkylthiazoles identified in pork arise from a Maillard-type reaction between amino acids and sugars. It has been demonstrated that the requirement for the formation of these compounds is severe heating. The mode of cooking may also affect the flavor formation via wet versus dry cooking.

From the mechanistic point of view, cooking conditions affect the generation of aroma compounds significantly. Volatile compounds formed from the reaction of 3-hydroxy-2-butanone/ammonium acetate at 25°C, 55°C, and 85°C were investigated. Six compounds were characterized. Among the volatile compounds identified, an interesting intermediate compound, 2-(1-hydroxyethyl)-2,4,5-trimethyl-3-oxazoline, was found. The formation pathway of these volatile compounds was proposed (Fu and Ho 1997). In these model systems, 2-(1-hydroxyethyl)-2,4,5-trimethyl-3-oxazoline was formed at reaction temperatures below 25°C. On the other hand, tetramethylpyrazine was the major component when the reaction temperature was higher than 85°C. The amounts of 2-(1-hydroxyethyl)-2,4,5-trimethyl-3-oxazoline and tetramethylpyrazine increased linearly with increased heating time at 55°C.

The reaction between 3-hydroxy-2-butanone and ammonium sulfide was studied (Xi and others 1999). In addition to oxazole, thiazole and thiazoline were formed in the presence of sulfide. Four well-known compounds, 2,4,5-trimethyl-3-oxazoline, 2,4,5-trimethyloxazole, 2,4,5-trimethyl-3-thiazoline, and 2,4,5-trimethylthiazole, were identified. On the other hand, tetramethylpyrazine was the major product with a reaction temperature higher than 100°C.

In a related study, volatile components in maize flour, extruded under different conditions obtained by varying product temperature (120°C, 150°C, or 180°C), moisture level (14%, 18%, or 22%), and residence time (35 or 60 s), were identified and evaluated (Bredie and others 1998). Increasing the product temperature, reducing the moisture level, or prolonging the residence times generally increased the numbers and quantities of Maillard-derived compounds, such as pyrazines, pyrroles, furans, and sulfur-containing heterocycles. In low-temperature (120°C) and high-moisture (22%) extrusions, the main volatiles were compounds associated with lipid degradation, with few compounds derived from the Maillard reaction. Increasing the temperature and reducing the moisture level to 18% gave rise to the formation of some pyrazines and thiophenones. A marked increase in quantities of 2-furfural, 2-furanmethanol, and alkylpyrazines occurred in the extrusions at 180°C and 14% moisture. Under these conditions, other nitrogen- and sulfur-containing heterocyclic compounds were also generated (Bredie and others 1998).

6.4.2 Effect of Buffer on Aroma Generation

Schiff base formation in meat aroma generation. The first step for the Maillard reaction involves the addition of a carbonyl group of the open-chain form of a reducing sugar to the primary amino group of an amino acid, peptide, or other compound. The elimination of water gives a Schiff base that cyclizes to give the corresponding *N*-substituted aldosylamine (van den Ouweland and others 1989). An important route to alkylpyrazines is from α-aminoketones which are formed in Strecker degradation or from the reaction of α-hydroxycarbonyls with ammonia. Condensation of two aminoketone molecules yields a dihydropyrazine which oxidizes to pyrazine (Vernin and Parkanyi 1982). The condensation between cysteamine and aldehyde or 2,3-butanedione leads to the formation of cysteamine-derived thiazoles and thiazolidines (Huang and others 1998a,b). The key step is the formation of a Schiff base.

Effect of phosphate on Schiff base formation. Buffer dramatically promotes Schiff base formation from amino and aldehyde groups. Phosphate-mediated flavor compound formation has captured the interest of food processors who are trying to use this technique to obtain a better quality flavor.

With regard to the enhancing effect of phosphate buffer on Schiff base formation, Schwimmer and Olcott (1953) have found a twelvefold increase in the brown color (420 nm) formed in a glycine/hexose model system in a phosphate buffer (0.33 M, pH 6.5) as compared with that in water. Saunders and Jevis (1966) have reinvestigated the role of buffer salts in nonenzymic browning and confirmed the findings of Schwimmer and Olcott (1953). Mori and Manning (1986) have performed a large-scale synthesis of a Schiff base from Ala–His and glyceraldehyde in a potassium phosphate buffer at pH 7, and the significant bifunctional catalytic effect of phosphate on the Schiff base formation in diketopiperazine from aspartame has recently been reported by Bell and Wetzel (1995). Phosphate buffer (pH 5.0) was used as a reaction medium for the formation of an intense roasty, popcorn-like odorant, 5-acetyl-2,3-dihydro-1,4-thiazine (Hofmann and others 1995). Phosphate buffer (pH 5.7) has also been utilized in the reaction system of L-cysteine and D-ribose to produce meat-like aroma compounds (Farmer and Mottram 1994).

The phosphate buffer system served as either a proton donor or acceptor, which catalyzed the Schiff formation. Extra phosphate may also have catalyzed the dehydrogenation reaction. This was due to the buffer effect of the phosphate system. A sufficient amount of phosphate ions serves both as a proton donor and acceptor.

The proton from hydrogen phosphate protonated the carbonyl carbon of 3-hydroxy-2-butanone, which facilitated the formation of an ionic intermediate. Subsequent rearrangement of this intermediate was catalyzed by phosphate and led to the formation of an aminoketone. Condensation of two molecules of the aminoketone and subsequent dehydrogenation produced one molecule of tetramethyldihdyropyrazine (TMP). Redox reaction catalyzed by phosphate ions results in the conversion of thiazolidine to the corresponding thiazoline through hydride transfer (Huang and others 1998b). The intermediate of TMP

formation was characterized as TMP using GC–MS. A 15*N*-labeled ammonium acetate/3-hydroxy-2-butanone model system was used to confirm the incorporation of a nitrogen atom in the molecule of tetramethyldihydropyrazine.

6.4.3 Effect of Water Content on Aroma Generation

Numerous meat-flavor compounds were formed in interaction between amino acids, pentose sugar, and phospholipids (Timon and others 2004). The volatile profile of the Maillard reaction formed in a system with excess water differs greatly from those of low-moisture systems.

Cysteine/ribose model reactions carried out in aqueous solutions with the concentrations of the reactants similar to those found in boiled meat were compared with that performed in the absence of water or with a relatively low-moisture content (5:1, water: solids) performed at 185°C, which is similar to the condition on the surface of grilled meat (Farmer and Mottram 1990). Timon and others (2004) analyzed pan-fried pork chops and identified approximately 150 volatiles, mainly hydrocarbons, aldehydes, ketones, and alcohols. Meinert and others (174) fried pork chops on a pan at 150°C and 250°C, and a total of 31 volatiles were identified. It was further shown that lipid-derived aldehydes dominated at 150°C, while Maillard-related aldehydes and ketones dominated at 250°C. In the sensory analysis, a more intense roasted flavor was found at 250°C compared with 150°C, and this is most likely due to the formation of Maillard-derived flavor compounds, which is accelerated by local dehydration of the meat surface at elevated temperatures (175°C).

In heated aqueous systems, the major volatile components were thiols such as 2-methyl-3-furanthiol, 2-furanmethanethiol, 2-thiolphenethiol, 2-methyl-3-thiolphenethiol, 2-mercapto-3-butanone, and two mercaptopentanones (Mottram and Whitfield 1994). However, in the corresponding dry or low-moisture systems, 3,5-dimethyl-1,2,4-trithiolane, 3-methyl-1,2,4-trithane, 3,6-dimethyl-1,2,4,5-tetrathiane, 1-(2-furanymethyl)-1H-pyrrole, and 5-ethyl-4-methylthiazole were the major components (Salter and others 1988). In the presence of large amounts of water, furan- and thiophenethiols, mercaptoketoness, and other oxygenated sulfur heterocyclics, such as dithiolanones, were formed from the action of hydrogen sulfide on ribose-derived Maillard reaction products (Zhang and Ho 1991). On the other hand, trithiolane, trithiane, and tetrathiane can be formed from the interaction of acetaldehyde, hydrogen sulfide, and mercaptoacetaldehyde, all which are produced by the thermal degradation of cysteine. At low-moisture levels, the thermal degradation of cysteine is much more prominent than reactions of ribose with hydrogen sulfide, which requires relatively high water content.

The addition of phospholipids to the dry and low-moisture system produced only small amounts of Maillard/lipid interaction products, and the only significant lipid oxidation products were a series of 2-alkanones. Under aqueous conditions, large quantities of Maillard/lipid interaction products, including 2-pentylthiophene, 2-hexylthiophene, 2-pentylthiapyran, and 2-pentylpyridine, were produced together with large amounts of lipid oxidation products such as pentylfuran, alkanals, and alkanols. The quantity of volatiles generated from the reaction of 2,4-decadienal with cysteine or glutathione was almost the same. However, it has been shown that cysteine degradation in an aqueous medium produces four times as many volatiles as glutathione (Zhang and Ho 1988).

As early as 1958, methanol was used as a reaction medium for the Schiff base formation between amines and glucose by Rosen and others (Rosen and others 1958), and a similar solvent effect has been observed by Herraiz and his coworkers (Herraiz and others 1993). The protic solvent attracted the water molecule, which led to completion of the Schiff base formation. The mechanism proposed in this chapter is directly applicable to nearly all reactions involving a Schiff base formation.

It has been demonstrated that the requirement for the formation of these compounds is severe heating. An investigation of the volatile components formed from the interaction of cysteine, xylose, and tributyrin at 200°C led to the formation of furans, or furanones, pyrroles, pyridines, thiophenes, thiazoles, and a number of other heterocyclic compounds containing sulfur or sulfur and nitrogen. The low-moisture content of the reaction mixture and the relatively high temperature of reaction (200–220°C) were the key factors in the formation of a large number of thiazoles (Ledl and Severin 1974). However, acetylthiazole was present in large amounts in the boiled pork suggesting that a different mechanism is involved in its production.

6.4.4 Role of Lipid in Meat Aroma Formation

The role of the lipid fractions of meat, both adipose tissue and structural phospholipids, has been the subject of a number of studies. Aqueous extracts of beef, pork, and lamb had similar aromas when heated, and when heating the fats, they yielded the species characteristic aromas. Interesting triangle taste tests reveal that the addition of 10% fat (beef or pork) enabled the panel to distinguish the lean meats more easily (Mottram 1979). The addition of pork fat to either lean beef or pork resulted in a substantial increase in hexanal but only small changes in most other volatiles (Mottram and others 1982).

It was suggested that lipids provide volatile compounds which give the characteristic flavors of different species, and the lean is responsible for a basic meaty flavor common to all species (Horstein and Crowe 1960). It is generally accepted that meaty notes come mainly from such sulfur-containing compounds as 2-methyl-3-furanthiol and bis-(2-methyl-3-furyl)disulfide, which are generated from water-soluble precursors in lean meat (Horstein and Crowe 1963). Phospholipids rather than triglycerides were important in determining meat flavor (Shahidi 1988). In mammalian tissue, structural phospholipids contain significant amounts of certain polyunsaturated fatty acids, in particular arachidonic (20:4), docosapentaenoic (22:5), and docosahexaenoic (22:6).

Foods cooked in lard usually have better flavor than foods cooked in vegetable oils. Pork fat was dry-rendered or wet-rendered to obtain crude lard. These two different lards were then refined resulting in dry-rendered lard and wet-rendered lard (Mottram and Edwards 1983). Results of sensory evaluation showed that crude dry-rendered lard had the strongest flavor intensity among the four lards. Crude dry-rendered lard also contained the highest amounts of trace components (phospholipids and amino acids) and flavor contributing volatiles after heating at 190°C. These results seemed to indicate that wet-rendering and refining of lard could reduce the trace components which might be important in producing lard flavor (Hwang and Chen 1994).

6.4.5 Effect of Fermentation on Meat Flavor

Generation of volatile compounds (via chemical or enzymic oxidation of unsaturated fatty acids and further interactions with proteins, peptides and amino acids, or Strecker degradation) during dry curing of meat products has been extensively discussed. Biochemical mechanisms involved in the development of dry-cured hams and other meat products including proteolysis (caused by calpains and cathepsins) and its effects on flavor; lipolysis by various enzymes at different stages of processing; and control of enzymic activities, for example, by controlling relative humidity (RH), temperature, and salting levels have been discussed as well (Toldra 1998). Numerous reports have indicated that carbonyl components make a significant contribution to the flavor of cured meat (Jacobsen and Koehler 1963; Langer and others 1970; Sanderson and others 1966). 2,3-Octanedione and 2,4-decadienal were found in pork, while 3-hexenal, 3-methylhexanal, 2-heptenal, octanal, 2-octenal, and decanal were found in chicken, and 3,3-dimethylhaxanal was uniquely identified in beef. The long processing of the dried-cured hams (12 months) provides ample time for the occurrence of lipolytic and oxidative degradation of unsaturated fatty acids. Aldehydes have been shown to be the major oxidative decomposition components. It is suggested that 2,4-decadienal may be derived from linoleic acid (St Angelo and others 1980). The unsaturated aldehyde, 2,4-decadienal, and 2-undecenal may undergo further oxidation to shorter-chain aldehydes during the long aging process (Drumm and Spanier 1991). Though these carbonyl components were detected in the cured meat flavor concentrate, they were absent in the cooked uncured meat. Two pyrazines, methylpyrazine and 2,6-dimethylpyrazine, were found in the headspace volatiles of 'Serrano' dry-cured ham. The temperature used during the dry-curing process is not as high as in cooking, therefore fewer pyrazines are found in the cured meat. 2-Oxopropanal is proposed to be the precursor of 2,6-dimethylpyrazine.

In addition, the chemical reactions during the curing process—lactic acid fermentation—deserve special interest. Potential and actual effects of lactic acid bacteria or Micrococcaceae on catabolism of carbohydrates, proteins and amino acids, and lipids and fatty acids in fermented meat products (such as hams and sausages), and their influence on flavor development, are discussed, covering: carbohydrate catabolism, proteolysis, amino acid catabolism, lipolysis, fatty acids oxidation, and ester production (Forss 1983). One of the investigations indicated that hexanal, a major lipid oxidation product, was found

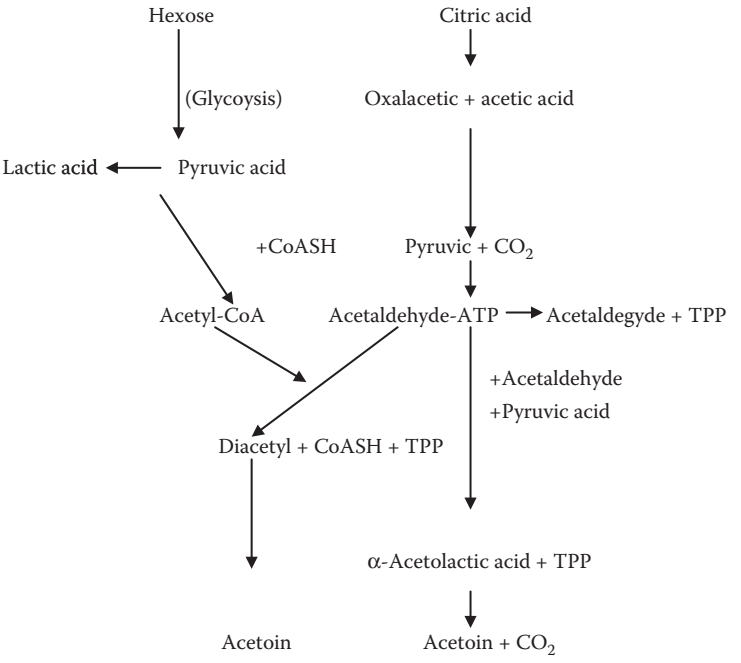

FIGURE 6.7 Formation of acetaldehyde, 2,3-butanedione, and 3-hydroxy-2-butanone.

to be present in uncured meat at a concentration of 12.66 +/– 0.08 mg/kg, while only 0.03 mg/kg was present in the cured product. Also, the concentration of other carbonyl compounds was higher in uncured pork, while they were either present in reduced amounts or not detectable in the cured meat. It is reasonable to postulate that aldehyde precursors for aroma generation may occur during the curing process.

Lactic acid bacteria produce significant amounts of acetaldehyde, 2,3-butanedione, 2,3-pentanedione, and 3-hydroxy-2-butanone. Formation of acetaldehyde, 2,3-butanedione, and 3-hydroxy-2-butanone is shown in Figure 6.7 (Montel and others 1998). Sterile pork loin tissue has been inoculated with *Lactobacillus plantarum* and *Lactobacillus fermentum*, placed in sterile sample bottles, purged with CO_2, and stored for up to 24 days at 3°C. *L. plantarum* grew more rapidly than *L. fermentum* under these conditions. Profiles of volatile compounds were similar for inoculated and sterile pork loin tissue (Ramarathnam and others 1991). It is expected that during the thermal treatment of the cured meat products, precursors may participate in the reactions leading to the formation of various heterocyclic meat aroma compounds following the pathways described previously.

Molds produce enzymes for the degradation of lipid- and protein-matter, but studies show that the proteolytic and lipolytic capabilities differ significantly between strains and is highly dependent on media, pH, and temperature (Sunesen and Stahnke 2003). A positive correlation between the aroma intensity in mold-ripened sausages and the degree of proteolysis was indicated by Geisen (1993). Proteolytic activity of *Penicillium strains*, *Mucor*, and *Aspergillus oryzae* against both myofibrillar and sarcoplasmic proteins in media has been studied (Trigueros and others 1995). Bruna and others (2001) found that *Penicillium aurantiogriseum* and a cell free extract of the same strain increased the concentration of free amino acids in sausages. The higher proteolytic ability of *Penicillium nalgiovense,* the more intense aroma formation was found. Concentrations of free fatty acids increased two- to threefold during a 22-day ripening period when sausages where surface inoculated with *P. aurantiogriseum*. Production of lipases is strain specific, depending on substrate, pH, and temperature. Significant differences in both production and action of the lipases among *Aspergillus niger*, *Rhizopus delemar*, *Geotrichum candidum*, and *Penicillium cyclopium* were reported by Iwai and Tsujisaka (1984). The influence of molds on meat aroma precursor production is mainly their ability to produce enzymes for the degradation of lipid- and protein-matter. Both exolipases and exoproteases are primarily produced by molds in the mold-microenvironment.

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7

Analytical Methods for Meat and Meat Products

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7.1 Introduction

In developed countries, it is difficult to buy packaged food that does not display information about energy, fat, protein, salt, or other components. Even when the food is relatively unprocessed, like fresh fruits, raw meat, or fish, advertising or packaging information often makes substantial claims about the product's composition. In recent history, several factors have contributed to a great awareness of food composition. These include a decrease in the cost of food production, reduced physical activity, and medical advances that have increased life span. A significant outcome has been an increase in obesity and associated diseases. As a consequence, the composition of foods has become a focus of regulatory bodies and activist groups leading to an upsurge of media attention.

For meat and meat products, issues of composition are particularly important. Most people regard meat as a part of their diet and overall enjoyment of food, but the public is bombarded with real and purported facts about the effects of fat, saturated fat, cholesterol, salt, and chemical residues in meat on health issues such as obesity, circulatory diseases, and cancers. There is no sign of interest in these issues abating, although there is an ever-shifting focus on different components.

The main components of interest in the chemical analysis of meat and meat products are moisture, protein, fat and fatty acids, cholesterol, iron, sodium, and calculated values like energy. Another less obvious component is qualitative rather than quantitative—species of origin. Several religions have strict dietary laws relating to meat origin. For example, pork is forbidden under Islamic and Judaic law. Even without religious edicts, many societies and groups within societies reject certain species as meat animals whereas other cultures regard meat from these species as edible without question. Horsemeat is not a big seller in the United States, but is a normal butchery item in France. Consumers want to know what species they are eating. Species identification is important for another reason: trade barriers are often based on quotas by species.

This chapter summarizes the analytical methods used to measure the composition of meats and meat products. An analytical method is a laboratory recipe to qualify or quantify some chemical compound or chemical class of interest. At the root of quantitative analysis are issues of statistics that should be understood before the recipes are followed.

7.2 Statistical Issues in Analytical Methods

7.2.1 Sources of Error

Each step in an analytical procedure introduces error. Some errors are random and some are systematic. In weighing for instance, a random error would be the variation in weight recorded for repeated measurements of the same object, perhaps arising from the balance circuitry or vibration. A systematic error might be a consistent overestimate of the weight. Both types of error can occur at the same time. The classic target example illustrates this well (Figure 7.1).

Random error is the scatter of hits (formally termed precision), whereas systematic error is the mean distance of the hits from the bullseye (accuracy). The degree of precision and accuracy that is required for a test will govern the effort applied to minimizing error. Replication and calibration respectively tackle the two sorts of error.

7.2.2 Replication and Calibration

Random errors are overcome by replication so that a meaningful average or mean can be obtained. The greater the random error, more replications are required to compensate for the error. For any analytical

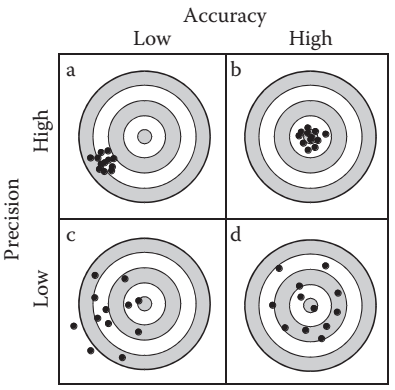

FIGURE 7.1 The target shooting example of precision and accuracy. Precision is high in targets a and b, but low in c and d. Accuracy is high in targets b and d, but low in a and c. It is easier to pick the difference between targets a and b than between c and d.

procedure, performing replicated measures at each step highlights where the most random error occurs. These are the steps where replication must occur in the method's application if higher precision is required. Systematic errors are minimized by regular calibration against reference standards. For example, the accuracy of laboratory balances should be routinely calibrated with a certified series of weights. Replication and routine calibration cost money, and a balance must be struck between the cost of better precision and accuracy and the needs of the client, commercial, or otherwise. The client will often be unaware of the distinction between precision and accuracy, but the importance of both in respect of cost should be made known, particularly where analysis is ongoing and the outcome is commercially important.

7.2.3 Sampling

The greatest source of random error in analysis of meat is the variability inherent in meat itself. If an analyst wanted to determine the relative proportions of fat, protein, and moisture (water) in a one ton container of meat pieces, analyzing the entire ton would give perfect answers from calibrated methods, but would destroy the meat. Therefore, a sample is taken. If the sample were small, say 50 g, and taken from a single site, the answer would probably bear no relation to the true proportions in the entire ton; fat or lean might dominate the sample. If the sample size were increased, say to 1 kg, the chances of obtaining a more representative answer would increase, and so on with larger samples. Better information could be obtained by taking a number of samples, say 20 replicates of 50 g, from randomly chosen sites. The proportions of fat, protein, and water would vary from replicate to replicate, but the mean of the 20 results would give a fairly precise result. Moreover, the spread of values around the mean would give the analyst an idea of how variable the meat was from site to site.

Sample size and replication are thus fundamental issues facing food analysts who are required to provide determinations with maximum certainty, but are expected to charge a minimum for the service. This tension between uncertainty and cost pervades all analysis and pivots on the relative heterogeneity of the food in question. For a stirred wine vat, a single 5 mL sample (~5 g) is sufficient to give an acceptably reliable answer for many analytes. As a solid derived from complex organisms comprising many tissue types, meat is inherently heterogeneous, requiring a more thoughtful approach to sampling. Experience helps resolve the conflict between uncertainty and cost.

7.3 Sample Preparation

Whatever the sample size, a preliminary comminution is usually required. There are two reasons for this. First, bulky samples of meat or meat products have a low surface-area-to-mass ratio making extraction of a particular analyte—moisture or fat, for example—kinetically inefficient. Comminution increases

the ratio making extraction much quicker, and also more predictable if all resulting particles are of similar size. Second, comminution reduces heterogeneity, so allowing small subsamples to represent the original bulky sample. Glassware items can thus be smaller and solvent volumes lower, so reducing costs and hazards to workers.

7.3.1 Equipment and Methods of Comminution

Whatever comminution method is chosen, it is critically important that it does not change the nature of the analytes. The most common cause of this is blunt equipment. Not only can blunt equipment cause variable comminution and analyte damage through heating, but it also makes comminution more difficult. The need for sharp equipment cannot be overemphasized.

The butcher knife is the most basic tool and much size reduction and tissue randomization can be accomplished quickly and easily. A laboratory grinder is essential equipment in an analytical laboratory for meat. The cutting plates typically have a diameter around 70 mm and hole sizes ranging from 2.5 to 4 mm. The so-called kidney plates are often included in the grinding assembly to aid comminution of large meat pieces. Where possible, the meat or meat product should be well chilled or even lightly frozen before grinding. Grinders work best when cool as even the sharpest grinder generates heat. Bowl choppers have rapidly rotating knives that cut meat to the required consistency. As with grinders, damaging temperature increases can occur. Liquid nitrogen can be used judiciously to control temperature when this is important.

Where an analyte is prone to oxidation from exposure to air, tissue can be comminuted as a solid in an excess of liquid nitrogen. Typically the tissue is pulverized by mortar and pestle while the nitrogen simultaneously displaces air, cools, and embrittles the sample.

When meat tissue is extracted with a solvent, aqueous or otherwise, the analyst can choose from a wide range of proprietary blenders. Traditional blenders by Sorvall and Waring have been joined by rotor/stator dispersing elements that operate in excess of 20,000 rev min^{-1}. Explosion proof blenders should be used with flammable solvents.

7.4 Overview of Analytical Methods

Four components of meat dominate analytical methods: moisture, protein, fat, and ash, the last a collective term for the minerals present in meat. These components are routinely determined by the so-called proximate analysis, the principal method by which the basic composition of meat is chemically described. As knowledge of food chemistry grew in the last century, methods were developed to determine the specific amino acids, fatty acids, and individual elements that are components of the protein, fat, and ash. Similarly, methods have been developed for vitamins and cholesterol in meat. Methods have also been developed for other analytes common in meat products, and, in recent decades, methods to identify species.

Whether analysis is simple, for example, moisture determination of ground meat, or more complex, for example, determination of geometric isomers of unsaturated fatty acids, the mission is fundamentally the same. The analyte of interest must be isolated or otherwise distinguished from other analytes and measured. This is achieved with standard methods that have been peer reviewed, tested and refined over decades. The Official Methods of Analysis of AOAC International provides statistically verified methods for meat and for foods (AOAC 2010). In the sections that follow, many of these methods are referenced by AOAC number, but details are omitted. Instead, the principles that underlie the methods are described. A variety of non-AOAC methods are also discussed, spanning noninvasive physical methods, meat quality attributes, and species identification. Finally, aspects of analytical laboratory management are examined.

7.5 Proximate Analysis

As noted above, the four major analytes of interest have to be isolated or otherwise distinguished from other analytes before determination. Moisture is the only analyte, that is, substantially volatile at

temperature just above 100°C, so the moisture content can be quantified by drying a sample at such temperatures. Similarly, protein is the dominant component in meat that contains nitrogen, and fortunately the nitrogen content of meat proteins is roughly constant. Thus determination of nitrogen is used to measure the protein content. Fats are the only component of meat that are substantially soluble in organic solvents and ash is the only matter in meat that is not oxidized and volatilized by temperatures above 500°C. Importantly, carbohydrate cannot easily be isolated, so it is calculated by difference. It is always a very minor component of meat but can be a significant component in meat products.

The expression "proximate" means that results are only close to being accurate. Thus, components of meat other than moisture are volatile during drying, nitrogen content varies slightly with the particular protein, and nitrogen is present in many vitamins. Not all fats are soluble in the solvent most commonly used to extract fat, and the solvent can extract some carbohydrate. However, provided methods are consistently followed, useful and widely accepted results are obtained.

7.5.1 Moisture

Moisture constitutes about 70–77% of raw muscle. The moisture content falls as the fat content of the meat increases, and can be markedly lower in processed meats, such as sausages that contain additional ingredients. In many processed products, reliable control of moisture content is important for preservation because a low moisture content lowers water activity (see later) and helps inhibit microbial growth.

Measurement of meat moisture content is determined by weight difference before and after removing the water from the samples. AOAC 950.46 dries a weighed sample in a forced air, convection, or vacuum oven between 95°C and 125°C for 2–16 h or until the sample attains constant weight, depending on the drying method used. Alternative drying methods include infrared, ultraviolet light, or use of a microwave oven (AOAC 985.14). Quick and effective moisture loss is aided by carefully mixing the samples with sand to increase the surface area of the sample and prevent clumping of the meat that might trap moisture during the drying step. Before reweighing, the dried sample must be cooled in a desiccator to prevent uptake of moisture from ambient air. Dried meats, particularly meat and bone meal, are hygroscopic and readily absorb moisture from the air.

More rapid moisture determinations can be accomplished by noninvasive physical methods that will be discussed later. These methods, which extend to other meat analytes, need calibration with standard chemical methods, which are the ultimate arbiter.

7.5.2 Protein

Nitrogen is in all amino acids, so most methods for determining protein determine the quantity of nitrogen in the meat and use a multiplication factor to calculate the quantity of protein. However, the relationship between nitrogen and protein depends on the amino acid composition: the percent weight of nitrogen in individual amino acids ranges from 8.6 for tyrosine to 35.9 for arginine. Fortunately, relatively constant proportions of myosin and actin dominate meat protein, and together contain ~16% nitrogen. Therefore, a multiplication factor of 6.25 is commonly used for meat.

Collagen in meat's connective tissue complicates the calculation because the factor for collagen is about 5.36, reflecting its higher proportion of nitrogen compared with other meat proteins. A lower multiplication factor should be used for meat containing high proportions of connective tissue (Benedict 1987). The situation becomes even more complicated in meat products, which frequently contain soy or milk protein. However, as long as the correct nitrogen–protein conversion factor is applied, protein determination by nitrogen measurement is the most accurate and reliable method currently available.

The traditional procedure for determining nitrogen in foods is the Kjeldahl method, which is named after its inventor. The food is digested at high temperature with concentrated sulfuric acid and catalysts to convert nitrogenous substances to ammonium salts. Addition of concentrated alkali then converts the ammonium salts into free ammonia that is distilled with steam and collected in either hydrochloric acid (AOAC 928.08) or dilute boric acid solution (AOAC 981.10) containing suitable colored pH indicators. In the boric acid procedure, the ammonia increases the pH and is titrated with acid to the original pH. In the hydrochloric acid procedure, excess hydrochloric acid is back titrated to neutrality with alkali.

To increase sample throughput, semiautomated equipment is available to analyze for protein in batches of samples (e.g., AOAC 977.14).

An alternative and faster method to determine protein nitrogen employs combustion. This Dumas method releases nitrogen into pure oxygen, which changes its thermal conductivity (AOAC 992.15). The Dumas method is suited to automation. However, thorough comminution is important with this method, as typically a very small sample is analyzed. As for Kjeldahl nitrogen, a conversion factor is used to relate nitrogen to protein.

7.5.3 Fat

Total fat and the fatty acid profile of fat are probably the most topical of all nutritional components in foods. This interest is engendered by obesity in Western societies and its associated degenerative diseases. Of fats in the human diet, those from animal foods have attracted the most adverse publicity because their fatty acid profile is usually high in saturated fatty acids, and because of the statistical association of animal fats with dietary cholesterol.

Fat measurements are usually made on the food product as sold to the consumer, but in a product's preparation the manufacturer is obviously concerned with the fat characteristics of the ingredients. Thus, the routine measurement of fat in a naturally variable ingredient like raw meat is important.

Fats as recognized by the public are a subclass of what are technically called lipids, but "fat" is often used interchangeably with "lipid." Although several classification systems have been proposed—usually based on the solubility of lipids in organic solvents—there is no agreed definition of the term "lipid." For nutritional purposes, however, total fat can be defined as the whole body of fatty acid-containing substances. Other organic compounds such as sterols, terpenes, and waxes that are dissolved by organic solvents are not considered fats, but if present in a food these compounds can be extracted along with the fat and contribute to the determined content.

Meat fats not only are mainly tri-, di-, and monoacylglycerols, but also include some phospholipids, cholesterol esters, and free fatty acids. Triacylglycerols are the dominant form of storage lipid in animals (typically 95% by weight). This class of compounds comprises glycerol esterified to three fatty acids. Mono- and diacylglycerols are similar to triacylglycerols, whereas phospholipids are diacylglycerols with the third glycerol alcohol group esterified to a phosphate or phosphate derivative.

The particular method used for determining fat defines the fat content. For instance, crude fat is defined as the petroleum ether* or diethyl ether-extractable portion, the two main solvents of choice. Extraction with a mixture of chloroform and methanol will extract crude fat plus other more polar compounds like phospholipids. Solvent polarity is a prime determinant of extraction, but other factors also influence extraction. These include acid hydrolysis to make the fat easier to extract, water washing to remove carbohydrate, extraction time, particle size, and moisture content. Subtleties of sample matrix also affect extraction. For example, if emulsion sausages are made from prerigor meat rather than rigor meat, fat in the resulting cooked meat gel is less accessible to the extracting solvent, so significant underestimates of crude fat can result unless the gel is pretreated by acid hydrolysis, as discussed shortly.

Crude fat, also known as the ether extract or the free lipid content, is the traditional measure of fat in meat and meat products (AOAC 960.39; AOAC 991.36). The most common method of extraction usually employs a Soxhlet apparatus that is named after its inventor. A comminuted meat sample is weighed into a Soxhlet thimble (either cellulose or glass fiber) and mixed with dry, acid-washed sand. The sand increases the surface area of the meat, allowing better penetration of the solvent. The thimble and sample is oven dried, and the fat is extracted by continuous solvent reflux. The extraction rate varies with the solvent and is rapid for diethyl ether and slower for higher-boiling-point solvents like hexanes. The solvent containing the extracted fat is evaporated, and the fat residue is dried to a constant weight. The fat content is calculated as a percentage of the original wet sample weight. Meat products containing water-soluble carbohydrates should be washed with water prior to drying and solvent extraction because the presence of carbohydrates may lead to erroneously high-fat values due to extraction of sugar.

* Petroleum ether is not an ether, but rather a mixture of alkanes, typically hexanes, of different boiling points. Diethyl ether is also a suitable solvent for crude fat determination but presents much greater safety risks.

Some sample matrices resist the extraction of fat. Acid hydrolysis is a useful way of making the fat more available for Soxhlet extraction (ISO 1973), and the name "total fat" reflects this improvement. Digestion with hydrochloric acid liberates the fat by hydrolyzing proteins and carbohydrates. The mixture is filtered, washed with water to remove acid and other water-soluble material, and then dried. A conventional Soxhlet extraction recovers the fat.

Although Soxhlet extraction is robust and proven, it is slow and requires large volumes of solvent. Moreover, it is a batch process unsuited to automation. Accelerated solvent extraction methods overcome these limitations. The speed of a Soxhlet extraction is limited by the boiling point of the solvent. By contrast, a pressurized solvent can be heated to well above its boiling point and this enables more rapid extraction with the use of less solvent, but with similar results. When hexanes are the solvent, the extraction temperature and pressure are 125°C and 10 MPa (about 100 atm). The total extraction time for a 3–4 g wet-weight sample is reduced about fivefold. A single accelerated extractor working sequentially and automatically through 20 samples can do the job in about 7 h. To achieve the same result by Soxhlet would require 20 extractors working in parallel at a high labor cost.

Mixtures of chloroform (or methylene chloride) and methanol extract lipids more thoroughly than other solvents. Chloroform:methanol mixtures are also gentle in the sense that lipids extracted at room temperature by this mixture undergo little chemical change, making this mixture the best starting point for detailed lipid analysis. Since the mixture is a good solvent for phospholipids, the following methods are particularly useful for tissues such as liver, kidney, and brain that are rich in phospholipids.

In the Folch method, the comminuted meat or meat product is mixed in a defined ratio with chloroform:methanol themselves in a set ratio of 2:1. Two phases result: a solid phase largely comprising of protein, and a single liquid phase containing all lipid and some water-soluble components. After filtration to recover the liquid phase, water is added to resolve it into two phases, a denser chloroform phase that contains the lipid, overlaid with a methanol/water phase. The chloroform phase is recovered, the solvent is evaporated, and the lipid is determined by weight (Christie 1984). Maxwell and others (1980) introduced a method where meat is ground with a drying agent, anhydrous sodium sulfate. The mixture is packed in a column from which the lipids are eluted by methylene chloride:methanol as a single water-free phase.

7.5.4 Ash

Ash is the inorganic residue that remains after combustion at high temperature (500–600°C). It is dominated by minerals such as potassium, phosphorous as phosphates, and others (see later). The ash content of raw meat is low and relatively constant but can vary greatly in processed meat products where minerals like salt and phosphates have been added, and in rendered meat and bone meal. AOAC 920.153 is designed for meat and meat products.

7.5.5 Carbohydrate

Raw meat contains very little carbohydrate, comprising glucose and sugar phosphates, residual glycogen and organic acids that are metabolic intermediates, and the terminal glycolytic product lactic acid. These organic acids are not strictly carbohydrates but are included as such because unlike fats, their molecular compositions have high proportions of oxygen. In proximate analysis, carbohydrate content is estimated by difference: [100%–(percent weight of fat + protein + moisture + ash)]. Since the combined four measured components in meat sum up to close to 100% of the original weight, small errors in any of the four measured components greatly affect the carbohydrate estimation. However, significant proportions of carbohydrates such as cereals, starches, and gums are often added to processed meat products, and where these carbohydrates are estimated by difference the result is inherently more accurate.

7.6 Detailed Analysis of Some Raw Meat Components

The preceding section discussed the concept of proximate analysis for meat. That level of detail is sufficient for many research and commercial purposes. However, in other situations more detailed

information is required, especially relating to protein, fat, and other meat components that are caught up in these two classes, such as vitamins and cholesterol.

7.6.1 Amino Acid Composition of Proteins

As a generic molecular species, protein is a linear polymer of different amounts of 20 or so amino acids. The possible combinations and permutations of the linear sequence are immense, but from a dietary perspective only the mass of protein in a unit mass of food and the amino acid profile (the relative proportions of the amino acids) are important. The amino acid profile is important because some amino acids cannot be synthesized by mammals and must be obtained from diet. Meat is rich in these so-called essential amino acids—lysine, leucine, isoleucine, and sulfur-containing amino acids—and in this sense meat is a source of high-quality protein. In the middle of the last century, the discovery of amino acids and their effects on growth and well-being led to the development of routine methods for determining amino acids as well as total protein.

The amino acids are joined by peptide bonds that must be hydrolyzed (broken) before the individual amino acids can be measured. Hydrolysis is accomplished by refluxing the meat sample with concentrated hydrochloric acid. This aggressive treatment causes partial or complete loss of some labile amino acids due to oxidation, notably tryptophan and cysteine/cystine, but most amino acids are resilient. Application of a vacuum or introduction of argon in the headspace of the digestion tube can reduce the degree of oxidative destruction. The acid conditions also deamidate asparagine and glutamine, resulting in apparent increases in proportions of aspartic acid and glutamic acid, which can be reported as the sum of asparagine and aspartic acid and of glutamine and glutamic acid. The sulfur-containing amino acids cysteine/cystine and methionine are usually determined separately by oxidation with performic acid of cysteine/cystine to cysteic acid and of methionine to methionine sulfone prior to acid hydrolysis. Tryptophan is also analyzed separately after alkaline hydrolysis. The loss of tryptophan, asparagine, glutamine, and cysteine/cystine during an unprotected acid hydrolysis limits quantitation to 17 amino acids. An aliquot of the hydrolyzate is evaporated to dryness, and the residue is dissolved in the buffer required for subsequent liquid chromatographic analysis. In the original chromatographic technology, the amino acids were resolved by ion exchange, and after elution were visualized colorimetrically with ninhydrin. In more modern methods, fluorescent complexes are prepared before chromatography. In these methods, separation is accomplished with reverse-phase columns largely on the basis of polarity and pH.

Hydroxyproline deserves special mention because it is an indicator amino acid of collagen. The collagen content of meat and meat products is often of interest to food processors because it alters batter gelation properties, and of interest to nutritionists because collagen contains a lower proportion of the essential amino acids (Zarkadas 1992). After acid hydrolysis, hydroxyproline is measured by a colorimetric reaction (Stegemann and Stalder 1967), and related to collagen by a conversion factor (7.14) because collagen in meat contains about 14% hydroxyproline.

7.6.2 Fatty Acid Composition

In the context of meat and meat products, the main aim of fatty acid analysis is determination of different fatty acids in the range 12 carbon atoms to 22. Fatty acids with 16 and 18 carbon atoms are the most prevalent fatty acids in meat. Beyond the length of the fatty acid's alkyl chain backbone, the number of double bonds is of particular interest, as are the positions of these bonds in the chain, so defining isomers. Drilling deeper still, each double bond can have a *cis* or *trans* geometric orientation, yet another level of isomer complexity. At varying depths of detail, this information—termed the fatty acid profile—is of interest to food manufacturers, nutritional and medical experts, government, and consumers. The fatty acid profile is best determined by gas chromatography. While some chromatographic columns can resolve free fatty acid in their protonated form, fatty acids are more often converted into methyl, ethyl, or butyl esters. As alkyl esters, the fatty acids are relatively nonpolar and volatile, and can be resolved to high precision on chromatographic columns where separation is based on chain length and polarity.

The choice of chromatographic column and chromatographic conditions is governed by what the analyst is looking for, because no one column and set of conditions will resolve all fatty acid esters and their isomers.

The details of fatty acid analysis, from fatty acid methylation techniques to chromatography, are not discussed here. Readers are referred to Christie (1989) and to AOCS (2009) that discuss methods that comply with U.S. food labeling regulations for total fat in foods including meat products. The nutritional definition of total fat is, not unreasonably, the sum of the fatty acids originating from all the lipid classes present in the food expressed as triacylglycerol equivalents. The labeling regulations also require the mandatory declaration of amounts of *cis*-monounsaturated, *cis*-polyunsaturated, total saturated fatty acids, and *trans*-fatty acids. The two major classes of polyunsaturated fatty acids, ω-3 and ω-6 fatty acids, can be declared voluntarily. In contrast to expressing the sum of the fatty acids as triacylglycerol equivalents, the fatty acid classes are expressed as free fatty acids (i.e., not-esterified-to-glycerol fatty acids).

7.6.3 Minerals

Minerals in meats and meat products are a collective term for nutrient elements often quoted in food composition tables. Depending on the method of isolation, the elements may include sulfur, which is retained by acid digestion but is lost as volatile sulfur oxides on ashing. Thus, ash and minerals are not synonymous but are related terms. Ash from raw lean beef contains (in descending order of abundance) ionic forms of potassium (about 350 mg 100 g^{-1}), phosphorus, sodium, magnesium, calcium, zinc, iron (around 3 mg 100 g^{-1}), and other trace minerals (USDA 2005). Although the color of meat is dominated by iron in the form of the heme protein myoglobin, iron is one of the lesser minerals in meat. Nonetheless, its determination is important, because heme iron is the most readily absorbed source of iron in the human diet. The iron content in meat can be measured spectrophotometrically after colored complexes are formed. However, inductively-coupled-plasma atomic spectroscopy procedures like AOAC 990.08 are now more common and convenient for use to determine the concentration of iron and other minerals.

7.6.4 Carbohydrates

As a very minor component of raw meat, the analytical composition of carbohydrates is not of dietary interest. However, as a component of prerigor meat, the glycogen content of muscle is important to abattoir operators who wish to predict the ultimate pH of meat. When the content is low due to physiological and psychological stress in preslaughter animals, typically bulls, the ultimate pH is higher than normal, resulting in dark-cutting meat whose properties are not suited to table cuts. The pH of rigor meat (raw meat as we know it) can be measured with a pH probe, but if muscle is hot-boned and boxed before rigor is attained, pH measures are meaningless. The Rapid pH technology (Young and others 2004a, b) measures glycogen in small muscle samples by rapid enzymatic hydrolysis to glucose, and subsequent determination of glucose with a diabetic meter. The method is widely used on-line in New Zealand hot-boning beef abattoirs.

7.6.5 Cholesterol

Historically, the development of arterial disease was attributed to the consumption of cholesterol from foods like meat and eggs, and this belief spawned analytical methods that defined which foods were "healthy" and which were "unhealthy." Although other factors like overall diet are more important in the etiology, the analytical methods are still used to satisfy labeling legislation, and to keep the cholesterol boogeyman alive for marketing purposes.

Cholesterol is a lipid, and in meat it is determined by direct treatment of samples with potassium hydroxide in alcoholic solution followed by the extraction of cholesterol into nonpolar solvents, typically hexanes. The hydroxide hydrolyzes fats to the potassium salts of fatty acids (soaps), which are insoluble in the solvent (Kovacs and others 1979). The plant sterol stigmasterol, which does not occur in meat, is

added as an internal standard prior to the determination of cholesterol concentration using gas chromatography. This procedure does not require derivatization of the extract prior to chromatography.

7.6.6 Vitamins

Meat is a significant dietary source of some B group vitamins. Vitamins B_1 (thiamine), B_2 (riboflavin), niacin (plus niacinamide), and B_6 (pyridoxine, pyridoxal plus pyridoxamine) can be measured in a single hydrolysate obtained by acid digestion and multienzyme hydrolysis of meat samples. Automated chemical procedures similar to those in AOAC 942.23, 981.15, and 975.41 can be used to determine the first three vitamins, and vitamin B_6 can be determined by paired-ion high pressure liquid chromatography (Gregory and Feldstein 1985). Vitamin B_{12} (cyanocobalamin) can be determined by the radioassay method of Millar and others (1984).

Meat is not a significant dietary source of vitamin E, but this vitamin is important for color maintenance in raw meat. Vitamin E (tocopherol) supplementation of cattle feed is now common practice. In the method of Vitamin E determination by Pfalzgraf and others (1995), meat is saponified with potassium hydroxide in a methanolic solution. The tocopherol is extracted into an organic solvent, resolved by liquid chromatography and detected by fluorescence.

7.7 Common Tests for Meat Products

7.7.1 Salts

The salts contained in meat products include common salt (NaCl), phosphate(s), and nitrite and nitrate, of which all impart useful properties to processed meat products. Salt is used to partially solubilize myofibrillar proteins, resulting in a more rigid gel when the salted meat is cooked. Salt also lowers water activity (see below). Polyphosphate in the form of pyrophosphate is an analog of ATP, with a parallel ability to dissociate myosin in rigor contact with actin. This lowers the viscosity of raw emulsion sausage, so assisting physical processing. Phosphates generally function as antimicrobials and antioxidants by chelating free transition metals ions like iron. Nitrite, the active ingredient in meat curing, is the reduction ion of nitrate, and is also antimicrobial and antioxidative. The concentrations of these additions are often measured for legislative reasons stemming from real or perceived health concerns, and for quality control purposes. This section outlines methods in common use, and also describes three other common tests applied to meat products: water activity, gel characteristics, and fat oxidation.

Added salt is usually measured as the chloride ion through its reaction with silver ion added as silver nitrate to extracts of meat products. The Volhurd back-titration method determines excess silver ions resulting in a colored ferrithiocyanate complex. The now more favored direct titration method employs a silver ion-specific electrode. A standard salt solution is used to calibrate both methods, which assumes that the chloride's counter ion is sodium. This is true for most meat products but in some products a proportion of potassium chloride (KCl) is used in place of NaCl for dietary purposes. If the analyst is not aware of this substitution, the concentration of sodium ion will be overestimated.

The cation counter to phosphates is either sodium or potassium, and the various phosphates used contain different proportions of water. To overcome this complexity, phosphates are expressed as either elemental P or phosphorus pentoxide (P_2O_5). In the most common analytical method, meat products are ashed, then dissolved in acid solution where the phosphorus is present as simple phosphate rather than the polyphosphates common in meat products. The phosphate is reacted with an acidic solution of molybdic and vanadic acids to yield a colored complex that is determined colorimetrically. A primary standard represents a known weight of P or P_2O_5.

Nitrite, and indirectly nitrate, is used in meat as a curing agent, where nitric oxide forms a stable pink complex with the heme ring of myoglobin. In one method, extracted nitrite forms a diazonium salt with the primary amine sulfanilic acid. In turn, this salt is coupled to an aromatic amine to form a colored azo dye. Nitrate is unreactive in this system. To determine nitrate, it is reduced to nitrite with cadmium metal prior to the determination of nitrite. The original quantity of nitrate is then calculated by difference.

AOAC 935.48 describes a method in which the nitrites in meat are oxidized to nitrates with $KMnO_4$, and the nitrate concentration is determined after reaction with *m*-xylenol that yields a yellow pigment, nitroxylenol, which is recovered by distillation and determined spectrophotometrically. In this method, the original quantity of nitrite is calculated by difference. The basis of reporting the concentration of nitrite/nitrate depends on convention and regulatory requirements.

7.7.2 Water Activity

Water activity (a_w) is a measure of how much of the water in a product is "free," referring to the water that is not chemically or physically bound. Rephrased, the a_w of a product is the water content that makes itself "noticeable" externally. Moisture content, by contrast, refers to all water that can be evaporated at high temperature in an oven. a_w is formally defined as the ratio of water vapor pressure above the sample to the water vapor pressure of pure water at the same temperature. It is equal to the relative humidity of air in equilibrium with the product. Products with absolutely no free water have an a_w of 0.00 while pure water has an activity of 1.00. The a_w of a saturated salt solution (NaCl) is around 0.75. The ingredients present in meat products, like salt, sugars, and phosphates, result in a lower a_w for the product than for raw meat ($a_w \cong 0.97$). For many products, a reduced a_w is important for their preservation because microorganisms will not grow below certain a_w levels (Rahman and Labuza 2007) and these levels vary with the type of microorganism. There are several technologies available to determine a_w. The temperature range of interest is between ~2°C and 40°C, the range that shelf-stable foods might experience during storage. For the purpose of preservation, determining the a_w is more useful than determining the moisture content. Besides, a_w determinations take a matter of minutes whereas conventional moisture determinations can take many hours.

7.7.3 Rheology

Heating of meat and meat products causes muscle proteins to denature, which in the case of myosin is converted from the soluble into the gel state. This latter state is most familiar to the consumer as texture of cooked meat or emulsion sausage, which has perceived sensory characteristics like hardness, springiness, fracturability, and others, characteristics that are immensely important in defining the eating quality of meat or sausage. Two rheological measures that correlate well with sensory evaluation for these characteristics in emulsion sausage are stress and strain to the point of fracture. Stress has units of force per unit area whereas strain has no dimensions, being expressed as the increase in deformation as the gel is deformed by the stress. Of the several methods to obtain these data from meat sausage gels, the torsion test to fracture is particularly useful (Hamann 1983). The fracture data are often highly variable even from very homogeneous sausage gels, so many replicates are needed to get meaningful results.

7.7.4 Fat Oxidation

In meat products, spoilage arising from fat oxidation often occurs prior to microbial spoilage. Fat oxidation is manifest as the rancid odors and flavors of aldehydes, short-chain fatty acids, and other compounds that are generated from the peroxidation of fatty acids. Of the very many oxidation compounds generated, malondialdehyde and some others have the property of forming a pink complex with 2-thiobarbituric acid that can be measured with a spectrophotometer. The thiobarbituric acid reactive substance test exploits this property. In the original execution of the test (Tarladgis and others 1960), a sample of meat product is blended with water, acidified, then distilled to volatilize the oxidation products of which malondialdehyde is the most abundant reactive substance. An aliquot of the distillate is reacted with the acid, and the light absorbance at a suitable wavelength is related to the concentration of the reactive substances by a calibration curve based on malondialdehyde.

7.8 Noninvasive Physical Methods

Noninvasive physical methods to determine meat composition and properties are sought after alternatives to wet chemistry methods. The test results are available within minutes at most, labor cost is

minimal, and systems are more amenable to automation. They exploit parts of the electromagnetic spectrum from x-rays through ultraviolet and visible light, near infrared (NIR) to microwave and radio frequency. While faster than wet chemistry, physical methods typically have higher capital cost, thus favoring high-volume applications. However, wet chemical tests remain the cornerstone of analysis against which noninvasive physical methods must be calibrated.

The simplest physical method is visual inspection (AMSA 1991), and while useful for qualitative judgements of meat grade and fat color, precise, and accurate data cannot be easily extracted from mental images. Moreover, visible light hardly penetrates the meat, so judgements can be made only from surface-reflected light. However, inspection is useful in monitoring of, for example, meat browning on supermarket display, and detection of postrigor high-pH meat (dark cutting meat). Less light is reflected from high-pH meat, so it is distinctly darker. Despite the inherent limitations of surface inspection, the advent of digital imaging has expanded the possibilities of surface inspection, because images can be recorded under controlled conditions and used to reveal information of interest. This requires the application of chemometrics to image analysis to sort useful information from "noise." For example, image analysis has been applied to carcasses to predict lean meat yields (Hopkins and others 2004; Rius-Vilarrasa and others 2009).

Whereas visible light hardly penetrates the meat, light of longer wavelengths, like NIR, can penetrate below the surface to some extent. NIR spectroscopy in reflectance mode is of most interest in analysis of meat because it does not require direct contact with the sample. The light that penetrates the sample is either absorbed or reflected. The absorption is quantitative and depends on the chemical composition of the sample. Absorption results from changes in the vibrational mode of functional groups in the meat sample (e.g., $-OH$, $-NH_2$, $-CH_3$, etc.), which makes NIR suitable for quantitation of the chemical composition of meat. Thus, the FoodScan™ analyzer by Foss (Denmark) has received AOAC approval (AOAC 2007-04) for determination of moisture, fat, and protein in ground meat. The absorbance and reflectance data also allow for determination of meat properties other than composition (Xia and others 2007). Modern instruments with detection that is based on photodiode array allow measurements to be taken over a wide visible–NIR spectral range (350–2500 nm) in less than a minute, making it suitable for online monitoring in processing plants. In reflectance mode, an NIR spectrum is formed by intensities of reflected light measured in several wavelengths that results in a data set related to the functional groups of molecules and macromolecules present in the meat sample. As with analysis of reflected visible light, chemometrics are applied to extract meaningful meat property information from the data. Beyond proximate analysis determinations, other applications of NIR spectroscopy to date have been experimental, with a focus on predicted composition, eating quality, color, ultimate pH, and drip loss in beef (Liu and others 2003; Andres and others 2008; Prieto and others 2009; Rosenvold and others 2009) and pork (Hoving-Bolink and others 2005; Savenije and others 2006).

Extending the wavelength into the microwave and radio frequencies allows for much deeper penetration of radiation. Equipment such as NIFT-D™ (AgResearch Limited, New Zealand) employs a range of frequencies to resolve the confounding effects of varying fat contents on the intensity of the reflected radiation. One application of NIFT-D™ confirms that cartoned meat is frozen or otherwise, based on the principle that ice transmits certain frequencies while water absorbs them. In another application of NIFT-D™, the temperature of cartoned meat can be determined throughout the chilled and frozen range based on the different dielectric properties of lean and fat at various temperatures.

X-rays penetrate meat to the point that absorbance of radiation rather than reflection is the basis of quantization. Dual energy x-ray absorptiometry (DEXA) methods—which are the basis of baggage security checks—are based on the differential absorption of two energy bands in the x-ray spectrum by the material under test. In the case of meat, DEXA is used to determine the fat and lean content, where the variable of interest is the so-called chemical lean, that is, derived from wet chemistry methods that were described previously. Chemical lean is calculated as 100—fat content percent (see Proximate analysis) and is particularly important in pricing meat and further processing. The early 2000s saw much international research cooperation between the United States and New Zealand leading to the availability of the Eagle FA™ from Smiths Group (U.K.), while Foss (Denmark) has independently developed the MeatMaster™. Both are used to determine chemical lean in cartoned meat and meat on conveyers.

7.9 Tests Commonly Used in Meat Research

In the value chain from grower to retail or food service, some of the important measures are those previously described, principally moisture, protein, fat, and fatty acid profile. While these measures are often important to the researcher, other attributes of meat are also routinely evaluated. These include pH, color, fluid loss, tenderness, protein solubility and emulsifying capacity, and proteolysis (aging) of meat.

7.9.1 pH

The pH of meat has major effects on meat color, microbial stability, and eating quality. pH is usually measured with a sharp, probe-style electrode which, after calibration, is inserted into a meat cut to obtain a reading. In an earlier technique, a 1 g sample is excised from the meat and homogenized in 10 mL of water. The pH is then measured in the slurry. Method details are well described in meat science research papers and recommended procedures have been described by Honikel (1998).

7.9.2 Color

Meat color is measured for many reasons including grading and quantifying the kinetics of color change on display. Although there is some spectral reflectance from the surface of wet meat, the color of meat is mainly determined by diffuse reflectance arising from light reflected from just below the surface. Importantly, because meat is partially translucent, samples that are assessed must be thick enough to ensure that no light is reflected from the background. As with any assay, the measurement of meat color demands a systematic approach to data collection, whether color is scored by a sensory panel or measured by an instrument, usually a reflectance colorimeter.

In the case of panel assessment, the usual techniques of sensory evaluation are used—discrimination tests, intensity, and preference. Any of these tests is complicated by the heterogeneity of meat and the test environment. For example, panelists scoring the color acceptability of lean meat can be unconsciously influenced by the color of the associated fat. Recommendations for visual appraisal of meat are described by AMSA (1991).

Reflectance colorimeters are usually set to record color in L*, a*, b* color space (CIE 1978), where color is represented as a globe. A vertical axis through the globe represents a measure of light reflection or lightness (L*) where 0 equals perfectly black and reflects no light (south pole), and 100 equals perfectly white and reflects 100% of light (north pole). The colors of the rainbow can best be visualized as compass points at the equator, where L* equals 50 and thus represents gray. The orthogonal axes, a* and b*, which together can radiate from the polar axis anywhere between 0 and 100, represent the complementary colors red (positive) and green (negative), and yellow (positive) and blue (negative), respectively. The compass direction and length of the radiating line for any one color determination defines the color (the hue) and its saturation (the intensity of color).

7.9.3 Fluid Loss

Determination of fluid loss is important at all points in the value chain. No one wants to buy meat that will suffer a significant loss in weight during their ownership. Ideally, fluid should not be released from meat until it is eaten, but in reality fluid is progressively lost from the time of slaughter. The pattern of loss from slaughter to consumption depends on species, genetic predisposition to rapid glycolysis, meat ultimate pH, processing variables, packaging, and time.

Drip is measured by recording the loss in weight of a suspended meat sample (80–100 g) during a period of holding, say 24 h, at a controlled chill temperature (Honikel 1998). Water-holding capacity refers to fluid loss where a force beyond gravity is applied to the sample to extract the fluid. (The word "water" is not an accurate descriptor but is in common usage.) Kauffman and others (1986) compared a number of methods. In one, fluid loss is generated by centrifugation. In another, a meat sample is placed

on a filter paper disk and squeezed between two flat plates. The area dampened by the expressed fluid and the area occupied by the squashed meat are used to calculate the water-holding capacity. Purge is another measure of fluid loss, and is a term applied to fluid loss when meat is vacuum packed in barrier bags. Cook loss, sometimes also referred to as purge, is as the name indicates.

7.9.4 Tenderness of Meat

Tenderness is probably the most researched quality attribute of meat. It can be measured by a number of compressive, shear, and tensile methods after meat has been cooked in a defined way and cut to a standard size with defined grain orientation. Some reference methods for tenderness are described by Claus (1995) and Honikel (1998). Texture profile analysis is a popular method that can be applied to any solid food (Bourne 1978). The meat or meat product is cut to a defined thickness. A flat-ended probe twice penetrates or crushes the sample at a set velocity to a final depth of 80% of the thickness. The two roughly triangular force–time curves yield the hardness (maximum force in the first stroke), and other data such as springiness and cohesiveness.

7.9.5 Protein Solubility and Emulsifying Capacity

Protein solubility, also known as protein extractability, is often used as a measure of muscle protein denaturation that can occur during frozen storage, for example. Denatured proteins are generally less soluble than native forms. There is no standard method for measuring protein solubility but many methods follow those of Helander (1957). Low concentrations of KCl dissolve mainly sarcoplasmic proteins, while high concentrations dissolve that fraction plus myofibrillar proteins. Extracted proteins are determined by conventional nitrogen-based methods (see earlier) or ultraviolet–visible spectroscopy.

The ability of muscle proteins to emulsify fat in comminuted meat products is an important quality. In general, a higher emulsification capacity indicates greater functionality in comminuted meat products. In the method of Swift and others (1961), liquid vegetable fat is progressively introduced to a salt-extracted solution of meat proteins continuously blended at high speed. When the oil-in-water emulsion can hold no more oil, the emulsion collapses to a water-in-oil state with a marked drop in viscosity. The quantity of oil required to reach this point is the emulsifying capacity. Several variations of the method have been devised, including the emulsion activity index proposed by Pearce and Kinsella (1978).

7.9.6 Proteolysis of Meat

Aging of meat is caused by proteolysis of selected meat proteins (Young and others 1980) that leads to increased tenderness. This phenomenon can be conveniently monitored by gel electrophoresis (Laemmli 1970) in the presence of a reducing agent like dithiothreitol and sodium dodecylsulfate that together break secondary protein structure, rendering proteins as negatively charged rods. Extent of migration in an electric field is related to molecular mass. This method is very well suited to the study of meat proteins at the molecular level. At the level of muscle structure, progressive proteolysis can be visualized as an increase in myofibrillar fragmentation of meat mechanically dispersed in a buffer, where the contiguous sarcomeric structure progressively breaks into oligomers and monomers of sarcomeres. Progress can be quantified by methods including phase contrast light microscopy (Takahashi and others 1967) and turbidity of samples in a spectrophotometer (Olson and others 1976). Researchers have refined and extended these methods in more recent years (see e.g., Karumendu and others (2009)).

7.10 Substitution, Species and Animal Identification, and Irradiation

7.10.1 Substitution and Species Identification

For high-value foods like fish and meat, species substitution for illicit financial gain is probably as old as commerce itself. Substitution is relatively easy because raw muscle tissue from different species is often

similar in appearance and in functional properties, and once incorporated in a comminuted product, identification based on appearance is lost.

At another level, declared or implied meat ingredients can be substituted with cheaper ingredients. These cheaper ingredients are either from the species declared (typically offals, blood, connective tissue) or are nonmeat ingredients. Sometimes, these practices break no laws but in the longer term condemn the product to a lower price category because eating quality is reduced. In many countries, however, meat products like sausages have zero tolerance for certain offals, and minimum/maximum limits for fat, lean meat, collagen, salt, added carbohydrates, and substitute proteins from plant or dairy sources. These ingredients in meat products are therefore often monitored. Ingredient substitution can also generate religious concerns. For example, the simultaneous consumption of dairy and meat items is prohibited for strict Judaics.

Religious strictures, perceived or real-health concerns, and cultural preferences are the main drivers of species identification for consumer protection. All methods to determine species are based on biochemistry because qualitative and quantitative biochemical traits set all individuals apart. Whatever marker is chosen, it must ideally show low variation within a species and higher variation between species. If the four targets in Figure 7.1 represent four species, distinguishing between the two top species (a and b) would be easier and more reliable than between the lower 2 (c and d). Comparisons between species thus involve statistical issues of accuracy and precision.

Methods to determine species have kept pace with biotechnology. Prior to the 1990s, the main methods of species identification were based on protein properties (Johnson and others 1986; Barai and others 1992), but DNA-based methods have dominated since the 1990s. Proteins are extracted from the meat or meat products with solvents, and subjected to electrophoretic or immunological analysis. Electrophoretic methods for proteins rely on differences in molecular weight and charge to generate patterns on gels that can sometimes distinguish species. The differences are often subtle, however, and immunology of proteins offers greater specificity if a unique antibody can be generated for a species. Basic immunological methods rely on diffusing the extracted proteins of interest on a gel against antibodies to proteins from a number of potential species. A white precipitate band on the gel indicates recognition of antigen of one protein in the extract for its corresponding antibody, thus identifying the species. The enzyme-linked immunosorbent assay technique is a refinement of the basic method, where an enzyme—usually peroxidase—is covalently linked to the antibody. The enzyme amplifies the precipitin complex by generating many more chemical equivalents of (colored) reaction product than are inherent in the precipitin band.

A major problem with protein-based methods is that protein expression can vary with factors such as tissue type, age, and physiological condition, and the methods do not work well with cooked meat. On heating, proteins tend to become insoluble, which means that they are difficult to extract and analyze on gels. Moreover, immunological methods are based on shape recognition, and the native shape of proteins is often lost when heated to cook temperatures.

Other non-DNA-based methods of detecting species substitution include amino acid and peptide composition (Plowman and Close 1988), a fatty acid unique to pork (Sawaya and others 1990), and branched-chain fatty acids common in goat and sheep but insignificant in other species (Wong and others 1975). However, methods to determine these defining amino acid and fatty acid profiles are complex and the results are equivocal. Unequivocal identification can, however, be determined from the genome (Teletchea and others 2005).

Whereas protein expression can vary between individuals within species, the genome is more static. Both nuclear and mitochondrial DNA can be used for species identification, but for a number of reasons the latter is favored. The gene for mitochondrial cytochrome *b* is the most common gene exploited for species identification, because it shows low variation within a species, as represented in Figure 7.1 where targets a and b represent the cytochrome *b* gene in two species.

As a chemical, DNA is also more stable to heat than proteins. It can be extracted from most tissues, even bone, and from cooked products even after retort cooking. To identify a species, the DNA is first chemically extracted from the sample in question. The next step is application of the simple but elegant technique of polymerase chain reaction (PCR), which employs temperature cycling of extracted DNA in solution with the so-called DNA primers. Primers are short sequences of single-stranded DNA that are complementary to the start and the end of a DNA sequence of interest within the extract. They are added

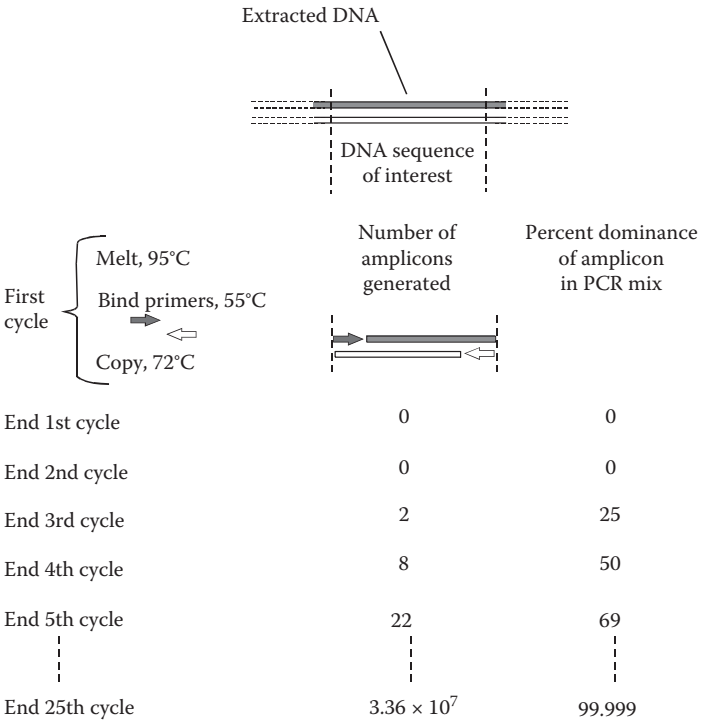

FIGURE 7.2 An outline of PCR. The extracted DNA is subjected to multiple cycles of melting, primer binding, and copying by DNA polymerase. Millions of copies of a particular DNA sequence—the amplicon—eventually dominate the PCR mix.

in excess to the DNA extract along with a DNA polymerase and an excess of the four nucleotides. For PCR, the extracted DNA in the now complex mixture is first made single-stranded by heating to 95°C (Figure 7.2).

When cooled to 55°C, the two primers respectively bind to the complementary strands of the particular DNA that the primers recognize. The temperature is then increased to 72°C, at which point the DNA sequence of interest, including and between the primers plus any adjacent DNA, is copied by the polymerase. By cycling through these temperatures, multiple copies of the selected sequence (called an amplicon) are produced in the manner of a chain reaction. The multiply-replicated amplicon soon completely dominates the PCR mixture (Figure 7.2), and can be analyzed in a number of ways to differentiate one species from another.

The amplicon is the basis of species identification. The most common way to identify a species is by gel electrophoresis, which resolves any generated amplicon according to molecular mass. If, for example, primers for pork DNA are included in a DNA extract of beef sausage and no amplicon is generated, then the sausage is free of pork; if an amplicon is generated that has the predicted molecular mass, then the sausage almost certainly contains pork. Electrophoretic methods are in common use in species identification analysis (Bartlett and Davidson 1992) and, along with two other DNA methods, dominate DNA methods with food.

In the conceptually simple method of forensically informative nucleotide sequencing (FINS) (Liu and Cordes 2004), the nucleotide sequence of the amplicon is compared to known sequences in a database. For example, the DNA sequence for the mitochondrial cytochrome *b* gene is known for many species. A variation of FINS is to fragment the amplicon with specific nucleases that result in different-sized fragments that yield a molecular mass pattern on an electrophoretic gel. This is called restriction

fragment-length polymorphism (Lockley and Bardsley 2000), and is analogous to peptide mapping of proteins (Cleveland and others 1977).

In fluorescence-linked DNA methods, generation of the amplicon is accompanied by the release of a fluorescent marker molecule into the temperature-cycling PCR mixture. With each temperature cycle step, more fluorescence is recorded. Using this technology, Laube and others (2007) and Kesmen and others (2009) differentiated many common animal species in raw and processed foods. The beauty of using fluorescence-based methods is that the amount of fluorescence can be measured quantitatively, which cannot be done with any accuracy using electrophoretic techniques. Thus the extent of pork contamination in a beef sausage could be determined, rather than just presence or absence.

DNA-based methods of fraud detection are unquestionably the best technologies, but these technologies can also be expensive. Much research is directed at reducing cost and making rapid techniques applicable to a food environment, rather than a dedicated laboratory, and using relatively unskilled labor.

7.10.2 Detection of Irradiated Meat

γ-Irradiation is a proven way of reducing microbial contamination of foods (Tritsch 2000), while causing minimal chemical change to the tissue compared with most other preservation methods. For reasons linked to the horror of nuclear weapons and accidents, there is widespread public concern about irradiation. While much of the concern is emotional, consumers in democracies maintain they have a right to know if their food has been irradiated, and in many cases this belief has been translated into law. In turn, such laws have spawned tests designed to detect irradiation. For any food, an unequivocal test would be the demonstration of a unique irradiation-generated (radiolytic) compound(s). This has proved difficult because the changes wrought in food due to irradiation are mediated by free radical mechanisms that occur naturally in raw foods, although usually more slowly. Meat contains about 80% water and a legal dose of γ-radiation generates about 5 mmol of free radicals per kilogram of food (Swallow 1988). These include superoxide and hydroxy radicals. These primary radicals are highly reactive and in the aqueous environment of meat combine with fats and proteins to generate secondary radicals, and downstream degradation products. Few if any degradation products are unique radiolytic products. Moreover, as detection limits drop due to improvements in analytical techniques, compounds once thought to be unique to irradiated foods are found to be present naturally.

In solid material like bone, some free radicals last for weeks rather than fractions of a second (Dodd and others 1988), and this longevity has been exploited with physical methods like electron spin resonance and applied to bone-in meats (Yang and others 1999; Chawla and Thomas 2004). In the aqueous environment of meat, where free radicals are short-lived, analysis of volatile hydrocarbons has been useful to detect prior irradiation. Radiolysis of fatty acids generates unusually high quantities of alkenes. Linoleic acid for instance gives rise to heptadecadiene and hexadecatriene (Kavalam and Nawar 1969; Nawar and others 1990). Another family of fat oxidation products, the cyclobutanones, are unique markers of irradiation (Crone and others 1992) and can be applied to a variety of fatty meats and other fatty foods (Horvatovich and others 2005; Priyadarshini and others 2005). In blind tests on irradiated chicken, Stevenson and others (1990) successfully identified irradiated samples through hydrocarbon analysis.

The so-called DNA comet assay relies on the fact that large DNA molecules are particularly sensitive to fragmentation by ionizing radiation, which indeed is one reason why microorganisms in food are killed by irradiation. The DNA in single cells is lysed in agar and subjected to a weak electric field. DNA fragmented by irradiation migrates as a comet-tail-like smear and this is detected by acridine orange staining (Ostling and Johanson 1984; Cerda and others 1993). It must be emphasized that the method is only presumptive of irradiation. For example, freeze/thaw abuse and longer-term storage of meat also result in DNA fragmentation. Likewise, cooking also fragments DNA.

Of several biological methods, the direct epifluorescent filter technique to aerobic plate count ratio (DEFT/APC) is the most useful (Betts and others 1988; Betts 1991). The basis of this method is as follows: irradiation does not reduce the fluorescence due to acridine orange staining of DNA in dead and live microbes isolated by filtration from meat, while the aerobic plate count due to live microbes is

markedly reduced through irradiation. A high ratio therefore indicates irradiation. The test is again presumptive, because there can be other causes of an unusual microbiological profile.

7.11 Laboratory Management

7.11.1 Costing Analyses, Calculating, and Reporting Results

Estimating costs is often difficult as each step in an analytical method has inherent error in costs of labor and materials. Moreover, costs are usually sensitive to the number of samples to be analyzed. In developing a useful costing system, a typical starting point would be a brief description of each of the steps for a particular analysis. The steps usually extend from sample receipt to presentation of the results, and must include early and late steps such as handling samples on receipt and cleaning up. Factors affecting each of these steps and their interactions need to be specified. This written description is then translated into equations for a spreadsheet. When the analyst is satisfied with the costing spreadsheet, cells are often protected to prevent under- or overcharging.

As a component of laboratory information management systems, spreadsheets are also used to calculate results. Again key cells are protected.

It is important to present analytical results in a form that the client understands and finds useful. In most cases, the value reported is some fraction (e.g., percent, parts per million, mmol kg^{-1}) of the sample as received, although this should not be assumed. The basis of presentation and units required should be established with the client at the outset. For many clients, the word "average" is readily understood but not so its synonym "mean." Whereas standard deviations are sometimes required for legal purposes, the expression often has no meaning to meat industry personnel. As an alternative, the spread of values can be shown by quoting the highest and lowest value, or by expressions that state what fraction of values lie between x and y on either side of mean z. The format of reports should be examined for clarity and readability. Above all, the key to good reporting is understanding the client's needs.

7.11.2 Quality Systems

When the analyst, and later the client, views a set of results that confirms preconceptions, analyst and client satisfaction are guaranteed. However, when results are at variance with preconceptions, problems arise unless the laboratory has a quality system in place. A quality system gives confidence that the results—no matter how unexpected or unwelcome—are believable.

A quality system is a formal way of minimizing the random and systematic error arising from equipment and procedures. To cite an example, a quality system for the determination of moisture might require that the temperature of the drying oven was always within a specified range. Every day, week, or other period (depending on the anticipated variability and the importance of the determination), the temperature of the oven is measured with a thermometer whose precision and accuracy can be traced through periodic audit to a national standard. The oven temperatures are recorded and monitored for trends. Likewise, the laboratory balances are calibrated routinely, usually by a certified agency with its own quality system. In quality systems, it is important that precision and accuracy are both controlled. When precision is controlled but not accuracy, the analyst could be justifiably accused of producing junk data, but happily consistent junk (Figure 7.1a).

Quality systems are tailored to the needs of the laboratory and its clients. The most basic of quality systems demands at least routine calibration of balances. In many cases, only some methods are fully monitored. However, systems are sometimes needed that demand fully calibrated glassware and routine inter and intra-laboratory comparisons. Thus a single batch of finely ground meat might be vacuum packed in small batches and stored at –35°C to be used as a reference standard for proximate analysis between and within laboratories. As with all systems, a sensible trade-off between benefit and cost must be struck. A common criticism of quality systems is that they are time and paper wasters. This appears true when things are going smoothly, but when results are unusual the analyst can look to the checks and balances of a quality system and sign the reports with confidence.

7.12 The Future of Analytical Methods for Meat

Measurement is fundamental to any technology, and in the case of meat science applications, the measurement of protein and fat, and in turn, amino acids and fatty acids, has been a cornerstone. In terms of human nutrition these are the macronutrients of meat, and the need for their future analysis is assured. Methods for their determination will evolve with an emphasis on increased speed and decreased cost, but without loss of precision or accuracy. As for the micronutrients of meat, like minerals and vitamins, some will be of enduring interest while others, like rare fatty acids and cholesterol, may cease to be of analytical interest as the concerns of societies move to fresh issues or obsessions. If and when irradiation of meat products becomes common, interest in its detection may wane. In contrast, guarantee of origin through traceability, and guarantee of species is likely to receive increased attention as nucleic acid-based methods reduce in price. Enduring concerns based on religion, and current societal concerns for animal welfare, environmental damage, food safety, and what we eat in general, will be powerful drivers of these DNA technologies.

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8

Recent Advances in Meat Quality Assessment

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8.1 Introduction

The meat sector, particularly the beef sector, has to contend with a major challenge due to the broad-ranging variability of raw material, which ultimately translates into high variability in product quality and low process control over the commercialized end product.

The meat industry needs reliable meat quality information throughout the production process in order to guarantee high-quality meat products for consumers.

From the consumer perspective, quality is tied to "functional" characteristics that range from sensory-domain taste and appearance properties to cost-domain storage and distribution criteria. The baseline characteristics concern food content in terms of nutrients, agrochemicals, heavy metals, pathogenic microorganisms, and any other substances that carry potential health risks. All of these criteria can be stratified into quality criteria indexed to physical properties that can be objectively quantified through

TABLE 8.1

Methods for Gaining Access to Meat Characteristics

Meat Quality	Quality Parameter	Target	Analytical Technique
Functional characteristics	Sensory characteristics	Dynamic properties (tenderness, toughness, etc.)	Mechanical, ultrasound, magnetic resonance elastography, electrical impedance, microwaves, front-face fluorescence spectroscopy
		Physical and structural aspects (texture, porosity, hardness, crispiness, viscosity, physical state of component ingredients, etc.)	Image analysis, thermography, thermo-acoustic analysis, etc.
		Composition	Ultrasound, image analysis, electrical impedance, microwaves, x-ray, infrared (IR), ultraviolet (UV)
		Appearance and morphological aspects (color, shape, size, etc.)	Image analysis, etc.
		Freshness	Electrical impedance, microwaves, electronic nose, NMR (nuclear magnetic resonance), front-face fluorescence spectroscopy, biochemical
	Chemical composition	Original ingredients, desired or undesired compounds formed during processing and storage	Dual x-ray energy imaging, IR, NMR, etc.
	Physicochemical properties	Water activity, pH, redox potential, etc,	Microwaves, impedance, NMR
	Health-protecting properties	Fiber content, antioxidant activity, probiotic bacteria, etc.	UV light, visible light, fluorescence, etc.
Basic characteristics	Nutritional characteristics	Micro- and macronutrients (protein, fat, amino acid, fatty acid, organic acid, vitamins, minerals)	Dual x-ray energy imaging, IR, NMR,. . .
	Safety	Hazardous materials (bones, wood, metal, glass, etc.)	Ultrasound, x-ray, image fluorescence
		Mineral contaminants (earth, metals, mineral oils, etc.)	X-ray phase contrast imaging, thermography, optical techniques, etc.
		Chemical contaminants (dioxin, acrylamide, mycotoxins, prions, etc.)	UV light, etc.
		Biological contaminants (parasites, microorganisms, virus, etc.)	UV light, visible light, x-ray imaging, image fusion, and image processing.
		Environmental contamination	Dual x-ray energy imaging, IR, NMR, etc.

physical measurements (Table 8.1). These measurements may be generated using cost-intensive high-tech instruments employed at cutting-edge labs, but oftentimes they can be produced using simple probes derived from these lab techniques. Industry agents outfitted with this kind of probe could gain tighter process control over these qualities and thus provide consumers with certified product quality assurances.

8.2 Sensory Characteristics

8.2.1 Dynamic Properties

The main criticism voiced by consumers concerns excessive variability in meat tenderness—especially regarding beef. This makes tenderness one of the most important quality factors to be characterized, not just for consumers but equally for industry players.

Meat tenderness, or indeed its corollary toughness, is essentially shaped by the quantity ratios and distribution patterns of two key muscle components: myofibrillar proteins and connective tissue.

8.2.1.1 Instrumental Methods

The most traditional measurement methods employed attempt to reproduce and measure the (compressive and shear) forces produced during chewing. There has been extensive research into Warner-Bratzler shear force (WBSF) procedures and how to interpret the results, although no clear consensus has been reached yet, WBSF testing remains the benchmark reference technique (Lorenzen et al. 2010). The same standardization issues plague the compression testing devices used to differentiate myofibrillar component impacts from collagenic component impacts on meat toughness (Lepetit and Hamel 1998). The major drawback of these shear strength and compression measurements is that they require destructive tests—tests that are not just time consuming but also tied to the lab. However, there are a handful of portable apparatuses available: the modified "Armor Tenderometer" (Timm et al. 2003), with six sharp needles, which gives pretty good predictive results on raw-meat toughness when compared to trained sensory panel (Stephens et al. 2004), the "G2 Tenderometer" from Carne Technologies Ltd. (New Zealand), which is based around a AgResearch MIRINZ (New Zealand research Institute) shearhead and the Tendertec mechanical penetrometer developed by the Australian Meat Research Corporation (Stephens et al. 2004), which gives a good correlation with meat toughness (Belk et al. 2001).

8.2.1.2 Recent Advancements

New methods of determining meat tenderness have recently been developed, some of which have derived their inspiration from techniques and hardware used in biomedicine.

Bioimpedance, ultrasound, x-ray scanning, and NMR/magnetic resonance imaging (MRI) are the prime examples.

Exploiting the fact that in-muscle proteolysis increases over the aging period, translating into changes in amino acid concentrations, high-resolution NMR spectrometry has been used to analyze beef samples by characterizing the changes in amino acids, nucleotides, and sugars during postmortem beef aging (Graham et al. 2010), while (Bertram et al. 2010) used high-resolution ^{1}H NMR (proton nuclear magnetic resonance) to investigate the relationship between stress, metabolite profiles, and water-holding capacity (WHC) in pigs.

Bioimpedance is used in a broad range of applications. It has a long history in characterizing biological tissue properties and proven particularly successful in assessing age-related meat tenderness (Faure et al. 1972; Lepetit et al. 2002). However, Byrne et al. (2000) reported that straightforward electrical measurements cannot be linked to WBSF-assessed tenderness, arguing that the amount of conjunctive tissue inducing the meat toughness cannot be assessed by electrical measurements due to overlap between myofiber and conjunctive tissue. Electrical impedance-based techniques for measuring tenderness therefore focus more on exploiting the potential of measuring the state of muscle fiber and shifts in its anisotropy (Castro-Giraldez et al. 2010). As meat ages, it tends to lose anisotropy, and there are studies and new instruments making it possible to spotlight this phenomenon and thus to characterize this myofibril profile-related anisotropy (Lepetit et al. 2007). One promising way forward is to use low-intensity polarized microwave measurement systems that also exploit this drop in anisotropy caused by time-course-related changes in state of muscle fiber (Clerjon and Damez 2009) but with the added advantage of enabling noninvasive zero-contact measures.

Biological tissues behave like viscoelastic materials, that is, presenting both fluid viscosity and solid elasticity properties. Since acoustic wave propagation is directly linked to these mechanical properties, following the through-tissue propagation of acoustic waves could be a solution for measuring viscoelastic properties of meat tissues.

Furthermore, acoustically propagated mechanical wave excitation of shear strain coupled with a nondestructive MRI technique (magnetic resonance elastography: MRE), optical coherence tomography, or transient elastography makes it possible to measure the localized viscoelastic properties such as tensile modulus (Gao et al. 1996). These techniques, which are used in medical imaging to detect breast tumors

or liver cancer, have recently been transferred over to studies on the viscoelastic properties of meat (Catheline et al. 2004).

8.2.2 Physical and Structural Aspects

As highlighted in Monin (1998), appearance is one of the main traits determining purchase decision and acceptability of meat. Appearance gives consumers signs of product condition, freshness (possibly through its color), taste (visibility and distribution of fatty tissue), and tenderness (amount and distribution of fat and connective tissues).

8.2.2.1 Macroscopic Imaging

Appearance can be determined by computer vision and image processing which, combined, can consistently characterize complex color, texture, and geometrical properties. Information delivered by image analysis can indirectly explain viscoelastic and other characteristics, and overlaying image texture features onto color and marbling features could be an efficient tool for tenderness prediction (El Jabri et al. 2010). Statistical processing is often a requisite step toward establishing correlations between the perceived and experienced sensory properties of muscle tissue. Tian et al. (2005) showed that a significant proportion of meat tenderness variability could be explained by image texture features analyzed by multiple linear regression, while Chandraratne et al. (2006) investigated the value of raw-meat surface characteristics (geometric and texture) in predicting cooked-meat tenderness using nonlinear regression and artificial neural network analyses. Li et al. (2001) used wavelet-based decomposition to extract features of fresh beef images and classify steaks into tough and tender groups. In a recent paper, Naganathan et al. (2008) developed and tested a visible/near-infrared hyperspectral imaging system to predict beef tenderness at 14 days postmortem.

8.2.2.2 X-Ray Measurements

Fat content and the intramuscular visual appearance of lipid structures (marbling) constitute an important factor for consumers. As highlighted by Swatland (1995), video image analysis could easily be used for meat cut quantification and could potentially be automated for in-line use. Pearce et al. (2009) showed that dual-energy x-ray absorption (DXA) is an accurate tool for measuring sheep carcass composition in both live animals and carcasses. As reported by Pearce et al. (2009), although DXA is too slow for commercial use, it may be used as a reference method in carcass composition studies. Another potentially valuable method is x-ray-computed tomography, which has given very accurate predictions of cut composition, intramuscular fat content, and fatty acid in primal beef cuts (Prieto et al. 2010), but is far less accurate for technological and sensory traits.

8.2.2.3 NMR Spectroscopy for WHC Assessment

Looking at meat appearance, especially pork, but not excluding beef and veal, WHC is a critical factor. Meat presenting defects tied to texture, color, and firmness (consistency), such as pale-soft-exudative (PSE) meat or dark-firm-dry (DFD) meat, correlate to low WHC and high WHC, respectively. Traditional measurements of WHC, such as drip, absorption by paper strip, filter pair, and centrifugation, are still in widespread use despite all their intrinsic known defaults. Fortunately, recently developed physical methods are providing deeper insight into the biological and mechanical events induced (postmortem myofibrillar shrinkage, water exchange in extracellular/intracellular compartment water exchange, and, finally, water expulsion) as a step toward curtailing the incidence of PSE or DFD defects (Monin 1998). Extending beyond a handful of techniques based on imaging, x-ray diffraction (Swatland et al. 1989), IR spectroscopy, and even Raman spectroscopy (Pedersen et al. 2003), which look for structural changes at cellular scale, low-field NMR remains the technique with the brightest future (Straadt et al. 2007). Bertram and Andersen (2007) concluded that proton NMR relaxation is a very powerful tool for determining WHC as it gives a direct measure of the proportion of in-meat water liable to be lost as drip. Recent studies

investigating WHC, water compartmentalization, and structural changes during the aging of meat samples have used NMR as a routine method (Stadnik et al. 2008).

8.2.2.4 Microscopic Imaging

As stated earlier, perception of meat tenderness is primarily shaped by the state, behavior, and distribution (structural organization) of two core components: collagen and myofibers. These tissues can be analyzed at cellular level, and their state studied. The histochemical properties of a muscle, such as fiber-type composition, fiber area, oxidative and glycolytic potential, and glycogen and lipid contents are all factors that have been found to influence meat quality. Histochemistry has been combined with several other staining protocols such as Sudan black-B, myosin ATPase, or NADH-tetrazolium reductase to classify fiber groups.

Immunohistochemical labeling is more selective, as it employs antibodies to visualize specific proteins and lipids. The secondary antibody is labeled with an enzyme or a fluorescent component. Several immunoenzymology applications have focused on collagen architecture and collagen typing in pork (Nakamura et al. 2003) and poultry (Oshima et al. 2007; Roy et al. 2006). Astruc et al. (2007) localized oxidized proteins in muscle to pinpoint the role of membrane proteins in oxidation. The occurrence of fast and slow myosin isoforms in fiber subtypes was also detected by immunohistochemistry in pigs (Fiedler et al. 1999, 2004).

When antibodies are labeled with fluorescent dyes, we are working with immunofluorescence—a common technique for visualizing the subcellular distribution of biomolecules of interest. Immunofluorescence-labeled tissue sections observed under a fluorescence microscope or by confocal microscopy (Nakamura et al. 2007) have been successfully used to study changes in bovine connective tissue according to animal feed (concentrate- and roughage-fed groups). The protocol uses confocal laser scanning microscopy coupled with immunohistochemical typing of collagen and assessment of protein structure in the perimysium and endomysium.

8.2.2.5 MRI for Fiber Typing and Studies on the Spatial Organization

Refocusing on fiber-type characterization (type I: slow-twitch oxidative; type IIa: fast-twitch oxidative glycolytic; type IIb: fast-twitch glycolytic), MRI offers elegant perspectives, looking at T_1 and T_2 values (Adzamli et al. 1989; Lerumeur et al. 1987), where T_2 can differentiate type I fibers from others with slightly higher values. *In vivo* studies on rabbits highlighted a higher T_2 for type-I-fiber muscles (Bonny et al. 1998), which the authors suggested was due to water structuration in muscle fibers, fat content, and myoglobin state.

Moreover, Sifre et al. (2005) investigated the spatial organization of the perimysium in beef meat using a combined histology and MRI approach. The technique provided complementary microscopic and macroscopic datasets on intramuscular conjunctive tissue structures, both of which are necessary for predicting sensory tenderness.

8.3 Nutritional Characteristics

Nutritional characteristics are high on the list of consumer interests. Here, we describe some of the more advanced methods for assessing the main positive or negative nutritional aspects of meat products.

8.3.1 Fat Content

Fat is considered a major factor in aggravating health risks such as coronary heart disease, cancer, diabetes, and hypertension. Fat content control is thus a highly relevant aspect of meat quality control, and again, recent advances in this field will be given in the following section.

8.3.1.1 X-Ray Measurements

The principle of x-ray is to obtain a measurement of the attenuation of penetrating energy. Different materials have different attenuation properties, and so depending on the level of penetrating energy delivered, it should be possible to obtain quantitative measurements—particularly for bone, lean meat,

and fat. A broad panel of technology tools have been developed using x-ray beams at different energy levels, making it possible to discriminate fat, bone, and lean meat according to the energy attenuation measured. The meat industry has been using low-energy x-ray systems like the Amyl-Ray system (The Kartridg Pak Co., Iowa) for well over 30 years now (Gordon 1973).

A novel x-ray technology dubbed DXA offers useful capabilities for meat fat assessment. Absorption at low x-ray energies (e.g., 62 keV) is dependent on both fat content and sample density, while absorption at higher energies (e.g., 120 keV) is essentially dependent on density only. Superposing the two measurements and subtracting one from another gives the fat content (Brienne et al. 2001; Hansen et al. 2003) with very good accuracy compared to chemical analysis (R^2 values from 0.7 to 0.97). As reported by Brienne et al. (2001) and Mercier et al. (2006), although DXA is too slow for commercial use, it may be used as a reference method in carcass composition studies.

8.3.1.2 NMR Spectroscopy and MRI

Studies by Foucat et al. (1997) and Renou et al. (1985) also provide insightful examples of meat fat content measurement using NMR spectroscopy.

Fat was quantified in ground beef by NMR (Foucat et al. 1997) and the results showed excellent correlation ($R^2 = 0.992$) with the Soxhlet method. For the test range of 5–15% fat content, actual fat content was determined with very good accuracy, making it possible to characterize the connective tissue structure of the perimysium (Figure 8.1) (Bonny et al. 2001; Laurent et al. 2000).

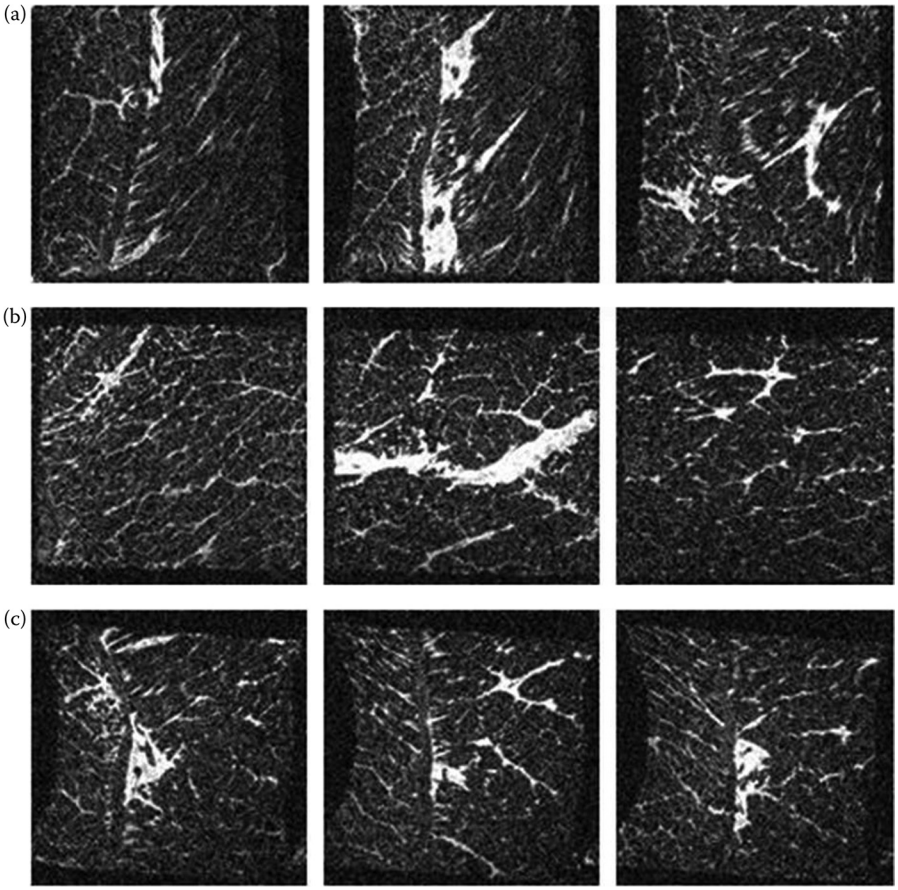

FIGURE 8.1 Successive planes obtained with MRI of the infraspinatus bovine muscle in three orthogonal incidences (a–c).

NMR microimaging on meat samples can be used to quantitatively characterize lipids. Fat content analysis by NMR involved selecting a sequence of impulses (Inversion-Recovery, Spin Echo, or others) to be applied to the product. The results highlight the versatility and practicability of the technique, since the equipment involved is compact and the method can be equally well deployed for controlling food composition as for checking food quality.

Moreover, diffusion-weighted imaging of muscle is another MRI method which makes use of the large difference between apparent diffusion coefficients of myofibers and lipids (intramuscular fat and frying oil). Using a sufficiently large *b*-value, the water signal of is attenuated so that only the lipid signal becomes detectable (Clerjon et al. 2010; Clerjon and Bonny 2011).

8.3.1.3 Ultrasound

Analyzing the acoustic parameters of waves propagating in a medium makes it possible to assess and characterize the propagation medium. Studies discriminating muscle samples on the basis of fat and collagen contents have reported better results than those obtained by straightforward analysis of chemical and mechanical properties (Abouelkaram et al. 1992, 2000; Morlein et al. 2005). Fat content has been reported to be correlated with ultrasound propagation speed, with fat and lean muscle showing reverse temperature dependencies on sound velocity (Abouelkaram et al. 2000; Benedito et al. 2001).

8.3.1.4 Optical Spectroscopies

Before being investigated in food science, Raman spectroscopy had been widely used in biomedical applications where it is well known for its ability to determine the degree of saturation in fatty acids—a highly significant aspect of nutrition. Furthermore, it has been shown that Raman spectroscopy provides structural information on the lipid changes (Beattie et al. 2006) that occur in muscle food during spoilage. This spectroscopy technique offers several advantages over traditional methods, since it is a direct and noninvasive technique that requires only small sample portions.

In the food science field, Marangoni (1992) used polarization fluorescence spectroscopy to determine microviscosity and structural order in complex lipid systems.

Rapid nondestructive methods to determine fatty acid composition are also being investigated as a solution for assaying the saturated, monounsaturated, and polyunsaturated fatty acids that influence human health. Near-infrared reflectance spectroscopy (NIRS) is used to rapidly evaluate fatty acid composition in dry-cured meat product (Fernandez-Cabanas et al. 2011) and is close to deployment at industrial level (Pérez-Juan et al. 2010).

8.3.1.5 Macroscopic and Microscopic Imaging

In meat science, image analysis consists in analyzing the texture of images produced from muscle and meat sections at one or more wavelengths. This imaging process makes it possible to clearly highlight the collagen and lipid structures of muscular tissue. Marbling score, which is a measurement of fat distribution density in the rib–eye region, is considered a texture pattern.

Multispectral image analysis on images acquired at different wavelengths in the UV–visible range was used to correlate meat components with sensory and physical properties, giving good results in terms of predicting lipid content ($R^2 = 0.87$) (Abouelkaram et al. 2006). The two key components of connective tissue—that is, fat and collagen—vary according to muscle, breed, and age, and both are clearly visible on photographic images.

Histology can be used to enhance lipid imaging: for instance, Thakur et al. (2003) worked on the effect of composition and deposition of Sudan dye-stained lipids on fish meat texture.

8.3.1.6 Impedance Measurements

Many studies conducted since the 1980s have attempted to use electrical properties to estimate fat content in animal carcasses or meat. As fat is an electrical insulator, it influences tissue impedance. Electric

impedance methods can obtain remarkable results. A simple electric conductivity measurement on a carcass immediately after slaughter can be associated with anatomical data to give fat content with remarkable accuracy ($R^2 = 0.95$). This could be explained by the fact that there are no membrane or extracellular compartment modifications occurring immediately after slaughter, and the measurements are made at a stable temperature. A patented electrical impedance system has been developed (Madsen et al. 1999) for measuring fat content in muscle. This portable apparatus uses electrodes inserted in the muscle, and fat content is estimated via measurements made at several frequencies. Measurements of fat content after rigor are not consistent, as impedance in this case is also influenced by membrane state.

8.3.2 Salt Content

In many industrialized countries, sodium intake is way over nutritional recommendations. Excessive sodium intake has been linked to hypertension and identified as a major risk factor for cardiovascular disease and osteoporosis (Afssa 2002). Salt content control is thus a priority for meat product manufacturers, creating a critical need for salt content and measurements.

Current methods for salt content measurement are time consuming, expensive, and sample destructive, and often require skilled personnel. These techniques are led by the hydrolysis-titration and electrochemical method described in detail by the Association of Official Analytical Chemists (AOAC) (1995), a chemical test kit (Huang et al. 2002) and a conductivity method (saltmeter) (Sarosi et al. 1989).

Other novel techniques have been proposed over the last few years and applied to different foodstuffs. Examples include NIRS (Ik-Soo et al. 1996), a selective electrode technique to measure Na^+ ions in raw hams (Palmia et al. 1997), and a microwave transmission technique (Shiinoki et al. 1998). Recent studies have shown that electrical impedance is a viable salt measurement in fish (Chevalier et al. 2006), minced meat (Labrador et al. 2010), and other food products (Masot et al. 2010).

Very recently, a pioneering new technique was described for the absolute quantification of double-quantum filtered spin-3/2 nuclei ^{23}Na spectra (Mouaddab et al. 2007). This method paves the way for absolute quantification of both bound and free fractions of Na^+, which are determinant factors in the characterization of salted/brined/dried meat products. Staying in the field of salted meat, Foucat et al. (2003) studied the interactions of sodium and chloride ions with meat products by means of ^{23}Na and ^{35}Cl NMR spectroscopy.

The ^{23}Na NMR spectroscopy protocol is also used in MRI studies. Salt ingress in muscle products is connected with structural integrity, as membranes act as barriers to ion diffusion. Guiheneuf et al. (1997) used ^{23}Na MRI to show that sodium ion ingress into postrigor pig muscle during brining follows a Fick's second law. Bertram et al. (2005) introduced the use of combined ^{23}Na MRI and ^{23}Na NMR spectroscopy for the study of sodium ion diffusion into meat during curing.

^{23}Na NMR spectroscopy here gives complementary information to MRI by identifying two sodium populations. ^{23}Na MRI quantification of sodium mobility in pork during brine was also investigated at different pH and postmortem ages (Vestergaard et al. 2005), and the results suggesting the diffusion coefficient was affected by changes in NaCl concentration, swelling, and degree of dehydration.

8.3.3 Freshness and Spoilage

Among the quality attributes, freshness is of special significance and can equally well be seen as a sensory characteristic or a nutritional (or food safety) characteristic. Consequently, the means used to determine meat freshness will usually depend on the quality attribute targeted.

Meat spoilage can be detected by gas sensor microarray, such as with the low-cost KAMINA Micronose microarray developed by the Institute of Instrumental Analysis at Karlsruhe (Forschungszentrum Karlsruhe, Institut für Instrumentelle Analytik, Postfach 3640, D-76021 Karlsruhe, Germany). This sensor has been comprehensively tested and its performance in qualifying fresh meat assessed. This technology is ready to be transferred to the food industry for evaluation of meat freshness (Musatov et al. 2010). Another aspect of freshness requires discrimination between fresh and frozen-thawed meat, as recently reviewed in Ballin and Lametsch (2008), and methods based on NMR relaxation times appear to offer a powerful solution (Bertram et al. 2007).

A noninvasive portable system has been developed that monitors meat quality in terms of soluble protein content, microbial load, and biogenic amine content (Jordan et al. 2009). This new device is based on standard analytical techniques coupled with Raman and fluorescence spectroscopy.

Moreover, a hand-held Raman sensor head using an excitation wavelength of 671 nm was developed as a tool for *in situ* characterization of meat quality. It has proven capable of detecting microbial spoilage on the meat surface even through the packaging foil (Schmidt et al. 2010).

Staying with spoilage, another promising recent study used synchronous front-face fluorescence spectroscopy (SFS) alongside chemometric methods to determine microbial load (Sahar et al. 2011).

8.3.4 Water Activity

Water activity is an important parameter in meat technology as a factor of microbial development. One original way to evaluate this parameter is to use dielectric relaxation spectroscopy. This technique determines the molecular motion response of sample polar molecules (mainly water) to a weak external alternative electric field. As electric field frequency increases, it reaches a frequency called the "relaxation frequency" where the polar molecule can no longer rotate with the electric field. Dielectric properties change markedly around this relaxation frequency.

The technique has been explored for measuring water activity in proteic gels (Clerjon et al. 2003). Water activity is closely connected to water binding, which is itself related to WHC in meat and thus to PSE and DFD phenomena. Moreover, fraudulently added water in meat products can be detected, thanks to its higher water activity value (Kent and Anderson 1996; Kent et al. 2001).

Dielectric properties are not only dependent on water binding in the food material, but also on food composition. For any given molecular composition, the dielectric spectrum will change with molecular binding. In real material, the complex interplay between molecular composition, presence of ions, electrical charges on proteins, and pH variations leads to a complex dielectric spectrum regulated by several phenomena.

Venturi et al. (2007) showed how NMR spectroscopy can also measure water activity in freeze-dried chicken breast meat, by studying the shape of the T2-relaxogram.

Moreover, as meat is highly organized into a fibrillar structure, water diffusion is anisotropic and can be modeled in three-dimensional (3D) using tensors. Diffusion tensor imaging is an MRI method that gives the mean diffusing water molecule displacement, at micron scale, in 3D. It is widely used in neuroscience to assess the intrinsic properties of water diffusion in the human brain (Pierpaoli et al. 1996), and its use is emerging in meat science, to access bi-multi-exponential water diffusion behavior expressing hindered and restricted diffusion of water in and out of fibers (Damez et al. 2010).

8.3.5 Authentication

There is mounting consumer concern over the quality of the food they are buying, and so attention is focused on product labeling. Meat and meat product authentication methods cover a spectrum spanning potential fraud to factors such as origin, ingredient substitutions, or the addition of nonmeat products, or nonregulatory-compliant processing or treatments. Most of these aspects are well covered in a recent review (Ballin 2010). "Fair-trade" meat products represent a specific aspect of food authentication that consumers are increasingly attached to. Qualitative species determination can be based on either DNA or protein or analysis. There are limits to the more conventional PCR or ELISA analyses, as they only apply to the meat in meat-based preparations (Ballin et al. 2009).

8.3.6 Contaminants

Another equally important aspect is food safety, where the aim is to screen for unwanted or even dangerous substances in meat-based food. The potentially dangerous elements may be endogenous (such as bone shards) or exogenous, being unintentionally introduced during handling (shards of glass, metal, plastics or wood, etc.). While x-ray techniques are viable, especially for detecting bone fragments (McFarlane et al. 2003), the industry has its own on-site techniques screening for metal fragments (such as the "Metal

Shark" from Cassel Messtechnik, Germany). Fraudulously added products, which the industry terms "adulterants" (such as liver, tripe, heart, or kidney), are easy to detect in raw products using chemometric methods that employ spectra acquired through midinfrared spectroscopy (Al-Jowder et al. 2002).

Another group of contaminants are the heterocyclic amines (HCAs), from the heating of meat and meat products.

The reference method for screening genotoxic heterocyclic aromatic amines (HAA) in cooked foods uses solid-phase extraction and high-performance liquid chromatography with UV and fluorescence detection, and is described in Gross and Gruter (1992).

A solid-phase extraction method was successfully optimized for the isolation and preconcentration of five mutagenic amines in beef extracts (Galceran et al. 1996).

HCAs are potent mutagenic and carcinogenic substances that can form when cooking muscle meat under perfectly ordinary conditions. A rapid solid-phase extraction procedure was used to purify samples, and liquid chromatography (HPLC)-tandem mass spectrometry with an ion trap mass analyzer was used as determination technique. A second well-established purification procedure was used to demonstrate the applicability of the method to the analysis of these kinds of samples (Toribio et al. 2007).

HCA levels have also been determined by Iwasaki et al. (2010) who modified a previously reported LC–MS method (Kataoka and Pawliszyn 1999), and by Messner and Murkovic (2004) who used HPLC analyses.

Murkovic (2007) gives an overview of recent HPLC improvements to HCA detection in heated meat.

More recently, the potential of SFS coupled with chemometrics was investigated for the determination of HAA in cooked-meat samples (Sahar et al. 2010). This study asserted that SFS along with chemometrics has the potential to be used as a rapid and nondestructive technique for the determination of HAA contents in meat.

A commercial sensor called Fluoralys (Spectralys Innovation, Romainville, France), also based on fluorescence spectroscopy, is proposed to measure neoformed acrylamide and furan components (Birlouez-Aragon et al. 2010).

8.4 Conclusion

Decades of research—including numerous in-lab studies—has targeted methods for investigating and controlling various different meat quality criteria. The past 10 years have seen the emergence of technologies borrowed from biomedical research. Computing-based analysis methods handling huge data throughput have found widespread use. All of the techniques employed provide progressively deeper insight into the different parameters and variables that, together, shape meat quality. However, in terms of capacity to directly qualify meat quality, few sensors and even fewer instruments have made the transition out of the lab. Research efforts need to push harder in order to take the huge potential of current lab-based analysis techniques and transfer this technology to industry practice.

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9

Beef Texture and Juiciness[*]

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9.1 Introduction

Texture of beef remains the most important aspect of eating quality of beef in North America (Brooks and others 2000) and has been extensively researched to understand, control, and predict this characteristic. Meat juiciness is also an attribute valued by most consumers. Although consumers routinely pay more for cuts of meat that are typically more tender (Thompson 2002), there is some expectation that the meat will also be juicy. Moreover, meat juiciness plays a key role in meat texture (Dransfield and others 1984b), probably contributing to its variability.

Properties of beef texture include both initial (first bite with incisors) and overall tenderness (after multiple chews) as well as more complex sensory attributes of chewing and mouthfeel with multiple descriptors such as fiber cohesiveness, adhesion, friability, chew count, mealiness, mushiness, softness, amount of residual connective tissue, rubberiness, and hardness. The complex interaction of the inherent properties of meat with the tongue, teeth, and salivary glands, as the meat is broken down during mastication and as it is interpreted by the brain, forms the basis of psychorheology (Muskowitz 1977) defined as the study of texture perception. In the mouth, textural properties are also not independent of other characteristics of meat, including water-holding capacity (WHC) and fat content, as they contribute to the necessary lubrication while chewing.

Given this complexity, researchers have sought to reduce the subjective multicomponent profiles of meat texture through psychometric analyses of the relationships among subjective texture descriptors. Early work by Harries and others (1972) determined that the texture of roast beef could be adequately described by only two attributes, "toughness–tenderness" and "juiciness" with the attributes of a more elaborate eight characteristic system not adding appreciably to the precision of measurement. In a more extensive study, Dransfield and others (1984a), using a variety of muscles from different species cooked by various methods, conducted principal component analyses using nine textural attributes and these collapsed to two independent dimensions, of greater complexity than those suggested by Harries and others (1972). The first component was comprised of the mechanical characteristic of force or work and chewiness, accounting for 63.9%, and the second component comprised a measure of juiciness or bolus formation, accounting for 19% of the variance. On this basis, the importance of including juiciness as an integral part of discussions on beef texture, as in this chapter, is immediately obvious.

While sensory evaluation of texture and juiciness remains the gold standard, the expense and time required for routine evaluation have led to the development of objective methods for assessing the texture. The most common method to measure meat texture, the Warner–Bratzler shear force (Bratzler 1949), uses a standardized blade, but has many other unstandardized aspects that limit absolute comparisons of results (Janz and Aalhus 2002). Relationships between objective and subjective measurements of texture vary and are study specific. Differences are greater if the cooking methods used for objective and subjective measurements differ, since cooking has a large influence on final texture. Given the fact that texture is composed of several characteristics (Dransfield and others 1984a), including mechanical resistance to force, the sometimes poor relationships between objective and subjective measurements are to be expected. However, the mechanical resistance to force, usually called "tenderness" and equating to the toughness–tenderness sensory attribute, is used routinely in the literature. Hence, this chapter relies extensively on beef tenderness literature, although the distinction between texture and tenderness is understood.

Meat juiciness is considered to arise from moisture released by meat during chewing, and moisture from saliva (Howard 1976; Christensen 1984), and is influenced not only by meat-related factors, such as fat content, but also by physiological and psychological factors inherent to individual tasters. Thus, early research separated initial and sustained juiciness. However, it is now established that both factors can also be combined into one factor (Dransfield and others 1984a; Thompson 2004). Despite the fact that it has been over 10 years since the comprehensive review by Winger and Hagyard (1999) where trials studying factors that have a primary impact on juiciness were compiled, many contradictions between objective measurements and their relationship with juiciness are still found throughout the literature. This illustrates that juiciness is still a poorly understood aspect of meat-eating quality. Unacceptable WHC does, however, ultimately translate into drip loss, which subsequently reduces the profitability in meat production and can cost the meat industry millions of dollars annually. Thus, this chapter includes the location and movement of water in relation to juiciness, and also considers the influence of fat.

9.2 Muscle Structure and Function in Relation to Texture and Juiciness

The development of meat tenderness is a complex process influenced by the architecture of the muscle, the integrity of the muscle cell, the activity of endogenous proteases, and influences of the extracellular matrix (McCormick 2009). For instance, the parallel organization of muscle fibers within a fascicle imparts unique mechanical properties to the tissue, such as the resistance to breakage depending on the angle of shear. Intramuscular fat content (Nishimura and others 1999), as well as the rate and extent of *postmortem* energy metabolism (Thompson and others 2006), indirectly influence meat tenderness. Thus, multiple interactions between these systems determine the final rate and extent of tenderization, making it difficult to separate the effects of individual factors. Additionally, other *pre-* and *postmortem* factors can impact on meat tenderness and juiciness. In this context, the structure and function of the connective tissue, muscle fibers and their proteins, muscle water, and intramuscular fat will be discussed.

9.2.1 Muscle Connective Tissue

Individual muscles have unique connective tissue infrastructure; muscles responsible for locomotion have a greater collagen content than postural muscles, and the nature of their function contributes to the distribution of collagen within the muscle (e.g., parallel, fusiform, pinnate, etc.). Extending from the tendons, connective tissue is the predominant component of epimysium (surrounding the entire muscle), perimysium (surrounding muscle bundles or *fasciculi*), and endomysium (surrounding individual muscle fibers; Harper 1999).

Collagens are the major protein constituents of endomysial and perimysial connective tissue and contribute to meat tenderness through both the total collagen content and the degree of development of heat-stable cross-links (Dransfield 1977). The distribution of perimysium to define the muscle fascicle size (muscle "grain" size) has also been reported to affect the meat tenderness (Purslow 2005). The effects of collagen on texture are thus generally considered to be established in the animal preslaughter (Harper 1999) and the greater the amount of collagen or number of cross-links, the tougher the meat (Bailey 1985). The cross-links become heat stable as an animal matures, increasing toughness in meat from older animals (McCormick 1994). On the other hand, connective tissue has been generally considered to be unaffected by *postmortem* factors, although there are spatial changes that might influence tenderness as the sarcomere shortens postslaughter (Lepetit and others 2000). Endogenous enzyme systems that are capable of breaking down connective tissue (e.g., metalloproteases) have been identified; yet structural weakening of perimysium and endomysium has only been observed during prolonged aging (Takahashi; 1996; Nishimura and others 1998). Hence, proteolytic breakdown of connective tissue during aging does not appear to have a clear effect on meat tenderness.

9.2.2 Muscle Cell Structure and Function

Individual muscles within the body have different functions and metabolic properties. Muscle fiber specialization has occurred in different muscles to match metabolic properties with required functions. Muscle fiber types can be generally classified as white or αW (fast contraction speed, low metabolic oxidative capacity, and high metabolic glycolytic capacity), red or βR (slow contraction speed, high metabolic oxidative metabolism, and low metabolic glycolytic capacity), and intermediate or αR (fast contraction speed, intermediate or high metabolic oxidative metabolism, and high metabolic glycolytic capacity). Many other types have been described based on their histochemical staining patterns, leading to the theory that the metabolic capacity of fibers is a continuous variable (Suzuki and others 1985) and their differentiation is a dynamic process (Swatland 1994). Being able to manipulate the fiber types through environmental or genetic means has numerous implications for meat quality (Aalhus and others 2009b). Information regarding the direct influence of fiber type on beef tenderness is still limited. However, cross-sectional area of muscle fibers (generally higher in white fibers) has been reported to be negatively correlated with beef tenderness (Calkins and others 1981; Seideman and Crouse 1986).

Regardless of the type of muscle fiber, its striated appearance (anisotropy) is due to the organization of myofibrils, the contractile cylindrical organelles, made up of repeating units called sarcomeres.

Current proteomic analysis estimates that sarcomeres are formed by over 65 proteins (Fraterman and others 2007) that interact with each other in a highly coordinated fashion. However, the actual number is likely far greater than this and some of these interactions are just now being discovered (Huff-Lonergan and others 2005). Most individual protein–protein domain interactions are weak, but the number of interactions is large, providing stability to protein complexes. Roughly, Z-lines, to which actin thin filaments are attached, delimit the sarcomere; A-bands, containing myosin thick filaments, are in the center of the sarcomere; and I-bands, containing actin thin filaments constitute the nonoverlap region of the sarcomere. Both filaments interact (actomyosin) via the dynamic head motion of myosin to produce contraction. The enzymatic activity of the myosin head hydrolyzes ATP to provide energy for contraction, which is resolved (actomyosin dissociation) when a new ATP molecule is bound to the myosin head. Bonds created and subsequent sarcomere shortening become permanent when ATP is depleted in post-*rigor* muscle. Consequently, the toughening that occurs during the first 24 h of *postmortem* is most likely due to sarcomere shortening (Koohmaraie and others 1996), and this shortening along with degradation of protein bonds during the aging process influences the final tenderness.

Myofibrillar proteins, such as titin, nebulin, tropomyosin, troponin, or desmin, also have important roles in muscle contraction. Titin is a giant muscle protein (~3000 kDa) extending from the Z-line to M-line (a cytoskeletal protein band at the center of the sarcomere). Because of its cytoskeletal role and large content in muscle, the link between titin *postmortem* degradation and meat tenderness development has been postulated (Boyer-Berri and Greaser 1998). Nebulin is another megaprotein (600–900 kDa) extending from the Z-line to the pointed ends of the thin filament. The C-terminal end of nebulin is embedded into the Z-line. Due to its function in the live animal, nebulin's *postmortem* degradation could be theoretically linked to the disruption of thick and thin filaments. In fact, nebulin degradation has been correlated to *postmortem* tenderization (Huff Lonergan and others 2010). Tropomyosin physically blocks the active sites on the actin filaments, preventing myosin head attachment. In the presence of intracellular calcium, troponin shifts the location of tropomyosin on the thin filament, exposing the active binding sites. *Postmortem* degradation of the troponin-T subunit has been reported to be associated with meat tenderness. Desmin is an intermediate filament protein surrounding the Z-lines of myofibrils. Desmin degradation seems to be correlated with meat tenderization. However, it is not clear as to whether it is simply an indicator or whether it is directly linked to the tenderization process (Taylor and others 1995). Other proteins, such as filamin, dystrophin, or spectrin, have also been reported to degrade during the *postmortem* processes and their role in meat tenderization is still under study.

9.2.3 Muscle Water

In mammals, the water content of muscle represents about 75% of its weight and it serves as a vehicle for nutrient transportation throughout the body. The water content varies inversely with the fat content, while the protein content is maintained constant within the muscle. Small amounts of water are in the extracellular space, while about 85% of the water is entrapped within cells. Within muscle cells, water is found within myofibrils, between myofibrils, and between myofibrils and the cell membrane (sarcolemma) (Offer and Cousins 1992). Therefore, key changes in the intracellular architecture of the cell influence the ability of muscle cells to retain water (Huff-Lonergan and others 2005). It has been theorized that the bulk of the water inside a muscle fiber is free (present in clefts and pockets) and that the less mobile water, about 4% of the total, is either loosely held through weak electrostatic forces or strongly bound to muscle proteins (Poulanne and Halonen 2010). Thus, very little water is held by the myofibrillar proteins themselves; it is the myofibril structure which holds the water (Offer and Knight 1988). Therefore, any factor that alters the spatial arrangement of the fibrillar network of the muscle may also influence its WHC (Offer and Knight 1988; Fennema 1990). In this regard, the swelling or shrinkage of myofibrils or muscle fibers can alter water distribution within the muscle, but this does not necessarily cause swelling or shrinkage of the whole muscle, and the uptake or loss of water from muscle depends mostly on changes in the volume enclosed by the perimysial connective tissue network.

In order to describe the state of water in muscle, several terms have been utilized in the literature. Water-binding capacity is often used, and it covers two properties of the muscle; the capacity to bind water by chemical interactions (bound water capacity), and the capacity to entrap water within its structure and

retain it upon the application of forces such as centrifugation or pressure (WHC). Bound water is defined in terms of its thermodynamic properties (freezing and boiling point, enthalpy and entrophy, etc.; Fennema 1977) and is the water that shows an extra resistance to diffusion and its properties in the vicinity of macromolecules differ detectably from those of the bulk water in the same system.

9.2.4 Intramuscular Fat

Intramuscular fat is composed of marbling (fat cells or adipocytes) located between bundles of muscle fibers and fat within muscle cells, which is largely comprised of the lipid in cell membranes and lipid droplets in vesicles (Cassens and Cooper 1971; Gandemer 1999). Regarding meat texture, an increase in fat could dilute the protein in meat, thereby lowering the bulk density, and fat within the connective tissue could reduce the force required to cut the meat. Fat composition is also important, since overall fat softness increases with fat unsaturation, which also reduces fat melting point. Dikeman (1987) reviewed the literature on the relationship between marbling and tenderness and reported that marbling accounts for only 5–10% of the variability in beef palatability. Thus, the minimum muscle lipid content to achieve an acceptable eating quality or palatability has been reported to be 3% (Savell and others 1986). In this context, although several studies (Smith and others 1985; Aalhus and others 2004b) have reported inconsistent results linking fat content and texture, in many countries, including Canada and the United States, premiums are paid to producers for greater levels of marbling in beef, as marbling is supposed to be associated with flavor, tenderness, and juiciness. In addition, fat provides lubrication between muscle fibers and could increase the perception of tenderness and juiciness (Calkins and Sullivan 2007). The lubrication effect of marbling relies on higher fat levels in marbled meat-stimulating salivation and giving the perception of increased juiciness of meat while chewing. Some of the early studies found that different fat levels play an important role in juiciness and acceptability (Cross and others 1980; Penfield and others 1989; Savell and others 1989). Thus, intramuscular fat, mostly in the form of marbling, has been found to contribute to the juiciness of meat (Gaddis and others 1950; Thompson 2004), although little is really known of the mechanism by which this occurs. Part of the complexity of studying the effect of intramuscular fat on texture and juiciness is due to interactive effects with aging and endpoint temperature, as well as psychological factors involved in the eating experience.

9.3 Meat versus Muscle: Important Contributors to Texture and Juiciness

There are important differences between muscle as a functional tissue in the live animal and the final meat that is consumed. These include the onset of *rigor mortis*, the proteolytic *postmortem* processes, and the effect of cooking (e.g., the effect of heat on muscle proteins).

9.3.1 The *Rigor* Process

The conversion of muscle to meat can be a lengthy process, requiring up to 48 h or more in beef cattle. During this time, any *postmortem* procedure has the potential to influence final meat quality. Protein synthesis and degradation rates in the live animal may influence tenderization *postmortem*, levels of stored glycogen may influence the rate and extent of *postmortem* glycolysis, and levels of fat in all the depots may influence the rate of temperature decline *postmortem*, thus affecting *rigor* development and proteolytic enzyme activities. Although the *longissimus thoracis et lumborum* muscle has been studied extensively to determine the effects of various *postmortem* treatments, it is well known that different muscles within a carcass and different parts within the same muscle respond variably to *postmortem* treatments. This variability can be partly explained by the white/red fiber ratio and by the differing cooling rates within and between muscles.

In general, as muscle is converted into meat, oxygen is no longer available for cells, producing a shift from aerobic to anaerobic metabolism. The increase in lactic acid reduces muscle pH from ~7.1 to ~5.6. The depletion in energy and the inability of ATP-dependent calcium, sodium, and potassium pumps to function lead to a rise in intracellular ionic strength. The cells, finally, are unable to maintain reducing

conditions (Huff Lonergan and others 2010). *Postmortem* muscle cells attempt to maintain ATP levels; however, the mitochondrial system stops ATP synthesis soon after *postmortem*, leaving anaerobic glycolysis as the only means to synthesize ATP. As well, the increase in intracellular calcium levels leads to an increase in the activity of myosin ATPase. Calcium binds to troponin-C, causing a spatial shift in tropomyosin, exposing active sites on the actin molecules that are then available to interact with the myosin heads. Myosin heads, without an ATP supply and in the presence of calcium, firmly attach to actin and no longer allow free sliding of thick and thin filaments. Macroscopically, the conversion of muscle to meat involves the transformation from being soft, pliable, and stretchable to a more rigid and inextensible state (Greaser 1986). *Postmortem* muscle metabolism is, however, highly influenced by temperature, with changes in temperature explaining much of the variation in pH decline among different muscles (Bruce and Ball 1990) and influencing the degree of muscle shortening. At high temperatures, sarcomere shortening is pH dependent, beginning at pH 6.1–6.3. Cold shortening occurs in muscle <11°C, being more rapid at lower temperatures and only in muscles with a significant proportion of red fibers, and is related to a rise in intramuscular calcium levels (Davey and Gilbert 1974a). Sarcomere shortening during *rigor* development is responsible for the toughening phase (Koohmaraie and others 1996). Thus, there is a strong negative relationship between sarcomere length and meat toughness when sarcomeres are shorter than 2 μm (Bouton and others 1973). The increase in toughening is related to the increased density of the thick and thin filament lattice, as well as the surrounding collagen fibrils.

Once actomyosin has been formed, the space for water to be held in the myofibrils is reduced and fluid can be forced into the interfilamental spaces, where it is more easily lost as drip. Lateral shrinkage of the myofibrils occurring during *rigor* can be transmitted to the entire cell if key cytoskeletal proteins, which link myofibrils together and myofibrils to the cell membrane, are not degraded. Therefore, manipulation of the net charge of myofibrillar proteins and the structure of the muscle cell and its components (myofibrils, cytoskeletal linkages, and membrane permeability) as well as the amount of extracellular space within the muscle itself are key factors affecting water movement and hence WHC (Huff-Lonergan and others 2005).

Aside from covalent protein bonding, once the pH reaches the isoelectric point (p*I*) of major proteins (i.e., myosin, p*I* = 5.4), there is a similar dominance of negative and positive charges neutralizing each other and conferring the protein a minimum net charge. In this situation, positive and negative groups within the protein are attracted to each other and result in a minimum swelling and consequently minimum capacity of meat to hold water (Diesbourg and others 1988; Offer and Trinick, 1983). During *rigor* development, the diameter of muscle cells decreases (Hegarty, 1970; Swatland and Belfry, 1985) which is likely the result of transmittal of the lateral shrinkage of the myofibrils to the entire cell (Diesbourg and others 1988). Recently, using a highly sensitive low field nuclear magnetic resonance, Bertram and others (2002) found that within the myofibril, a higher proportion of water is held in the I-band than in the more protein dense A-band. Therefore, during *rigor*, and I-band shortening, the loss of volume in the myofibrillar region combined with the pH-induced lateral shrinkage of the myofibril could lead to expulsion of water from the myofibril into the extramyofibrillar spaces within the muscle cell, and ultimately out of the muscle (Bertram and others 2002). For this to occur, the linkages between myofibrils and the cell membrane (proteinaceous connections that make up or are associated with the intermediate filaments and costameres) have to remain intact so that the reduction in diameter of myofibrils transfers to the muscle fiber (cell) volume (Diesbourg and others 1988; Offer and Knight 1988; Kristensen and Purslow 2001). Therefore, the *rigor* process could result in mobilization of water out of not only the myofibril, but also of the extramyofibril spaces as the overall volume of the cell is constricted (Huff-Lonergan and others 2005). The water that is expelled from the myofibril, and ultimately the muscle cell, eventually collects in the extracellular space. Several studies have shown that gaps develop between muscle cells and between muscle bundles during the post*rigor* period (Offer and others 1989; Offer and Cousins 1992). These gaps between muscle bundles are the primary channels by which purge flows from the meat, and some researchers have actually named them as "drip channels" (Bertram and others 2004). In this regard, it has been observed that disruption of the intermyofibrillar and costameric connections by degradation of proteins such as desmin early in the *postmortem* period has the potential to affect WHC that is hypothesized to occur by limiting the extent of lateral shrinkage of the myofibrils and overall shrinkage of the entire muscle fiber caused by *rigor* shortening and denaturation of myofibrillar proteins (Zhang and others 2006; Bee and others 2007).

9.3.2 *Postmortem* Proteolysis

Ultimate meat tenderness is highly dependent on the degree of alteration and weakening of myofibrillar structures, explaining the increase in tenderness in aged meat compared to unaged meat. Breaks at the junction of the I-band and Z-disk, as well as the degradation of myofibrillar and cytoskeletal proteins, such as titin, desmin, troponin-T, or nebulin, have been consistently reported to be associated with meat tenderization. Three endogenous proteolytic enzymatic systems have been traditionally considered responsible for such myofibrillar degradation: the lysosomal cathepsins, the calpain system, and the multicatalytic proteinase complex (Koohmaraie and Geesink 2006). Cathepsins, a group of enzymes comprised of both endo- and exopeptidases located in the lysosomes, were the first enzymatic system considered by the scientific community to be linked with *postmortem* mechanisms of meat tenderization. However, that initial interest was later discarded on the basis of numerous observations (Kemp and others 2010). The role of the 20S proteosome or multicatalytic proteinase complex on meat tenderization is still unclear. Although its proteolytic activity has been observed *in vitro* for several myofibrillar proteins, such as nebulin, myosin, actin, and tropomyosin, the degradation patterns are not the same as those seen in *postmortem* muscle. Thus, the calpain system or potentially another, such as the caspase system, is apparently responsible for proteolysis of key myofibrillar proteins during the *postmortem* period (Koohmaraie and Geesink 2006).

The calpain system consists of at least three calcium-activated proteases, μ-calpain, m-calpain, and calpain 3, and an inhibitor of μ- and m-calpain, calpastatin. Both μ- and m-calpain are composed of two subunits with molecular weights of 28 and 80 kDa. Calpain 3 is a single polypeptide of 94 kDa with sequence homology to the large subunits of μ- and m-calpain. Unlike μ- and m-calpain, calpain 3 cannot be easily extracted from skeletal muscle due to its association with titin. Calpastatin is the endogenous-specific inhibitor of μ- and m-calpain, containing four calpain-inhibiting domains in its most common isoform. An important characteristic of μ- and m-calpain is their calcium-activation (micro- and millimolar concentration of calcium, respectively) and autolysis in the presence of calcium. A remarkable stability of m-calpain activity in *postmortem* muscle, in contrast with the decrease in the activity of μ-calpain, however, suggests that m-calpain may not have a real effect on muscle tenderization. Moreover, the levels of calcium in muscle *postmortem* are enough to activate μ-calpain but not m-calpain. On the other hand, several studies with knockout mice indicate a great influence of μ-calpain but not calpain 3 on protein degradation and meat tenderization (Kemp and others 2010). Calpastatin also requires calcium to bind and inhibit calpains at levels close or below those required to activate calpain. Calpastatin is also a substrate for the calpains, although resulting fragments retain inhibitory activity (Goll and others 2003). Variations in the calpastatin levels and its inhibition of calpain in the first hours of *postmortem* have been widely reported to affect meat tenderness, with increased toughness in meat from carcasses with higher calpastatin levels. Interaction between μ-calpain and calpastatin within 3 days after slaughter would appear to be a key factor in *postmortem* proteolysis and meat tenderization.

Despite the general assumption of the importance of the calpain system on ultimate meat tenderness, new approaches suggest that other processes and systems could contribute, such as apoptosis mediated by the protease family of caspases (Ouali and others 2006). Programmed cell death or apoptosis naturally occurs in living animals, mediated by complex biochemistry. After slaughter and exsanguination, cells are deprived of nutrients and oxygen, initiating apoptotic pathways in which caspases are the main enzymes. The role of caspases during *postmortem* apoptosis and its influence on the initiation of meat tenderization are still under study. The fact that calcium is a central element of the apoptotic process, the *in vitro* studies showing degradation of a number of myofibril proteins by caspases and the results suggesting a caspase-mediated decrease in calpastatin support the hypothesis of an important interaction between the calpain and caspase protease systems. However, the real contribution of caspases to *postmortem* proteolysis and meat tenderization as the first stage of meat aging needs further research (Kemp and others 2010).

9.3.3 The Effects of Heat on Muscle Proteins

Cooking is the final *postmortem* step applied prior to consumption and it is the action of heat on the muscle tissue that gives meat its characteristic flavor, texture, and aroma. It is a commonly held belief, and scientifically supported fact, that the cooking procedure can have more to do with the final eating

quality of meat than any other production or processing factor (Aalhus and others 2009a). The action of heat on meat results in changes to the myofibrillar proteins and connective tissue, resulting in changes in the WHC, all of which contribute to the texture and juiciness of the final cooked product.

Initially, on heating, meat undergoes a three- to fourfold toughening between 40°C and 50°C, followed by a decrease in toughness between 50°C and 60°C, and further doubling of toughness occurs beyond 60°C (Davey and Gilbert 1974b). However, there have been contrasting theories regarding the underlying cause of these toughening phases (Davey and Gilbert 1974a; Bouton and others 1977; Harris and Shorthose 1988). In an elegant study that looked at the changes in shear force in whole muscles in addition to the changes in tensile strength of individual muscle fibers and isolated perimysial connective tissue, Christensen and others (2000) concluded that the first phase of toughening is due to an increased strength of the perimysium, possibly due to an increase in the concentration of collagen fibers in a given cross section as denaturation alters the tertiary structure (Lewis and Purslow 1989). In contrast, the second phase of toughening, above 60°C, corresponds to an increase in the breaking strength of individual muscle fibers, at the same time when actin and other sarcoplasmic proteins undergo denaturation. The period of tenderization which occurs between 50°C and 60°C is associated with the initiation of collagen solubilization which is thought to be a causative effect (Lewis and Purslow 1989). Recently, Okitani and others (2009) have shown that an irreversible dissociation of actin from myosin can occur in this temperature range, and it is possible that this also contributes to the brief period of tenderization.

At the same time, between 40°C to approximately 55°C, myosin denatures and coagulates, causing shrinkage of the myofilaments, a tightening of the microstructure of myofilaments (Cheng and Parrish 1976), and sarcomere shortening (Hostetler and Landmann 1968). Further heating (>60°C) results in collagen shrinkage to approximately one-third of its original length, and contracture of the collagen sheath (Davey and Gilbert 1974b). Actin denaturation occurs between 70°C and 80°C (Cheng and Parrish 1976), while collagen gelatinization occurs above 75°C (Bejerholm and Aaslyng 2004). As collagen begins to gelatinize, it forms channels in the meat through which melted intramuscular fat can mobilize and translocate that may act as a barrier to moisture loss during cooking. Consequently, highly marbled meat shrinks less during cooking and remains juicier than when the level of marbling is low. As well, meat that has a significant amount of connective tissue will become more tender if cooked under conditions of moist heat for extended periods. On the other hand, meat that has very little connective tissue will toughen as it is heated due to denaturation of the myofibrillar proteins (Cross 1987).

Taken together, these changes result in a decrease in immobilized water with an accompanying increase in evaporative and drip losses. Hence, cooking losses start at about 30°C, go through a more rapid period of loss between 50°C and 70°C, and then continue at a slower rate as the meat continues to be heated. Meat that becomes less juicy because of extended cooking is also less tender (Cross 1987). Thus, the final tenderness and juiciness of cooked meat results from a combination of intrinsic (amount of collagen, denaturation of the myofibrillar proteins, and water loss) and extrinsic (temperature and length of cooking) factors.

9.4 Factors Affecting Beef Texture and Juiciness

The four important factors that determine meat tenderness are background toughness (determined *antemortem*), the toughening phase during *rigor* onset, the tenderization phase (during the *postmortem* aging period), and the denaturation/solubilization of proteins during cooking. Logically, *antemortem* factors that influence the moisture or fat content of meat, as well as the effects of the toughening, tenderization, and cooking phases on proteins, also affect meat juiciness (Winger and Hagyard 1999). Thus, numerous ante- and *postmortem* factors may have a significant influence on the final tenderness and juiciness of meat.

9.4.1 Preslaughter

9.4.1.1 Species and Domestication

As the fraction of *Bos indicus* inheritance increases, tenderness decreases (Seideman and others 1987; Barkhouse and others 1996). The reduced proteolytic potential in *Bos indicus*, caused by a greater

calpastatin activity, is well documented and explains the lower tenderness values reported for meat from this subspecies (Wheeler and others 1990b; Shackelford and others 1991). On the other hand, differences in overall carcass leanness between *Bos taurus* and *Bos indicus* cattle contribute to a limited extent to overall variation in tenderness (Wheeler and others 1994).

In general, domestication has led to the selection of increased muscle mass primarily through an increase in fiber size, rather than number (Rehfeldt and others 2008), and through fiber-type shifts from oxidative to glycolytic fibers with an accompanying increase in cross-sectional area (Essén-Gustavsson and Lindhölm 1984; Weiler and others 1995). Since the effects of selection for lean growth are muscle specific, with muscles that have a higher proportion of βR fibers being less affected than muscles with a higher proportion of αR and αW fibers, there have been more effects in the propulsive muscles than in the postural muscles. Early work suggested that the effects of selection and domestication on lean meat yield may simultaneously promote qualitative alterations to meat quality (Ashmore and others 1972) based on the inherent characteristics associated with different fiber types. Thus, shifts to larger αW fibers in the process of domestication may cause an increased coarseness in perceived fiber grain and less susceptibility to cold shortening due to both their extensive sarcoplasmic reticulum (Wanson and Drochmans 1972; Goldstein and others 1985; Xu and Becker 1998) and paucity of mitochondria (Ogata and Yamasaki 1985; Essén-Gustavsson and others 1992). Recent studies have also reported differences in the content of total and insoluble connective tissue in highly selected and less selected beef breeds (Christensen and others 2011).

9.4.1.2 Breed/Biotype

A large number of breeds of cattle are used for beef production, and this genetic diversity can produce meat with many different qualities (Albertí and others 2008). Meat texture of different beef breeds has been reported to vary in numerous studies (Mandell and others 1997; Dikeman and others 2005; Christensen and others 2011). Some breeds of cattle have anecdotally been described as having a finer muscle grain, related to the macroscopically visible muscle fiber bundles, which is favored by consumers. However, while Albrecht and others (2006) confirmed differences in fiber bundle size with corresponding differences in connective tissue and muscle fiber architecture among several breeds, a relationship to tenderness was not confirmed.

With the advent of advanced genomic techniques, gene associations with tenderness have been reported. Thus, several single-nucleotide polymorphisms in the μ-calpain (CAPN1), Calpain 3 (CAPN3), and calpastatin (CAST) genes have been reported to be associated with tenderness in beef cattle (Vieira and others 2007; Warner and others 2010). Also, the downregulation of a gene with a significant role in apoptosis (DNAJA1) was reported to explain 63% of the variability in tenderness in a population of Charolais bulls (Bernard and others 2007). Also, double-muscled syndrome in beef originated by a mutation in the myostatin gene (MSTN or growth differentiation factor 8, GDF8). Meat from homozygote animals has been reported to be more tender, possibly due to a mutation in the Q204X gene, related to a lower collagen content (Allais and others 2010), and to a higher activity of cathepsins (Caballero and others 2007; Sierra and others 2010). Indeed, Bocard (1981) reported a thinner and more open-meshed connective tissue network and larger, macroscopically coarser bundles in double-muscled cattle compared to normal cattle.

Genotype can also have an effect on tenderness due to variations in glycolytic rate, driven through differences in muscle fiber type among genotypes (Thompson and others 2006). However, genotype can also affect tenderness indirectly as a result of changes in other growth, carcass, and meat quality traits. Since fatness and live weight are moderately to highly heritable (Johnston and others 2003), genotypic differences in overall carcass size or propensity to fatten can result in carcass interactions with the cooling environment. These interactions can affect rates of cooling and have subsequent effects on tenderness, inflating its additive genetic variance if glycolytic rates are not controlled (Thompson and others 2006).

On the other hand, extensive studies with different genotypes have revealed very little difference in meat juiciness (Wheeler and others 1996). The fact that limited differences in juiciness have been found among breeds is not surprising given that it has not been a trait which has been specifically selected. Bernard and others (2007) found 215 genes differentially expressed in Charolais bull calves related to

tenderness, juiciness, and/or flavor and, when combined, 18 of these could be used to explain up to 60% of the variation in both flavor and juiciness.

9.4.1.3 Rate of Growth, Diet, and Age at Slaughter

As animal age increases, meat tenderness decreases, mostly due to the increase in total collagen and decrease in soluble collagen (Reagan and others 1976). Cross-linking of collagen in older animals is generally viewed as a ''background'' contributor to meat texture, although definitive links between mature cross-link content and cooked meat toughness have been difficult to prove (Purslow 2005). In contrast to general knowledge, several studies have reported no differences between groups of animals differing in age (Riley and others 1986; Shackelford and others 1995a). This variation can be explained by the large number of factors interacting with the chronological age of the animal. Depending on the breed, diet, gender, exercise, or production system, animals show different rates of growth and, therefore, similar ages can actually reflect different maturity stages. In addition, intra- and intermuscular fat are late-developing tissues, increasing their relative content at a slower rate than bone or muscle. This explains increases in tenderness observed between 9 and 18 months (Lopez 1977), due to the link between intramuscular fat and texture (Calkins and Sullivan 2007). Compensatory growth as a means to enhance beef tenderness has been shown to improve sensory characteristics without modifying shear force in a muscle-dependent fashion (Therkildsen and others 2008). Furthermore, feeding cattle with high-energy concentrate versus forage-based diets is known to increase levels of marbling, but again relationships between marbling and juiciness have not always been found (Dinius and Cross, 1978; Crouse and others 1984; French and others 2000). Thus, in order to obtain a difference typically requires a large difference in feed quality and/or quantity, affecting growth rates, carcass fatness, and marbling levels (Basarab and others 2007). Finally, as discussed in relation to genotype, the increase in fatness of older animals is responsible for an interactive effect during carcass chilling, since the extra fat accumulated in the carcass provides added insulation and protection from rapid temperature decline during *postmortem* (Lawrie 1998).

9.4.1.4 Hormonal Growth Promotants

Hormonal growth promotants (HGPs) are commonly used in multiple countries to increase the growth rate and feed conversion of beef cattle and to reduce the carcass fat content. Although there is variation in the interpretation of the literature, using HGPs is generally considered to increase beef toughness (Thompson and others 2008). A recent meta-analysis of available published results and unpublished data (Watson 2008) showed that *longissimus thoracis et lumborum* muscle from cattle implanted with HGPs had greater shear force values and lower tenderness scores than carcasses from nonimplanted cattle. Koohmaraie and others (2002) proposed that the decreased tenderization associated with HGPs may be related to a reduction in protein degradation, consistent with the increase in lean, possibly as a result of increased calpastatin activity in the live animal. Increases in calpastatin activity would result in lower aging rates and less tender meat after the animal had been slaughtered. For that reason, the effects are most evident in muscles with the greatest aging rates, or highest calpain-to-calpastatin ratio (Thompson and others 2008). If slaughtered at a similar age, implanted animals show an advanced physiological maturity, including an increase in ossification (Morgan 1997), which could lead to an increase in mature collagen cross-links (Bailey and Shimokomaki 1971). Moreover, the different effect of using single or combined HGP treatments or the type of HGP (oestrogenic and androgenic hormones) on beef texture needs further research (Hunter 2010).

As indicated by Winger and Hagyard (1999), zeranol implants have either had neutral or slight effects on juiciness. Ouali and others (1988) found a slight reduction in juiciness (0.5 on a 10 unit scale using a trained taste panel) with a combined oestradiol and trenbolone acetate implant in Charolais steers. In a comprehensive study using commonly used steroid implant strategies in North America, however, Barham and others (2003) found no difference in juiciness when evaluated by trained sensory or by consumer panels. Allen and others (2009) also found no effect of feeding ractopamine on meat juiciness

after a 90 day feeding trial of cull dairy cows. Growth promotants, therefore, appear to have very limited, if any, effects on meat juiciness.

9.4.1.5 Stress

The effects of animal stress on meat quality have been studied for over 50 years. Hedrick (1965) reviewed the effects of *antemortem* stress on meat quality and indicated cattle, when subjected to stressful conditions for several hours preslaughter, usually have lower muscle glycogen, higher *postmortem* pH, and improved tenderness and juiciness. In the past 45 years, these observations have held true and animals undergoing long-term stress and glycogen depletion produce meat referred to as dark, firm, and dry (DFD). Pale, soft, and exudative (PSE) beef tends to occur in deep muscles of the hip and results from a lack of heat dissipation and a resulting elevated temperature as the pH falls, causing protein denaturation (Aalhus and others 1998). PSE meat shows reduced WHC. Although Wulf and others (2002) observed higher shear force and decreased sensory tenderness scores in DFD beef, variation in tenderness and juiciness within DFD and PSE meat is still, however, a matter under consideration.

Early work by Penny and others (1963) in beef showed that simulated stress by adrenalin injection can lead to more tender and juicy meat. Meat produced would be currently classified as DFD or "dark cutting," with an ultimate pH >6.0 and greater water retention through the muscle-to-meat conversion and cooking process. Mixing, transportation, and environmental conditions are the most important stressors in the preslaughter period, but establishing codes of practice so that meat quality is not negatively affected is an ongoing concern. Villarroel and others (2003) transported bulls up to 6 h, but there was no effect on meat juiciness. Fernandez and others (1996) compared 1 h versus 11 h of transport of veal calves and longer transport increased live weight loss and dressing percentage and reduced glycolytic potential. In addition, pH, 24 h postslaughter, was increased and there were no differences in drip or cooking losses but there was a trend for reduced juiciness. In an unpublished study from the Lacombe Research Centre, steers were transported for 0, 8, or 48 h and longer transport periods resulted in greater losses of live weight (12% for 48 h transport) and increased dressing percentages due to loss of gut fill and dehydration. None of the meat from steers transported for 0 or 8 h was classified as dark cutting, but meat from 17 of 31 steers transported for 48 h was dark cutting. Meat from steers transported for 8 h and 48 h was rated lower in juiciness than 0 h, although the difference in juiciness was less than a panel unit on a 9-point scale. It is thus apparent that DFD meat might not always lead to juicier meat, particularly when stressful conditions promote weight loss and dehydration.

9.4.2 Postslaughter

9.4.2.1 Carcass Suspension

Traditionally, carcasses are suspended by the Achilles tendon during *postmortem* chilling. However, it has been clearly demonstrated that this method of carcass hanging allows considerable *rigor* shortening and a resulting decrease in tenderness in some of the major muscles of the loin and hind limb in cattle (Herring and others 1965; Hostetler and others 1972; Jeremiah and others 1984). When a load of sufficient magnitude (such as the weight of the carcass when carcass suspension is altered) is applied to a muscle during the *postmortem* period, despite undergoing the same *rigor* process, the muscle is unable to shorten (an isometric contraction occurs) and the sarcomere length remains the same, or is lengthened (Aalhus and others 1999). Although various methods of altering carcass suspension have been attempted, such as horizontal, neck-tied, hip-tied, and hip-free by the obturator foramen in the pelvic or aitch bone, most reports (López-Campos and others 2010) indicate that the latter method, also called Tenderstretch, results in longer sarcomeres and improved tenderness in the *longissimus* and posterior muscles of the hind limb (*semimembranosus*, *semitendinosus*). Another method, called Tendercut, consists of cutting bones and connective tissues at two positions in the mid-loin and the round/sirloin junction of beef carcass sides. According to Claus and others (1997), sarcomere lengths and tenderness increases for several muscles in the loin and round. However, other authors (Beaty and others 1999) observed improved tenderness only in the *longissimus* muscle.

Aitch-bone suspension also allows the same overhead system of moving carcasses, and leaves more floor clearance by the carcass, reducing drip-loss. In North America, the objection to aitch-bone suspended carcasses in the meat industry appears to be the altered shape of the carcass, which influences grade measurements (e.g., rib–eye area and fat depth) and the appearance of retail cuts when the carcass is broken down.

9.4.2.2 Chilling Regime

Low temperatures are required during carcass storage to prevent microbial spoilage. However, cold shortening or cold toughening depends on the difference between hot carcass and environmental temperatures. Carcass size and degree of fatness are other factors that influence carcass temperature decline. Therefore, regarding beef texture, several strategies have been proposed to increase tenderness by reducing the sarcomere shortening during *rigor* development.

Delayed chilling has been demonstrated to decrease toughness in beef (Fields and others 1976; Smith and others 1979a,b). Chilling carcasses at temperatures >15°C for 15–20 h leads to a decrease in sarcomere shortening and to an increase in proteolysis. However, the higher risk of microbial proliferation makes this method unsuitable for commercial purposes.

Ensuring that muscle temperature is not <11°C before muscle pH reaches 6.1–6.3 minimizes cold shortening. Based on that premise, slower chilling procedures have been developed to reduce carcass temperature in 36–48 h cycles instead of the traditional 24 h cycle. Thus, for the past 20 years, commercial chilling regimes have been designed to try to avoid having carcass meat temperatures fall to <11°C within the first 10 h *postmortem*. That modification has been shown to increase tenderness acceptability between 10% and 40% in different muscles (Aalhus 2002).

On the other hand, several reports indicate that very fast chilling, namely the attainment of –1°C within approximately 5 h *postmortem*, can result in improvements to tenderness in species other than beef, such as pork and lamb (Davey and Garnett 1980; Jones and others 1993; Joseph 1996). Low temperatures, obtained by very fast chilling, bring about a considerable release of calcium from the sarcoplasmic reticulum to the myofibrils (Jaime and others 1992). This early supply of free calcium, together with a high muscle pH, could result in an advanced and increased activation of calpains. In beef, applied blast chill temperatures of –20°C or –40°C for ~3 h can lead to deep hip temperatures of only 11°C by 4 h postslaughter if carcass backfat thickness is increased (Aalhus and others 2000). Thus, some studies report an increase in toughness after very fast chilling, perhaps due to an insufficient decline in the internal temperature of the muscle (Van Moeseke and others 2001). In order to obtain the targeted temperature, extreme blast-chilling conditions (7 and 10 h at –35°C) need to be used. Although these conditions result in more tender beef after 6 days of aging, differences disappear after 21 days of aging (Aalhus 2002). Therefore, the main advantage of very fast chilling is a reduction in the necessary aging time to achieve an acceptable product.

9.4.2.3 Electrical Stimulation

Originally developed to prevent cold shortening, electrical stimulation has been a commercially successful mechanism for improving beef tenderness. In general, shear force values are decreased by 15–30% by electrical stimulation. Taste panels rate electrically stimulated meat as 10–50% more tender than nonstimulated meat (Chrystall and Devine 1985; Locker 1985). Studies comparing the efficacy of high- and low-voltage stimulation (Bouton and others 1980; Aalhus and others 1994) conclude that the largest improvements in tenderness are associated with high-voltage stimulation. Electrical stimulation tends to be more effective in lower-grade carcasses, and is sometimes more effective in short-fed grain- or grass-fed animals when compared with long-fed grain-fed animals (Smith 1985). However, despite significant amounts of research, the underlying mechanism and the correct commercial application (voltage, amperage, and frequency) have remained elusive, particularly given the range in individual carcass characteristics that can have influence (e.g., carcass size, electrical sensitivity, glycogen levels, etc.). A recent review by Simmons and others (2008) has refocused attention on the underlying mechanisms and

commercial application. As discussed by Aalhus (1995), several mechanisms of action have been proposed in the electrical stimulation literature, including physical damage due to tetanizing contractions (Takahashi and others 1987), prevention of cold-induced shortening (Davey and Gilbert 1975; Honikel and others 1983), or due to an earlier onset of proteolysis (Simmons and others 2008) either through nonenzymatic weakening of the myofibrillar structure (Takahashi 1992) or mediated by the calpains (Ducastaing and others 1985), or the lysosomal enzymes (Dutson and others 1980). With the recent literature published on apoptosis, the caspase enzymes may also play a role (Kemp and others 2010). The possibility also exists that more than one mechanism may be contributing to the development of tenderization in electrically stimulated carcasses at any one time.

The reported effects of electrical stimulation on juiciness have been variable, with some authors reporting an improvement (Hwang and Thompson 2001), others reporting no effect (Jeremiah and Martin 1982), and still others reporting a reduction in juiciness (Ferguson and others 2000). The reported differences are likely to result from the extent to which physical damage to the muscle fibers occurs, the extent to which stimulation affects ultimate pH in relation to the isoelectric point of the fibers, and the extent that proteolytic activity is accelerated by stimulation. In addition, the backfat thickness of the carcasses, and/or the amount of intramuscular fat, may play a role in the perception of juiciness in electrically stimulated carcasses. The insulative effects of fat, combined with a rapid pH drop during stimulation, may result in additional denaturation of the myofibrillar proteins and a reduction in WHC. Indeed, Ferguson and others (2000) reported that stimulated sides from feedlot-finished cattle had a much larger decrease in juiciness compared to stimulated sides from pasture-finished cattle.

9.4.2.4 Aging Methods

As already explained in detail at the beginning of the chapter, the process of meat aging is largely a consequence of enzymatic degradation of key myofibrillar and cytoskeletal proteins that, under *in vivo* conditions, maintain the structural integrity of myofibrils. Therefore, the effects of most factors previously explained in this chapter could be minimized by using correct aging procedures. Although shear force is generally accepted to decline over time *postmortem*, with the rate of decrease being rapid initially and decreasing over time, almost all studies limit the time of aging to a maximum of 20–30 days (Mies and others 1999; Gruber and others 2006b). There are several reports in the literature, however, that indicate the pattern of tenderization for individual muscles (e.g., the *longissimus thoracis et lumborum*) can be quite variable over 30 days of *postmortem* (Parrish and others 1969; Stuby-Souva and others 1994; Aalhus and others 2004a). Some studies suggest that it is possible to select meats that have aged rapidly by 48 h of *postmortem* (Lepetit and Hamel 1998; Damez and others 2008) and avoid long periods of storage and the related costs. If aging does not improve tenderness in certain muscles, the unnecessary storage of these muscles could be avoided and the economic saving from storage could be even higher. In fact, although previous US guides (Mies and others 1999) recommended to industry a universal aging time of 12 days, current recommendations (Gruber and others 2006a) focus on the differences among beef subprimals and individual muscles, based on the belief that aging of different muscles will not universally result in an improvement in tenderness (Juárez and others 2010). For a given aging time, the intermuscular variations in texture can be explained by their different fat and moisture content (Aalhus and others 2004a; Moreno and others 2007), shape (Janz and others 2006), sarcomere length (Willems and Purslow 1996), fiber type (Lefaucheur 2010), and connective tissue (Light and others 1985). However, the evolution of different texture parameters during aging is more related to the rate of *postmortem rigor* shortening (Olsson and others 1994), myofibrillar proteolysis (Koohmaraie and others 1996), moisture loss (Aalhus and others 2004a), and collagen breakdown (Purslow 2005), as well as to interactions among these factors.

Many upscale restaurants dry-age beef before serving it to customers, based on the perception of improved flavor and tenderness (Warren and Kastner 1992; Campbell and others 2001). Dry-aging is a traditional method of aging beef, which involves direct exposure to air flow and humidity in a cooler for a variable number of days followed by trimming of spoiled or oxidized meat prior to cooking. It is believed to produce a unique and desirable flavor, compared to wet-aged beef (DeGeer and others 2009), due both to the development of different compounds in the presence of oxygen and to the reduction of moisture in meat due to evaporation, with the subsequent concentration of the flavor-responsible

compounds. The greater evaporative moisture loss seems to have no effect on perceived juiciness compared to wet-aged beef, but it increases the shrink losses and, therefore, the costs associated with this practice. Regarding meat tenderness, several authors have reported a decrease in tenderness in dry-aged beef (Parrish and others 1991; Laster and others 2008), limiting the positive factors of dry-aging to the theoretical improvement in beef flavor.

Current theory suggests that meat stored at higher temperatures has a more rapid decline in shear values (Minks and Stringer 1972; Herrera-Méndez and others 2005), although many of these studies used temperatures outside the range of normal storage temperatures (ranging from 20°C to 30°C), which may have a negative impact on palatability of several muscles. King and others (2009) indicated that increasing the temperature from –0.5°C to 3.3°C had substantial effects on proteolysis, and subsequently on shear force in *longissimus lumborum* and *gluteus medius*. When normal storage temperature ranges (1°C–5°C) are used during meat aging, the overall effect of temperature on tenderness follows the same trend, as previously reported by Kim and others (2007) for *longissimus dorsi* (0°C and 4°C over 49 days of aging).

9.4.2.5 Artificial Tenderizing

Due to the importance of texture in meat quality, different approaches have been attempted to make beef more tender. These strategies include infusion of substances into the carcass immediately after slaughter, blade and needle tenderization, marination, the use of proteolytic enzymes, or high-pressure processing (HPP).

Calcium chloride injection of pre-*rigor* hip muscles reduced aging time needed for normal tenderization to 1 day *postmortem* (Wheeler and others 1991). This effect is due to an early activation of the calpain system, resulting in a greater proteolysis rate and more tender meat. However, the development of off-flavors in meat from carcasses with calcium chloride infusions (Morgan and others 1991) reduces the commercial applications of this method in beef. Other combinations of substances have been tried in postexsanguination vascular infusion, such as "glycolytic rate enhancers" (Farouk and others 1992). However, they have been reported not to be effective for improving beef tenderness (Smith and others 2008).

Blade/needle tenderization consists of passing beef cuts through machines with reciprocating banks/rows of very sharp blades or needles, to disrupt the structural integrity of myofibrils, muscle fibers and bundles, and fibrils of collagen, reticulin, and elastin (Smith and others 2008). Numerous studies report a decrease in shear force after blade tenderization in several muscles (Seideman and others 1977; Pietrasik and Shand 2004), although the fracture planes created by the tenderizer blades could lead to an overestimation of shear force differences (Bowling and others 1976). Nevertheless, data from sensory evaluation supports the increase in tenderness and a decrease in the perceptible connective tissue after blade/needle tenderization, increasing the effect with successive passes through the machine (Pietrasik and others 2010). Blade/needle tenderization tends to reduce juiciness, but without adversely affecting other palatability attributes. Therefore, it can be used as a means of improving tenderness in those muscles with high content of connective tissue (Jeremiah and others 1999).

A large number of substances have been used for marination or injection to improve beef tenderness. Acids, such as vinegar and lemon juice, disrupt hydrogen bonds within the collagen fibrils and cause the connective tissue to swell (Lawrie 1974). Introducing an alcohol to an acid marinade may also increase marinade penetration since fats are soluble in alcohol, thus enhancing final tenderness (Uttaro and Zawadski 2008). Recent studies have reported that seasoning with salt (sodium chloride) before cooking (0.3% w/w) is beneficial, enhancing tenderness, juiciness, and browning (Pérez and others 1998; Aalhus and others 2009a). Injection of sodium chloride solutions, especially those including phosphates, have been reported to be highly effective in improving beef tenderness and juiciness (Baublits and others 2005), partly due to an enhanced WHC. Injecting calcium salts into the muscle, as previously mentioned, stimulates the calpain system but can result in the development of off-flavors. The injection of solutions containing ammonium hydroxide seems to have effects similar to those reported for sodium chloride plus phosphates, without negative effects on flavor (Hamling and Calkins 2008). One of the drawbacks of mechanical treatments (i.e., blade tenderization or injection) is that pathogens can be translocated to the normally sterile interior of meat (Heller and others 2007).

An approach that has been used for centuries by different civilizations to improve beef tenderness is marination with proteolytic enzymes. Enzyme preparations typically degrade meat proteins, tenderizing the meat and increasing peptide and free amino acid levels (Weir and others 1958). Fungal or bacterial enzymes (e.g., fungal amylase, protease) degrade muscle fiber proteins, while tropical plant enzymes (i.e., bromelain, papain, ficin) act on collagen and elastin. However, time and temperature considerations are important to prevent overtenderization and mushy texture. The effect of these tenderizers, as well as other marination substances, unless the meat cuts are very thin or are somehow placed inside the meat, is mostly limited to contact surfaces (Rao and others 1989). Therefore, enzyme solutions may need to be injected.

HPP is a popular nonthermal method of food preservation where the food is subjected to intense isostatic pressures between 100 and 600 MPa near room temperature to enhance safety and shelf life by inactivating microbes and viruses while maintaining the fresh qualities of food products. During HPP (>200 MPa), certain macromolecules can change their native structure, affecting protein solubility and color (Marcos and others 2010; Tintchev and others 2010), in a manner analogous to thermal treatments, leading to a cooked appearance in meat (Carlez and others 1995; Cheftel and Culioli 1997). The effect of HPP is different from that of thermal processing since HPP is said to affect only noncovalent bonds (Bak and others 2009). These structural changes can be positive for the processing of meat products, reducing cook loss and improving texture (Sikes and others 2009). As well, hydrodynamic pressure (HDP) technology, high-pressure shock waves in water for an extremely short-time interval, has also been reported to improve tenderness and decrease variation in beef texture (Spanier and others 2000; Claus and others 2001).

9.4.2.6 Freezing

When meat is frozen, the cell membranes are damaged, which results in a lower WHC and a higher cooking loss (Wheeler and others 1990a), and consequently, a risk of less juicy meat. On the other hand, for similar reasons, it is known that freezing beef also influences tenderness. Thus, beef that is aged and then frozen and thawed has been reported to have lower shear force values compared to chilled meat aged the same time (Shanks and others 2002; Enfält and others 2004). However, the effect of freezing and thawing on instrumental shear force has not been corroborated by consumers, while the negative effects on juiciness were confirmed by trained panel analysis (Lagerstedt and others 2008).

9.4.2.7 Meat Cookery and Degree of Doneness

In addition to the complex effects of heat on meat, many other aspects of meat cookery can affect the texture and juiciness of beef, including the endpoint temperature, rate of heat transfer, and method of cooking. In beef cookery, endpoint temperature is extremely important to the consumer. As would be anticipated by our knowledge of the effects of heat on meat proteins, beef cooked to a rare endpoint (63°C) tends to be more tender and juicy than meat cooked to a well-done endpoint at 71°C (Obuz and Dikeman 2003; Aalhus and others 2009a). In a beef customer satisfaction study carried out in the United States (Brooks and others 2000), the degree of doneness had a greater effect on instrumental tenderness, and possibly juiciness, than marbling levels.

Many aspects of beef fabrication can ultimately affect the rate of heat transfer and, in general, when meat is cooked at a slow rate of heating, tenderness and sometimes juiciness are improved, although cooking losses usually increase. As examples, the rate of heat transfer is decreased in multimuscle cuts, when fat cover remains, and when the meat cut is bone-in. Also, when equal weight roasts of uniform cylindrical and square shape were fabricated from the same muscle and cooked under similar conditions, cylindrical roasts had a more uniform rate of heat transfer and rated higher for tenderness and juiciness (Aalhus and others 2009a).

Home cookery methods can also influence the tenderness and juiciness of beef, and can be classed as dry (e.g., broiling or roasting), moist (e.g., stewing or braising), and dielectric (e.g., microwave) heating. Juiciness is affected by moisture losses, which can occur by evaporation in dry heat cookery and by exudation and diffusion in moist heat cookery (Hernández and others 1999). In general, the effect of cooking on tenderness depends mostly on the structural composition of the muscle. Muscles with high

connective tissue content benefit from moist cooking due to gelatinization, while muscles with low connective tissue content benefit from dry cooking (Cheng and Sun 2008). Microwave cooking reduces cook times by 30–50% which is generally associated with negative effects on tenderness and juiciness, although results in the literature have been variable (Korschgen and Baldwin 1978; Hawrysh and others 1979; Moore and others 1980; Choun and others 1986; Howat and others 1987). Modern microwave technology has improved and often includes convection heating to improve the rate of heat transfer and promote surface browning.

9.5 Measurement of Texture and Juiciness

Texture is a complex attribute as evidenced by the large number of characteristics used to describe it, and it depends on diverse factors, such as chemical composition, structure, physical properties, processing methods, shape, and many others (Szczesniak 2002). Depending on whether meat is cooked or raw, and on the rheological test performed, the contraction state has different effects on the mechanical characteristics measured (Lepetit 1989). Because of the complexity of texture, numerous objective measurements have been developed in raw and cooked meat to describe texture parameters including, but not limited to, tenderness, cohesiveness, firmness, and elasticity.

The complexity of juiciness also causes difficulties in performing objective measurements. WHC can be used as an objective parameter related to meat juiciness (Grau and Hamm 1953). Usually, meat is consumed after cooking and is the reason why the WHC of cooked meat is of central interest. However, to date, the only reliable and consistent measure of juiciness is achieved using sensory methods (Winger and Hagyard 1999).

9.5.1 Instrumental Texture and Juiciness Measurements on Raw Meat

The penetrometer method simulates the process of mastication and is considered a measure of the ease of the first bite between the teeth. This method uses a cylindrical flat-ended plunger in a test frame, driving it vertically 80% of the way through a 1-cm-thick meat sample cut so that the fiber axis is perpendicular to the direction of the plunger penetration. The plunger is driven twice into the meat at each location and the work and the force–deformation curves are recorded. Hardness, cohesiveness, and gumminess can then be calculated (Honikel 1997). Lepetit (1989) developed a modified compression device to avoid lateral deformation and measure the mechanical properties of muscle fibers. In addition, a modified penetrometer (Tendertec) was developed as a moderately invasive procedure to predict tenderness in beef carcasses (Belk and others 2001).

Although sensory analysis is the only reliable method to directly measure juiciness, WHC is commonly used as an indicator in raw meat. There are three main ways to measure the WHC in raw meat: applying no force, applying mechanical force, and capillary action methods. Drip loss is the main method to measure WHC in meat in which the only force on the meat is gravity. Drip loss is a very sensitive method but is time and space consuming. After cutting, the samples are immediately weighed and suspended or packed in retail packages, and then held at 0–4°C for either 24 or 48 h. The drip in this method is thought to result from lateral shrinkage of myofibrils *postmortem*, causing water to be expelled into the extracellular space (Honikel 1997).

Other methods applied to measure WHC in raw meat require the use of some sort of pressure to encourage fluid removal (Fennema 1990). Thus, in the filter paper press method, the pressure is created by applying a vertical weight (Grau and Hamm 1953) on a small sample between two preweighed filter papers. Several modifications have been developed to better control the pressure (Lee and Patel 1984). The water which is squeezed out is absorbed by the filter paper and the amount of water released from the sample is measured either directly by weighing the filter papers or indirectly by measuring the area of the ring of expressed fluid. This method has the advantage of small samples, easy operation, and rapidity, but it has disadvantages in that the measurements of meat and liquid areas with a planimeter are tedious and nonpermanent.

High- (5000–40,000 × g) and low-speed (200–800 × g) centrifugation can also be used to remove the loosely bound water. After centrifugation, the amount of water released is determined either directly, by weighing the water released, or indirectly, removing the sample from the tube and carefully drying and reweighing it to determined liquid loss (Trout 1988). Finally, capillary suction pressure generated by filter paper is used to quantitatively the unbound water from the samples (Labuza and Lewicki 1978). Some other modifications have made the method faster (Kauffman and others 1986a; Kauffman and others 1986b) or more complex by combining compression and capillary suction (Hofmann 1975).

9.5.2 Instrumental Texture and Juiciness Measurements on Cooked Meat

Tenderness is one attribute embodied in cooked meat texture. The most common instrumental measurement methods simulate the mechanical forces (shear, compressive, and tensile) acting on meat in the process of mastication. For these reasons, the numerous methods may be grouped into shear and biting, compression, and tensile methods.

The shear and biting methods are based on the fact that to eat meat it is necessary to bite through pieces with the front teeth and then grind them with the molars (Boyar and Kilcast 1986b). Thus, there are a multitude of instrumental tests that attempt to imitate biting or chewing. The Warner–Bratzler shear force (WBS) test, characterized by a triangular hole in a shear plate, is the most widespread method used to indicate meat sensory tenderness (Lepetit and Culioli 1994; Honikel 1997), as it is the instrumental method that arguably has shown the best correlation with sensory panel scores for meat toughness (Monin 1998). The Allo–Kramer test cell, consisting of 10–13 blades guided between bars through a square box containing the sample, was originally used to measure the texture of particulate items, and was later modified from its original design (Timbers and others 1985) for testing beef and pork. Finally, the flat blunt-ended blade for the slice shear force was designed to replace the WBS on a universal testing machine (Shackelford and others 1999a; Shackelford and others 1999b) and was more strongly correlated with tenderness assessed by a trained taste panel than was WBS. For all these methods, the shear force is calculated as the maximum force recorded in newtons or kilograms, whereas the shear energy is calculated as the area under the force deformation curve from the beginning to the end of the test in Newton mm. Some authors (Bouton and Harris 1972; Moller 1981; Pietrasik and others 2010) have attempted to partition the curve into myofibrillar and connective tissue-related segments with some success.

Texture profile analysis (TPA) is the most popular compression method. TPA was developed for the General Foods Texturometer (Szczesniak and others 1963) and involves compressing a sample, at least twice, using a small flat-faced cylinder and quantifying the mechanical parameters from the force–deformation curves generated (Szczesniak 2002). The main advantage of TPA is that one can assess many variables such as fracturability, hardness, cohesiveness, adhesiveness, springiness, gumminess, and chewiness in a single testing. Alternatively, Bourne (1968) adapted the Instron Universal Testing Machine to perform a modified texture profile test by compressing standard size pieces of food two times. This method was later adapted for determining meat tenderness (Bouton and others 1971; Bouton and Harris 1972) and was further refined by Bourne (1978). Caine and others (2003) compared both texture profile analysis and WBS force to sensory analysis and concluded that texture profile analyses explained more of the variation in subjective sensory tenderness of the rib steaks than WBS.

Tensile methods have been used for the structural investigations of beef and pork (Tornberg 1996). These tests can be performed with either cooked or raw samples. Testing may be conducted either transverse or parallel to the fiber direction. The sample is normally gripped with pneumatic clamps at pressures sufficient to maintain a firm grip without obvious slippage, yet minimizing sample damage (Honikel 1998). Samples are extended at different rates of speed, until completely broken. Width and thickness of the samples after cutting is measured with a calliper. The peak load is measured and the results are reported in pascals (Honikel 1997; Honikel 1998).

Regarding juiciness, after samples are cooked to a defined end temperature, cooking losses can be determined by weight loss (Gault 1985; Honikel 1998). Meat cooking shrinkage has also been determined by calculating the difference between the raw and cooked areas of the meat sample, expressed as a percentage of the raw area (Barbera and Tassone 2006).

9.5.3 Sensory Analysis

As defined by Stone (1993), sensory evaluation is a scientific method used to evoke, measure, analyze, and interpret those responses to products as perceived through any of the senses. Three primary kinds of sensory tests focus on the existence of overall differences among products (discrimination test), specification of attributes (descriptive analysis), and measuring consumer likes and dislikes (affective or hedonic testing). The different types of sensory tests require a suitable degree of selection and training of the panel members, who will have a particular sensory qualification depending on the sensory test. In this sense, depending on the extent of their skills the panel can be a trained (expert), laboratory-trained, or consumer panel. According to Risvik (1995), there is a fundamental difference in sensory evaluation of meat when performed by trained and consumer panels. In the first case, the attention is focused on meat as an object of scientific research, providing a complete description of the different characters within the texture and juiciness profile, such as surface properties, partial compression properties, first bite properties, mastication properties, and after-feeling properties (Jeremiah and Gibson 2003), while the consumer reacts very differently, mainly focusing on how meat can contribute to his/her personal satisfaction.

Sensory evaluation, regardless of whether it involves expert, laboratory-trained, or consumer panels, is both time consuming and expensive. However, the use of humans in sensory evaluations is necessary, because currently no laboratory analysis or mechanical device exists that can approximate all the actions of biting and chewing, and that can measure or duplicate the human perceptions. The advantage of sensory methods over instrumental methods is in the complexity of human perception, enabling immediate analysis, integration, and interpretation of a large number of single sensations at the same time. On the other hand, it is important to know how effective objective measures of meat are as predictors of sensory characteristics. In the literature, the correlations between objective and subjective methods are very variable and conflicting. While some researchers have demonstrated weak correlations (Bouton and others 1971; Shackelford and others 1995b), others have shown stronger agreement between panel scores and objective measures (WBS) of the tenderness component of texture (Poste and others 1993; Otremba and others 1999; Peachey and others 2002). Similarly, the results of studies comparing subjective measurements of juiciness to objective measures of WHC or to quantitative or qualitative measures of muscle fluid are contradictory. Some studies suggest close relationships, whereas others show little relationship (Van Oeckel and others 1999). Linking instrumental and sensory analyses is complicated by the fact that the human subject measures and integrates sensory chewing perceptions on a material that undergoes continuous transformation, being influenced by the mechanical destruction of the original structure, the biochemical conditions in the mouth, and the psychological perception of texture and juiciness (Kapsalis and Szczesniak 1976). Thus, instrumental and sensory investigations are often conducted simultaneously.

9.5.4 Alternative Predictive Methods

Near-infrared reflectance (NIR) spectroscopy is a sensitive, fast, noninvasive, and nondestructive method with simplicity in sample preparation allowing simultaneous assessment of numerous meat properties. NIR has been widely used in the meat industry to predict fat, moisture, and protein level in meat and meat products (Tøgersen and others 1999). Also, NIR has been used in attempting to predict WHC (Geesink and others 2003) and tenderness. However, no conclusive evidence of its success has yet been published (Rødbotten and others 2000; Geesink and others 2003). Numerous studies have shown that NIR spectral readings have the potential to predict tenderness and WHC, whereas other studies have shown the contrary, with poor predictions (Hildrum and others 1995; Swatland 1995; Forrest and others 2000; Bowling and others 2009; Prieto and others 2009).

Electromyography (EMG) is used to measure the activity and chewing rate of the muscles used in mastication (Boyar and Kilcast 1986a), as influenced by the physical properties of the food. EMG is a relatively noninvasive method that only needs surface electrodes placed over the relevant jaw muscles. EMG has been used in texture studies over the past 25 years (Boyar and Kilcast 1986a; Frances and others 1993; Duizer and others 1994; Peyron and others 1996).

In past years, numerous image collection and analysis technologies have been proposed for objectively evaluating beef with varying results (Park and others 1994). There continues to be interest in developing

technologies such as ultrasound image analysis (Nam-Deuk and others 1998; Abouelkaram and others 2000), images captured under visible light and ultraviolet light using the computer vision system and the BeefCam System (Allen 2004; Woerner and Belk 2008), hyperspectral imaging that includes light across the spectrum from visible to infrared (Cluff and others 2008), and nuclear magnetic resonance (Trout 1988; Ablett 1992; Beauvallet and Renou 1992). Currently, the most important issue is raising confidence in the assessments and determining what muscle parameters they are measuring. For this reason, further development of these technologies to improve their robustness, accuracy, and precision is required.

9.6 Future Direction

The complexity of beef texture and juiciness has provided fertile grounds for research endeavors over the past century. While some gains have been made in our understanding of the underlying structure and biochemistry, and the measurement and prediction of tenderness and juiciness, the control of these important attributes in commercial production is still elusive. Newer genomic technologies, which allow the identification of multiple genes and supported by advanced bioinformatics, may lead to marker-assisted selection for multivariate traits, such as tenderness and juiciness, in the foreseeable future. However, the multitude of environmental factors that can impact on these traits will necessitate continued fundamental research into the understanding of texture and juiciness, as well as the factors affecting them.

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10

Sensory Evaluation of Muscle Foods

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10.1 Sensory Evaluation

Sensory evaluation is the analysis of product, materials, and their attributes as they are perceived by the senses of sight, smell, taste, touch, and hearing (Anonymous 1975). Sensory evaluation of food products has been conducted as long as human beings have been evaluating the goodness and badness of food (Meilgaard and others 2007) and is often used in the meat industry for two important purposes. First, consumer testing must be conducted along with marketing tests to help determine if consumers will purchase a new or substantially modified product (Ramirez and others 2001). Second, objective sensory testing must be performed in order to relate the consumer's acceptance of a product to the sensory descriptors that define that product and their intensities. This allows companies to run consumer acceptability testing when they are making a significant change in the product or launching a new product, but allows companies to use objective sensory testing to compare modified products to standard products that have been evaluated using consumer acceptability testing. Muñoz and Chambers (1993) reported that relating consumer data to trained/descriptive analysis data addresses the limitations of consumer

panels. This is because only consumers can provide information on a product's acceptance or its perceived integrated attributes, and only laboratory methods can provide the technical, precise, and reliable information that researchers need to describe individual product attributes and indicate their impact on consumer acceptability. Numerous books and book chapters have been written on sensory evaluation (Meilgaard and others 2007; Resurreccion 1998; Stone and Sidel 1993) and the sensory evaluation of muscle foods (Miller 1994a; Lyon and Lyon 2001; Coggins 2007a,b). The information in this text will include the sensory properties of the majority of meat species, sensory testing that is important in industry and academia, statistical techniques that are important in sensory evaluation, and the relationship between instrumental, trained sensory and consumer acceptability data.

10.1.1 Sensory Attributes

Owing to the utilization of live human panelists, sensory experimentation is much more complex than research where instrumentation is used and requires much greater care in experimental design and interpretation of results. In most experimentation, variability is due to the treatments and error in the model. In sensory testing, there is variability among panelists, and variability due to treatments and error. Even in trained panels, variability among panelists is a concern and must be minimized to describe the sensory properties of a food product and differentiate food products in a product class. One of the most important parts of sensory testing is the management and understanding of panelist variability in both trained and consumer panels. Meilgaard and others (2007) reported that we perceive attributes of a food item in the order of appearance, odor, texture, and flavor. Appearance is generally the only attribute in which consumers base a decision to purchase or consume a meat product and includes but is not exclusive of color, size, shape, and surface texture (Fox 1987; Young 1996). Odor refers to the sensation that occurs when volatiles from a product enter the nasal passage and are perceived by the olfactometry system, and these sensations are most often associated with the formation of compounds during the cooking process (Meilgaard and others 2007). Texture can be defined as the sensory and functional manifestation of the structural and mechanical properties of foods, detected through the senses of vision, hearing, touch, and kinesthetics (Szczesniak 1963) and consists of hardness, cohesiveness, adhesiveness, denseness, springiness, perception of particles, and perception of water. These attributes are perceived by sensors in the mouth both before and after chewing and have been described extensively (Brandt and others 1963; Szczesniak 1963; Szczesniak and others 1975). Tenderness and other textural characteristics are often the defining factors for the acceptability of the American consumer whereas flavor is often more important to other nations and cultures. Flavor includes aromatics, tastes, and chemical feelings. Tastes consist of salty, sweet, sour, and bitter perceptions caused by soluble substances in the mouth, and chemical feelings include astringency, spice heat, cooling, and metallic flavor. Flavor is crucial in the acceptance of food, and the use of flavor-related words is a very important aspect of marketing (Amerine and others 1965; Phillip and others 2010). The National Research Council (1988) reported that the three most important factors that consumers look for in meat products are nutrition, price, and taste. However, if taste is not acceptable, then nutrition and price are irrelevant.

Thousands of new products are unsuccessfully introduced to the retail market every year. With the continued influx of new products into the market, sensory evaluation has become increasingly important to food companies in the creation of new food products as well as in determining the quality attributes of products within a competition category. Marketing and sensory evaluation departments must work together to bring the appropriate new product to market. Both groups have equally important roles that must be understood by each other as well as by upper management to perform the appropriate market research, trained panel sensory evaluations, and consumer testing.

10.2 Practical Objectives of Sensory Evaluation

In dealing with subject's sensory responses, there is a three-step mechanism (Meilgaard and others 2007). First, a sensation results from a stimulus that is detected by a sense organ and travels to the brain as a nerve signal. The brain is then utilized to transpose the sensations into perceptions, and finally, a response is

formulated based on the subject's perceptions (Carlson 1998). This causes much greater variability when utilizing humans as instruments since this three-step process exists in comparison with a one-step process that exists in other instrumental tests. The complexity of sensory evaluation makes it imperative that a specific process be taken in the design of sensory evaluation experiments. Pangborn (1979) states that there are three common problems to sensory evaluation that include lack of test objective, adherence to a single test method regardless of application, and improper subject selection procedures. These three details must be addressed in all sensory analyses. Erhardt (1978) reports that the role of a sensory analyst can be divided into seven tasks. The following is a paraphrase of these seven steps that are discussed in detail in that paper. The first step in conducting a sensory experiment is to determine the project objective. The next step is to determine the test objective to assure that the resulting data will be relevant to the overall objective of the sensory project. The third step of the analyst is to screen samples. This allows the analyst to become familiar with the responses that might be expected, minimizes the evaluation of obviously different samples, and helps the analyst design the test. The next step for the sensory analyst is to design the test. The correct time to consider experimental design and statistical analyses is after a test is planned and not at the conclusion of the work. When conducting a test, it is imperative that the procedures are strictly adhered to in order to prevent nontest variables from influencing panelist responses and/or perception. The last two responsibilities of the sensory analyst are to analyze data according to the predetermined statistical analyses and to report the results to the entity that requested the research. The analyst must make it clear to the requester what test results mean, the conclusion that can be drawn from the results, and the next step to be taken is based on the initial project objective.

10.3 Shelf-Life Testing and Product Cuttings

Use of a trained and/or expert sensory panel to evaluate a product over its shelf life and compare it to a gold standard is a very important tool that can be used to understand the distribution and shelf life of a product. Physical methods can be used instead of sensory testing to determine when color or flavor deteriorates, such as Tbars to evaluate lipid oxidation, volatile compound composition for off-flavor determination, texture analyzers to evaluate product texture, and bacteria counts to determine spoilage. These physical methods are all important indicators of sensory quality, but having a good trained or expert panel allows a company to understand the sensory properties or defects that cause a meat product to be out of specifications and how they relate to instrumental tests.

It is often the goal of a company to substitute an ingredient in their product to decrease costs, increase shelf life, or make marketing claims. However, any ingredient substitution can change the sensory quality of the product, both initially and at different time periods during the shelf life of the product. It is important to evaluate these products throughout the shelf life to determine how it compares to the gold standard or original formulation of the product. This is especially important in the current market with a high demand for products that can be labeled as natural or low-sodium. Natural and low-sodium products can often be produced but not without sacrifices in sensory quality, yields, and shelf life. These sensory limitations need to be understood in order to determine if the product meets quality and shelf-life expectations. In addition, product cuttings such as these are becoming even more important as the field of culinology evolves and research chef/food technologists match the gold standard of a product that a chef would make in the kitchen and make it so that it can either be reheated in a restaurant or by a consumer who buys it from the grocery store.

10.4 Types of Sensory Evaluation Testing

10.4.1 Determining Test Objective

Lyon and Lyon (2001) stated that there are six fundamental questions that need to be asked to determine whether to use a difference/discriminative test method or an affective test method. These questions include: (1) Do the samples differ? (2) If the samples differ, what do the sensory parameters differ in?

(3) Can the difference be quantified? (4) What is the direction of the difference? (5) How does the product compare to similar products? and (6) Does the difference have importance at the consumer level?

10.4.2 Difference Testing

Difference testing can be used in both consumer and trained sensory testing when the objective is to determine if either an overall sensory difference or an attribute difference exists between two products. Difference testing is a very important tool for in-house testing when an ingredient substitution is made, a product is being matched, an all natural formulation is being developed, or the price of the formulation is being minimized. There are many types of difference testings that are discussed in depth in Meilgaard and others (2007). The most common tests that are used with muscle foods are the triangle test, the duo–trio test, and the paired-comparison test.

10.4.3 Triangle Test

The triangle test is commonly used in the muscle foods industry with either a trained or consumer panel. This test is often used with only 8–12 trained panelists, to determine if differences exist between a product and a control (gold standard). In a triangle test, panelists are asked to evaluate three samples for either an overall difference or an attribute difference(s), in which two samples are the same and one is different. Though not statistically significant, it is logical to conduct further testing if at least half of these panelists (6 out of 12) can pick out the odd sample and are able to describe what they believe a difference between the products. It is also a great idea to conduct multiple replications of the triangle tests with your 8–12 trained panelists.

Though triangle tests are appropriate tests when minor product changes are made, such as using a different supplier for an ingredient to save costs, Ennis (1993) demonstrated through Thurstonian methods that a 3-alternative forced choice (AFC) often has more power (which reduces type II error) than a triangle test. In a 3-AFC test, the panelist knows that two samples are different and then has to match the treatment sample to one of the known samples. Meilgaard and others (2007) gives a great explanation of setting up and evaluating data for simple difference testing, duo–trio tests, triangle tests, and other difference tests. Ennis (1998) describes applications of using 2-AFC and 3-AFC tests and demonstrates their increased power (in some situations) in comparison to duo–trio tests and triangle tests. If a trained panel can tell a difference between the gold standard and a product with an ingredient substitution (either through an informal or a formal test), the next logical step is to conduct a difference test, a 2-AFC, or a 3-AFC with a consumer panel ($n > 100$) to determine if consumers of your product can detect differences in the sample. Here, you have a small chance of making a type I error but have a substantial chance of making a type II error (do not determine a difference when one exists). In addition, it is crucial to make sure that the panelists are consumers of the product and are representative of the panelists that consume the products at all locations within the area that the product is sold.

10.4.4 Other Difference Tests

In a duo–trio test, three samples are given to a panelist. One is marked as "reference" and the other two samples are marked with a random three-digit number. The objective of the panelist is to choose the sample that is identical to the reference. There is a 50% chance that the panelist will select the correct sample by random selection. Two out of five tests can be used with appearance evaluations but is not useful in tasting or sniffing due to panelist fatigue. In this test, a panelist is presented with five samples, two of which are the same. The panelist must choose the two samples that are alike. This test has greater power than many other difference tests since the probability of guessing correctly is only 0.10. When the objective of the test is to determine if samples differ in a specific attribute, a paired comparison difference test can be conducted. In this test, panelists are presented with two samples and asked to indicate which sample has a greater or lesser intensity for that attribute. Ranking tests can also be used to determine differences in an attribute for multiple samples. In this testing, panelists may be asked to rank samples based on an attribute, such as ranking samples from most tender to least tender or most juicy to least juicy. This testing can also be used with consumers to rank samples in order of acceptability. Category scaling can also be used for specific attributes to rate the amount or intensity of a characteristic.

10.4.5 Descriptive Testing

Trained panels are utilized to provide accurate and repeatable data pertaining to the quality of meat products. Cross and others (1978) reported that there are four steps that should be taken in the selection of a trained panel which include recruitment, screening, training, and performance evaluation. A sensory study should only be initiated after all of these steps have been taken (AMSA 1995; Cross and others 1978). Panelists must be recruited that are interested in participating in the panels. Panelists must then be screened to determine their ability to perceive sensory attributes and determine known differences between muscle foods products. After sufficient training, the performance of the panel must be evaluated. In this evaluation, the panel must be evaluated for panelist × treatment interaction to make sure that panelists are calibrated and follow the same trends from treatment to treatment. If panelist × treatment interaction exists, this means that specific sensory attributes may not be important, that the panelists need more training, and/or that certain panelists should be removed from the panel. Trained tests in muscle foods include ranking and scaling of samples, magnitude estimation, and descriptive sensory analysis. The latter of these methods has limitless applications. It has been utilized to relate to physical and chemical analyses, product formulations, preferences, and other kinds of consumer measures of concepts, pricing, and so forth (Stone and Sidel 1998).

Descriptive analyses are very important to the meat industry since they are useful in investigating treatment differences, monitoring ingredient process control criteria, and defining sensory properties of a target product (Bett 1993). This is helpful in matching products and determining if product changes cause sensory differences. In small product changes, it is often unnecessary to run consumer tests because a good descriptive panel is able to determine if differences exist in a food product. There are many techniques that are used for descriptive analysis that are covered in depth in Meilgaard and others (2007). These tests include the flavor profile method, texture profile method, quantitative descriptive analysis, spectrum descriptive analysis, and time–intensity descriptive analysis. In most descriptive analysis techniques, it is very important to use references and enough training to make sure that the panel is calibrated and to meet whatever the objective is for that specific test. Spectrum™ analysis is an excellent method because it provides reference scales that can be used for all types of samples.

After a panel has been selected, training is necessary that may be as short as 5–10 h for fresh cuts in which the researchers are only interested in product differences with respect to tenderness, juiciness, flavor intensity, and off-flavors to 40–120 h for more complex meat products such as dry-cured ham and processed products. During training, the panel leader works with the panelists to develop the terminology for the food products that are of interest. It is helpful here to use descriptors and references from literature and then adapt previously used terminology as needed for the specific project and project objective. The panelists are then introduced to descriptive scaling in which references are used to help anchor and calibrate the panelists with respect to different sensory descriptors. In this stage of testing, samples are presented to the panelists that are vastly different in attributes and attribute intensity in order to build the confidence of panelists. Training will then be conducted such that samples within the product class with small variations will be presented to the panelists so that they are able to detect differences between products with small differences in attributes. The last stage of training should be similar to real-world testing situations with products similar to what will be evaluated in the panels. This stage of training should be conducted similarly to how the panels will be conducted. Part of the final training is to evaluate the panelist performance. Panelist standard deviation, panelist bias, and panelist × sample interaction plots are important and should be evaluated to determine how well panelists are trained and to determine if the panel is adequately trained or if there is anyone on the panel that either needs more training or should not be on the panel.

10.4.6 Consumer Testing

Affective tests are used for product maintenance, product improvement/optimization, development of new products, determination of market potential, product comparisons in a category, and to make advertising claims. With product maintenance, trained sensory panels can often be used to determine if sensory attributes or quality changes over shelf life. Baseline data need to be compared with consumer affective testing to determine what is acceptable to consumers, but quality standards can be evaluated by in-house testing to see how products compare with this standard over time.

Product improvement/optimization usually includes enhancing one or two attributes that consumers would like improved in a product. Examples could be the texture of deli meats or frankfurters or tenderness and juiciness of certain meat cuts. Improvements in these attributes are determined by a descriptive panel and consumer testing is conducted to perceive how changes in sensory attributes impact product acceptability (Meilgaard and others 2007). Category review is often conducted with descriptive analysis and consumer acceptability testing to evaluate the sensory descriptors and intensities that describe products and how that impacts the acceptability of these products for different consumer groups. For product maintenance, employees and local residents can be used to conduct consumer testing. However, the evaluators still need to be consumers of the product. In addition, for other sensory testing such as product development and product optimization, consumers of that type of product need to be tested in demographic areas that are representative of where the product is sold. In all of these tests, the environment needs to be controlled as well as possible to minimize the variability due to anything other than potential differences in the products that are evaluated.

Kauffman (1993) stated that meat quality includes seven variables: wholesomeness, nutrition, processing yield, convenience, consistency, appearance, and palatability. Palatability has five components: tenderness, texture, juiciness, and flavor (odor and taste) (Kauffman and others 1990). Booth (1990) stated that people eat foods they like, including meat, and sensory properties impact on those likes. However, Logue and Smith (1986) reported that liking fresh meat was not related to liking fish and neither of those was related to liking restructured meat products. This demonstrates that consumers expect and emphasize different sensory characteristics for various meat products (Chambers and Bowers 1993). For example, consumer studies have revealed that tenderness is the most important attribute of beef (AMSA 1978) and chicken, but that this attribute is not as important in other species since it is not so variable. This has led researchers to determine the relationship between consumer acceptability and objective measurements of tenderness in meat products (Lyon and Lyon 1990; Schilling and others 2003). These factors are measured objectively, but the most important perception is evaluated by sensory panels. Since meat cookery has a significant influence on sensory characteristics, its selection is an integral part of sensory evaluation (Cross 1987). The sensory properties that consumers want depend on species as well as whether the food is being purchased, stored, cooked, or eaten (Chambers and Bowers 1993). To fully understand consumer acceptability testing in meat products, it must be understood what attributes are important to consumers for each particular study. Objectives will often be similar when testing different muscle food products, but quality attributes that are important to consumers may differ among different muscle food products.

10.4.7 Quantitative Tests

Quantitative tests are used to determine overall preference or liking of food products and their properties as well as to determine intensities of certain descriptors. Preference tests are simple and indicate preference between two products. Rating tests determine acceptability, can easily be used for two or more products, and can also infer preference if one exists among the products that are being tested. Preference tests include paired preference tests (two samples), rank preference (three or more samples), and multiple paired preference tests (three or more samples). The most commonly used consumer test is the 9-point hedonic scale that was created by the U.S. Army Quartermaster Food and Container Institute in response to unacceptable rations that were served to troops in World War II (Peryam and Pilgrim 1957). Another type of consumer test that can be used is just right scales. These tests indicate if a sample has the right amount of an attribute such as saltiness or if there is too little or too much of this attribute. Some important parts of consumer tests are random number assignments to treatments and randomization of sample order to prevent biased data.

10.5 Sensory Characteristics of Meat

For most meat products, the characteristic flavor is due to the lipid composition of foods since most meat products have fairly similar volatile aroma compositions with the exception of the break down products from the lipids. The majority of meat products have a characteristic flavor that is species dependent.

However, the most important sensory properties for most meat products are tenderness, juiciness, and flavor that are dependent on chemical characteristics such as pH, water-holding capacity, aging time, and antemortem factors such as nutrition, stress, and breed

10.5.1 Pork

Pork appearance is a very important sensory attribute. If pork is too pale or too watery, then it will be undesirable to most consumers and will likely be dry and tough once it has been cooked (Kauffman and Marsh 1987). Pork color is also indicative of the functionality of pork meat in either a marinated or processed meat product. If a raw product is pale with denatured proteins, then it will not have functional protein and therefore will not allow for the production of acceptable marinated or processed meats. Sensory appearance attributes that should be evaluated for pork include color and marbling scores. Brewer (1998) indicated that consumers' purchase intent and acceptability of pork chops increase as color becomes redder and darker. These researchers also stated that more consumers selected low or medium marbling levels when compared to high marbling levels. Aaslyng and others (2007) reported that all consumers in their study preferred tender and juicy meat with no off-flavors. In addition, these researchers reported that consumers over 30 years of age put more emphasis on tenderness than panelists under 30. Cannata and others (2010) reported that as marbling score increased, sensory juiciness and tenderness increased. This indicates that raw pork with marbling that has a pinkish-red color and minimal exudates provides the best eating experience for the consumer (Jeremiah, 1998; Brewer and others, 1998).

Raw pork has minimal aroma, but the nonvolatile components break down during cooking to produce volatile compounds that contribute to the aroma of cooked pork. These chemical reactions include the oxidation of lipids, reactions between amino acids and carbohydrates, and interactions between these reactions (Chen and Ho 1998) that are discussed in detail by Shahidi and others (1986), Whitfield (1992), and Reineccius (1994). Lipid oxidation breakdown is important because these compounds can also form during storage (prior to cooking) and can lead to off-flavors that occur in the meat in both raw and processed pork products. The most significant group of compounds that form from lipid oxidation is aldehydes, of which hexanal is commonly used as an oxidation indicator. Addition of nitrite to a product vastly reduces the formation of aldehydes since it prevents nonheme iron from catalyzing the oxidation reaction. Pyrazines, pyradines, thiazoles, thiophenes, furans, oxazole, and pyrroles can be formed due to Maillard browning in grilled pork and contribute to the flavor that is associated with grilling.

Aroma/flavor of pork is affected by antemortem factors such as age, breed, sex, nutritional status, stress level, and fat composition. Postmortem factors include slaughter, carcass handling, aging, and cooking method (Imafidon and Spanier 1994). The cooked aroma of pork can be evaluated through descriptive analysis and the common aroma descriptors that are used for pork or ground pork products include cooked pork/brothy, cooked pork fat, lean pork, serumy/bloody, grainy, cardboardy, painty, fishy, livery/organy, soured, browned, burnt, and others (Miller 1998). Other descriptors could also be used depending on the product that is being evaluated.

Terms that are used to define aromatics can also be used to define pork flavor. Other common terms that are used include the basic tastes: salty, sour, sweet, and bitter. Additional terms that are commonly used but are not necessarily considered flavors are metallic, astringent, and aftertaste. According to AMSA (1995), whole-pork products and whole-beef products that have been cooked are often evaluated for flavor, texture, and water-holding capacity using an 8-point scale for juiciness (1 = extremely dry, 8 = extremely juicy), muscle fiber and overall tenderness (1 = extremely tough, 8 = extremely tender), connective tissue amount (1 = abundant, 8 = none), flavor intensity (1 = extremely bland, 8 = extremely intense), and off flavor intensity (1 = extremely bland, 8 = extremely intensity). In addition, off-flavors can be characterized by descriptors such as acid, liver, metallic, fish-like, old, rancid, bitter, and sour.

10.5.2 Beef

According to AMSA (1995), trained descriptive analysis of beef usually includes initial juiciness, sustained juiciness, tenderness (first impression/overall impression), amount of connective tissue, and flavor intensity. These attributes are important since beef flavor, tenderness, juiciness, and connective tissue are

generally the most important sensory attributes to consumers. Miller (1994b) indicated that the odor attributes that describe beef include cooked beefy/brothy, cooked beef fat/serumy/bloody, grainy/cowy, cardboard, painty, fishy, livery/organy, grainy, medicinal, and putrid/soured. Sensory perception of beef quality is dependent on various muscles, animal age, breed, sex, amount of intermuscular fat, cookery, and degree of doneness. Researchers at Kansas State University and Texas A&M University recently developed a beef flavor lexicon in an effort to understand the beef aromas of value-added cuts that are currently offered by the U.S. beef industry. These researchers reported that beef flavor and aroma could be characterized by 41 attributes, but that the major sensory descriptors or notes included beef flavor, browned/roasted, bloody/serumy, fat-like, metallic, liver-like, green-haylike, sweet, sour, salty, bitter, and umami. These researchers indicated that this beef lexicon will allow the beef industry and researchers to characterize and differentiate flavor attributes among beef products and cuts (Phillip and others 2010).

Similar to pork, beef flavor is predominantly formed due to the volatile compounds that form during cooking due to Maillard browning and lipid oxidation (MacLeod 1998). Beef flavor has been studied extensively and a synopsis of this work is reported by MacLeod (1998). The volatile compounds that contribute the meaty aroma to beef can be defined by approximately 10 different odor qualities that are a result of volatile compounds in beef (Galt and MacLeod 1983), including buttery, caramel, roasted, burnt, green, sulfur, fragrant, fatty, and nutty qualities. In addition, sweetness has been associated with l-amino acids and monosaccharides that are present in the meat. The savory taste of beef is linked to monosodium glutamate, 5′-inosine monophosphate 5′ guanosine monophosphate, and other peptides. These compounds are also responsible for flavor enhancement in the meat. The difference between beef flavor and other species such as pork and lamb is primarily due to different fatty acid profiles and the volatile compounds that break down from those fats.

10.5.3 Poultry

Raw broiler and turkey breast should be pinkish white. Similar to pork, poultry breast meat can be pale, soft, and exudative, which occurs more frequently at times of increased environmental stress. Leg meat is made up of a high percentage of red muscle fibers and therefore does not have the same quality problems that can occur in breast meat during times when environmental stress levels are high. The aroma of chicken broth is very similar to that of beef and is predominantly characterized by 16 odors. This is very important because it indicates that the meaty note of beef and poultry broth is due to the same compounds and that the fat component of the broth or meat is what makes beef broth flavor different from chicken broth flavor as well as meat from other species. This is important because it helps flavorists understand how to synthesize various meat flavors.

Fanatico and others (2007) reported that chicken breast could be evaluated by basic tastes (sweet, salty, sour, and bitter), cooked white meat, white meat fat, blood serum aromatic, sweet aromatic, astringent, metallic, and aftertastes. Thigh meat was described using similar attributes with the exception of using cooked dark meat and dark meat fat in the place of white meat descriptors. Other researchers (Zhuang and others 2009) have also included brothy, barnyard/wet feathers, chickeny, cardboardy, cohesiveness, hardness, tenderness juiciness, chewiness, and a few other textural attributes. One of the most important determinants of consumer acceptability and sensory quality is chilling and deboning time. It is recommended that broiler breast meat be properly chilled through water or air chilling and left on the bone for at least 4 h postmortem to make sure that rigor mortis has reached the point that the muscle will not contract when it is taken off of the bone (Sams 1999). This ensures that the meat will be tender and juicy as long as the breast meat is not pale, soft, and exudative.

10.5.4 Lamb

Lamb meat is widely consumed throughout the world and is often imported to European countries. In Mediterranean countries such as Spain and Italy, lambs are generally raised to light carcass weights and are usually reared on milk and/or concentrated feed. In contrast, in more Northern and Central European countries, lambs are generally raised on grass and slaughtered at much heavier weights (Font i Furnos and others 2006). From a sensory standpoint, lamb can be described and evaluated by lamb aroma, lamb flavor,

tenderness, juiciness, and flavor intensity (Goodson and others 2001; Hoffman and others 2003). In addition, diet has a large impact on lamb meat quality. Resconi and others (2010) matched volatile compounds and sensory properties for meat from lambs that were fed by pasture, pasture and concentrate, and concentrate and Lucerne hay. Lambs that were only fed through the pasture could be described as more rancid, acidic, and metallic than other samples and had higher concentrations of aldehydes such as hexanal and octanal. When lambs were fed concentrate and Lucerne hay, they had more intense fat and lamb flavor and had higher concentrations of some ketones and other aldehydes that are generally not as offensive as hexanal, octanal, and nonanal. Hoffman and others (2003) evaluated six cross-bred lamb types and determined that breeding wool-type ewes to mutton rams did not impact lamb meat quality when other factors such as diet and age remained constant. In Goodson and others (2001), differences in sensory quality were evaluated between Dorset × Suffolk crossbred lambs with and without the callipyge gene. The Callipyge phenotype was superior in feed efficiency and carcass traits. However using similar animals, Hoffman and others (2003) reported that the Longissimus muscle was negatively impacted when the Callipyge gene was present, with respect to tenderness and juiciness, but that there was no difference between the tenderness and juiciness of biceps femoris muscle from lambs with and without the Callipyge gene.

10.5.5 Goat

Goat meat is eaten throughout the world and is becoming a more common meat choice in many parts of the United States. There are many species of meat goats, but four common types of goats are produced in the United States and include the Spanish Meat Goat, Tennessee Meat Goat, South African Boer Goat, and New Zealand Kiko Goat. Carlucci and others (1998) evaluated the sensory properties of meat from goats that were intensively and extensively reared and either castrated or intact. These researchers reported that extensively reared goats had more tender and juicy meat than the intensively reared goats that were defined as more stringy. In addition, meat from the castrated goats had a more intense meaty flavor than meat from the intact goats, and meat from the intact goats had a more goaty and bloody aroma and flavor when compared to the castrated goats. Rodbotten and others (2004) evaluated the sensory properties of meat from many types of meat animals and indicated that goat meat from the loin was more similar to beef than any other species. Sensory descriptors that matched goat meat in the principal components analysis were sweet, metallic, liver, and gamey. Rhee and Myers (2003) and Rhee and others (2003) also evaluated ground goat meat and meat loaves and reported the following aromas: cooked goat, nutty, grassy, grainy, gamey, sour, musty, and cardboard. Other sensory attributes included the basic tastes and gamey, musty, nutty, and cardboard aftertastes. Rhee and others (2003) also reported that meat consumers could distinguish between meat loaf, and chili that was made from either beef or goat meat.

10.5.6 Venison

Venison refers to the meat from different types of deer that are in the *Cervidae* family and can also refer to meat from moose, elk, caribou, and reindeer. Limited literature is available on the sensory properties of venison with the exception of research reported by Wicklund and colleagues. These researchers have published numerous papers on the sensory properties of venison between 2000 and 2010. Hutchinson and others (2010) reported on the sensory acceptability of red deer and determined that color, aroma, aroma strength, flavor, flavor strength, game flavor, tenderness, and juiciness were the most important characteristics to consumers when they evaluated *gluteus medius.* These researchers reported that pelvic suspension at the aitch bone prior to slaughter for 24 h improved tenderness which made the venison more acceptable to the consumers. In addition, Wicklund and others (2003) reported that red deer meat could be profiled by grassy flavor, game flavor, sweetness, tenderness, juiciness, livery flavor, bloody flavor, and astringency. These researchers evaluated the effects of grass-feed versus pellet-feed (commercial deer feed) on venison sensory quality and reported that meat from deer that were fed the pasture-fed diet had a greater percentage of omega-3 fatty acids in the *Longissimus* muscle and that the only sensory difference between the meat was that the meat from deer that were fed the pasture diet had a more intense grassy flavor than meat from the pellet-fed deer.

10.5.7 Processed Meats

The sensory properties that are used to evaluate and determine the quality of processed meat are different than those of raw meats. Processed meats are often defined by texture since deli meats and emulsion-type products need to have enough protein bind to be sliced thinly and/or have acceptable texture and yields. Processed meats are commonly evaluated for springiness, cohesiveness, hardness, chewiness, juiciness, fracturability, and tenderness and can be related to instrumental texture analysis. Other aspects of processed meats that are important are the impact of salt in meat products since chloride ions improve yields in marinated products, and lead to protein extraction that is necessary to form emulsion and deli-type products. The sodium in salt provides a salty flavor but also enhances flavor and can enhance the shelf life of products due to preservation. Nitrite is often added to processed meats along with salt to cure meat products. Nitrite is a very important ingredient because it inhibits *C. botulinum*, but it also reduces to nitric oxide and binds to myoglobin. When it is heated, nitrosylhemochrome is formed. This prevents heme iron to be liberated from myoglobin which prevents warmed-over flavor in meat products, due to the inhibition of lipid oxidation. Addition of nitrite inhibits the formation of hexanal and other carbonyl compounds that are responsible for oxidation and rancid flavors in meat products.

Flavors in processed meats are predominantly due to the spice blends that are added for certain meat products, especially sausage, but additional flavors occur in aged meats such as dry-cured ham and bacon due to aging of the meat after salt preservation. These flavors are due to raw material, breed, animal diet, age, curing ingredients, and aging methods. In the United States, dry-cured hams are aged anywhere from 3 to 12 months and vary in flavor among companies. However, hams have many of the breakdown products from lipid oxidation and amino acid breakdown due to calpains and cathepsins. Some Spanish dry-cured hams such as Iberian have a very different flavor profile when compared to other dry-cured hams because of the different feed (acorns and free range for Iberian vs. grain fed in the United States), aging time (2 years for Iberian vs. 3 to 12 months for U.S. hams), and covering of the lean with lard (in Iberian ham) late in the aging process to allow cathepsins to continue to break down proteins and give the desired flavor. The differences in feed cause lower concentrations of linoleic and linolenic fatty acids in the Iberian hams, which lead to a lower concentration of the oxidation compounds such as hexanal (Toldra 2010).

10.6 Relationships between Volatile Flavor Compounds, Sensory Descriptors, and Consumer Acceptability

The meat industry and academia have made significant contributions to the study of flavor which has become invaluable for the increased understanding of the desirable flavors in meat. Flavor is an essential parameter that contributes to the overall acceptance and marketing of meat and meat products. A product must live up to its flavor expectations for continued customer patronage (Drake 2004). Each customer is unique and an understanding of their flavor expectations is needed to bring about the desired flavors.

Flavor can be accurately and reproducibly determined by the composition of its volatile compounds. Currently, gas chromatography mass spectrometry (GCMS) is the technique of choice to quantify volatile compounds in meat and meat products (Elmore 2009; Flores 2009). To obtain an increased understanding of product flavor, relating particular flavors to volatile compounds is required. Establishing significant correlations between specific chemical compounds with particular flavors requires extensive instrumental and sensory analysis; nevertheless it is a combination of both techniques that will generate valuable results (Rothe 1988; Marsili 2007). Once specific links are positively established between volatile compounds and sensory data, powerful information for relating flavor to consumer acceptability and for monitoring flavor quality can be made (Drake 2004).

An approach to relating analytical and sensory data and eventually to consumer preference consists of these steps: (1) determining the volatile compounds that impact flavor using GC techniques such as gas chromatography olfactometry (GCO), which can help identify volatile compounds that play important roles in overall flavor by selecting those compounds present at their sensory threshold range; (2) developing a lexicon and conducting descriptive sensory analysis to identify the sensory characteristics that

describe and differentiate the product; (3) conducting consumer acceptability of the product; (4) utilizing multivariate analysis (MVA) to explain the relationships between volatile flavor compounds, sensory descriptors, and consumer acceptability of the product; and (5) carrying out model systems as similar as possible to the actual product to verify the role of the identified volatile flavor compounds on sensory perceived flavor and to confirm that these are the compounds in the product responsible for the flavor notes preferred by consumers.

A considerable amount of research on meat volatiles has been focused on their identification and quantification with limited approach to their true contribution to meat aroma and flavor. However, current studies have concentrated on linking flavors to specific volatile compounds as well as isolation and concentration techniques which provide an extract with an aroma representative of the original product (Calkins and Hodgen 2007). GCMS is widely used for the identification and quantification of volatiles in meat and has greatly contributed to the success of meat flavor characterization. Deciding on methods which efficiently extract target compounds from the sample matrix is one of the most important steps since the extraction technique will influence the volatile profile and also this is where analyte loss often occurs (Steffen and Pawliszyn 1996). Oftentimes this is not a clear-cut approach, and using an inappropriate sample preparation method often results in failure. Much has been written on the advantages and disadvantages of various extraction techniques as well as selecting the appropriate method for certain types of analytes and samples (Parliament 1997; Wampler 1997; Werkhoff and others 2002). Even under ideal conditions, it is impossible to resolve all components considering the complexity of sample matrices. Alternately, multidimensional gas chromatography (MDGC) may result in a more meaningful separation of target analytes (Qian and others 2007). Additionally, the use of gas chromatography time-of-flight mass spectrometry (GC-TOFMS) in combination with sophisticated peak deconvolution software can also achieve the desired resolution of chromatographic peaks (Marsili 2007). With conventional MDGC, only particular segments of the effluent are transported from a preseparation column onto an analytical column while comprehensive MDGC (GC × GC) involves the transfer of the entire effluent from the first column onto a second orthogonal column through a modulation interface so that complete two-dimensional data can be obtained for the entire run of the first column (Qian and others 2007). A more accurate quantification is obtained due to a reduced occurrence of coeluting peaks and greater sensitivity is achieved by fast-GC analysis with the second column (Phillips and Beens 1999; Qian and others 2007). Marsili (2007) recommended investing in GC-TOFMS using a peak deconvolution software to avoid missing important analytes and accurately determine peak area. Ultimately, we are still more interested in the capability of an analytical method to accurately identify and quantify the analytes responsible for flavor. A number of volatiles may be identified in a food sample but not all of these compounds contribute to the characteristic aroma and flavor of the product. In addition, it is known that some volatile compounds are present at very low concentrations in the parts-per-million (ppm) or part-per-billion (ppb) levels, which may be below the detection limits of conventional GCMS (Qian and others 2007). More attention should be given to compounds with sensory significance instead of merely identifying volatile compounds even though they may or may not contribute to a particular aroma (Qian and others 2007).

Gas chromatography olfactometry (GCO), also known as "GC sniffing," has emerged as a valuable analytical tool in flavor research because it characterizes the aroma of single compounds or complex mixtures of volatiles eluting from the sniffing port (Mistry and others 1997). GCO has been useful in further screening of the volatile compounds that play key roles in overall flavor and aroma by identifying compounds that are present in the sensory threshold range. The effluent is split into two portions, one portion proceeds to an instrumental detector such as flame ionization detector (FID) or mass spectrometer (MS) and the other portion goes to a sniffing port (Bett-Garber 2007). In this method, the human nose is also used as a detector which has a theoretical odor detection limit of about 10^{-19} moles, making GCO a very sensitive tool for the detection of aroma active volatiles (Reineccius and Heath 1999). Another advantage of this method is that volatile compounds with different aromas and those that have small variations in their retention indices can be differentiated from one another by GCO (Acree 1993). Much has been written about GCO methods, which can be divided into four categories: dilution methods (aroma extract dilution analysis (AEDA)), combined hedonic aroma response measurements (CHARM), detection frequency methods (olfactometry global analysis), and intensity methods (Osme) (Acree and others 1984; McDaniel and others 1990; Grosch 1993; Ott and others 1997; Pollien and others 1997).

Recently, GCO has been coupled with MDGC or GC × GC—Olfactometry for the advanced analysis of aroma impact compounds in the essential oils area (Gomes da Silva and others 2008).

Once aroma impact compounds have been identified, it is easier to focus on these compounds and determine their chemical origin or flavor influences. Using this approach, GCO has been utilized to characterize the aroma impact compounds in roasted Mini-pig pork (Xie and others 2008) and cold smoked salmon (Jónsdóttir and others 2008) and has proven its use in the identification of the volatile compounds that contribute to the overall flavor of these products. Correlations have been found between the green, roasted, meaty, moldy, and fatty flavors of roasted Mini-pig pork with the identified aliphatic aldehydes, benzene-derived aldehydes, ketones, terpenoids, furans, oxygenous benzene derivatives, sulfur compounds, pyrazines, and aromatic hydrocarbons. In the case of cold-smoked salmon, guaiacol has been related to its smoky aroma while microbial metabolites 3-methylbutanal and 3-hydroxybutanone were associated with sweet aromas.

Another factor of utmost importance in analysis of aroma impact compounds is panelist training and calibration. Training sessions usually consist of practice runs by sniffing original samples and prepared samples extracted by the specific method of choice. These sessions are carried out to familiarize panelists with the aroma of the compounds, to practice proper breathing techniques, and to establish verbal descriptors for the samples (Rousseff and others 2001). The number of hours of training differs with each sample as well as the number of terms used. Once trained, panelists can operate as with any analytical instrument where data can be replicated for each sample.

Correlating GCO data with sensory descriptive analysis is not a technique without limitations. It seems that the intensity ratings of GCO compounds would correlate better with descriptive data rather than their corresponding threshold concentrations (Blank 1997; Boylston 2007). Threshold concentrations can change with experimental conditions causing intensity function changes brought about by relative proportions of volatile compounds in the sample. Synergy problems wherein aroma impact compounds are perceived differently when they are smelled together or individually may also be encountered (Marsili 2007).

Descriptive sensory analysis has developed into a robust tool for flavor characterization. It is perhaps the most powerful of all sensory tools and is the technique of choice for qualitatively and quantitatively differentiating foods as well as for defining the relationships between sensory and instrumental perception (Drake 2004; Marsili 2007). Descriptive analysis is a sensory method used by human subjects who have been specifically trained for the purpose of identifying and quantifying the attributes of a food or product (Hootman 1992; Meilgaard and others 2007). The analysis may be focused on certain aspects such as flavor, aroma, texture, and aftertaste or it may encompass all the parameters of the product. A defined sensory language or lexicon is often developed to describe the sensory properties of the product. Variations of this method include the Flavor Profile Method, Quantitative Descriptive Analysis® (QDA) and Spectrum Method all of which are reviewed in detail elsewhere (Stone and Sidel 1993; Keane 1992; Muñoz and Civille 1992).

In all of these methods, establishing a trained panel to perform descriptive analysis is a critical matter. As with GCO panelists, extensive training and calibration of sensory panelists cannot be exaggerated. The primary goal of this training is for panelists to operate in unison as an instrument where each individual serves a function equivalent to an individual sensor on an instrument (Drake 2007). Thus, the sensory instrument or trained panel should be as precise and reproducible as possible. It is also important for the panel to be trained and maintained by a sensory professional with experience in the analytical method being applied so as to produce sensitive and meaningful results (Hootman 1992). Finally, panel and panelist performance have to be monitored throughout the training to identify the problem areas and to track the discriminatory ability of the panel.

An optimized flavor lexicon is said to be both discriminative and precise such that it has the ability to readily relate both consumer and instrumental data (Drake and Civille 2003). Thus, when developing a lexicon it should comprise of precise sensory descriptors that are easily understood and replicated in order to better define the sensory characteristics of the product. It is also helpful to develop the sensory language from a wide-ranging representative sample set that shows all the potential variability within the product. This step can be followed by selecting the most representative attributes from the developed lexicon for further analysis. For example, Flores and coworkers (1997) developed a lexicon for dry cured

ham flavor where they selected the following attributes as most representative of the desirable and off-flavors of the product: fat complex, boar taint, barnyard, haylike/musty, brown spice, pickling spice, smoky, pork, serum, pungent, sour, salty, bitter, astringent, and metallic.

We have seen how developments in analytical and sensory methods have contributed to a better understanding of the complex relationships between the volatile composition and sensory perception of meat flavor. However, the ultimate objective is to relate analytical measurements to sensory perception and eventually to consumer preference. This final step is deemed important since what is desirable to one consumer may not be to another. Also, knowledge of the target flavor profiles can help pinpoint target consumer market segments.

Consumer acceptability is an affective test which helps evaluate the differences in consumer liking of products. A minimum of 100 individuals who are regular consumers of the product is recommended when conducting this type of test so that statistical validity of the results can be obtained. Untrained panelists are preferred because the typical consumer does not sequentially identify and quantify specific attributes in their foods (Drake 2007).

MVA is a statistical tool that is widely used by flavor chemists and sensory professionals alike to simplify and help in the interpretation of descriptive, consumer and analytical data (Karow and others 2007). This technique starts with the application of exploratory algorithms such as principal component analysis (PCA) and cluster analysis which are designed to reduce large, complicated data sets into a more comprehensible format. In PCA, the goal is to reduce the dimensionality of the original data set since a small set of uncorrelated variables is easier to grasp and utilize in further analysis compared with a larger set of correlated variables (Dunteman 1989). This method allows as much as 75–90% of the total variability of a data set with 25–30 variables to be explained with as few as two–three principal components. It acquires data from numerous sample analyses and generates a vector made up of the weighted contributions of each of the monitored volatile compounds or sensory descriptors (Eaton and others 2007). The PCA biplot consists of the projection of the vector onto a two-dimensional plane. Samples with the same composition will contain vectors whose projected points cluster together on the biplot. These representations can only be considered reliable if the sum of the variability percentages associated with the x and y axes of the principal components biplot are greater or equal to 80%. Conversely, cluster analysis groups together observations based on the degree of similarity among their ratings in the same way that PCA groups together attributes/compounds based on their degree of correlated behavior (Meilgaard and others 2007). An important application of cluster analysis in sensory acceptance testing is the identification of groups of respondents that have different patterns of liking across products (Meilgaard and others 2007). Agglomerative hierarchical clustering (AHC) is a cluster analysis algorithm which is used to group homogeneous objects (classes) on the basis of their description from variables that indicate either similarity or dissimilarity between the objects. Compared with PCA which has long been used as a stand-alone method in sensory and instrumental analyses, AHC is seldom used in sensory studies aside from external preference mapping (Schilling and Coggins 2007). This method allows a better understanding of the hedonic scaled consumer data by making the results easier to interpret (Ares and others 2006; Schilling and Coggins 2007). Another advantage of AHC is in the graphical presentation of its results using a dendrogram which shows the progressive grouping of the data. Schilling and Coggins (2007) coupled AHC with traditionally used analyses in the evaluation of hedonic scaled consumer data relating to chicken nuggets, retorted ham, fluid milk, and cooked shrimp. The authors concluded that combining AHC with traditional analyses was successful at grouping consumers together based on product preference and liking. Once PCA and AHC show distinct patterns, external preference mapping and flavor mapping can be performed to reveal the similarity and differences among the clusters in how the perceived attributes or monitored volatile compounds relate to liking. External preference mapping is used to explain consumer preference data by descriptive sensory information and/or instrumental data (Ebeler 1999; Schlich 1995; McEwan 1996; Murray and Delahunty 2000). Individual consumer preferences are regressed onto the first two principal components of the covariance matrix of descriptive or analytical ratings across the products such that the dimensions of the descriptive or instrumental analysis space are the predictor variables while consumer acceptability is the response variable (Schlich 1995; Guinard 1999, 2002). An important contribution of this technique is that it can guide product

optimization and development by identifying the main market segments and drivers of liking (McEwan 1996; Guinard 2002). External preference mapping has been applied in a number of studies with various products such as cheddar cheese (Casapia and others 2006), dulce de leche (Ares and others 2006), and vanilla ice cream (Guinard 2002).

It is now possible to formulate a theory as to which volatile compounds are responsible for specific flavors and whether they impact consumer liking based on analytical, sensory, and statistical data. At this point, one should try to validate their hypothesis. One approach is to perform aroma recombination or model system studies. This process involves combining the key aroma impact compounds determined from your sample in specific concentrations followed by evaluation of the reconstituted food aroma by a trained sensory panel (Mistry and others 1997). In aroma recombination studies on tomato paste, Guth and Grosch (1999) identified and quantified the key odorants using AEDA, GCO of the headspace (GCOH), and stable isotope dilution assay (SIDA). The key odorants were then incorporated into an aqueous model system for tomato paste and evaluated by a trained panel. In this way, contributing odorants of tomato paste such as 3-hydroxy-4,5-dimethyl-2(5H)-furanone (HD3F) were identified with the model mixture closely resembling the original product. Similar studies using model systems have been applied for stewed beef juice, wine, olive oil, French fries, and boiled beef (Guth and Grosch 1994; Guth 1997; Reiners and Grosch 1998; Grosch 2001).

10.7 Conclusion and Future of Sensory Evaluation

As food technology becomes more complex, the basics of sensory evaluation need to be remembered. The goal of sensory evaluation is to explain the consumer acceptability of food products. This can only be done through utilization of the sensory methodologies listed in this chapter and working together with the market research department to make sure that the appropriate questions and problems are being answered and solved. Education on appropriate utilization of sensory analysis must be continued. It is clear that most companies are utilizing sensory analysis, but quite often, the wrong methods are being utilized for the stated objectives of the studies (Stone and Sidel 1993). Simple testing can be used for most test objectives in the industry, but use of instrumental analysis, descriptive analysis, and consumer testing can help understand the relationship between volatile composition, sensory properties, and consumer acceptability of muscle food products.

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11

Meat and Functional Foods

Francisco Jiménez-Colmenero, Ana Herrero, Susana Cofrades, and Claudia Ruiz-Capillas

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11.1 Introduction

Meat and meat products are an important group of nutritionally dense foods in the diets of many consumers around the world. Although meat consumption is relatively high in developed countries, the amount of meat consumed in developing countries is increasing rapidly. By 2020, the share of developing countries in total world meat consumption will have expanded from the current 52–63% (Delgado 2003). Because of the amount consumed (Table 11.1) and because of their composition (Table 11.2), meat and meat products are important sources of a wide range of nutrients and contribute considerable proportions of the dietary intakes of various nutrients that are essential for optimal growth and development (Tables 11.3 and 11.4).

TABLE 11.1

Total meat[a] Intake (g/day) in Several European Countries[b] and Red Meat Consumption (g/day) in Different World Regions[c]

	Total Meat			Red Meat	
Country	**Men**	**Women**	**World Region**	**Men**	**Women**
Denmark	141.1	88.3	North America	85.9	57.7
Germany	154.6	84.3	Argentina, Uruguay, Paraguay	168.1	122.0
Greece	78.8	47.1	North Africa	30.0	21.7
Italy	140.1	86.1	Sub-Saharan Africa	20.7	15.0
Netherlands	155.6	92.7	Australia, New Zealand	125.7	84.1
Spain	170.4	99.2	Middle East Asia	21.6	15.7
UK	108.1	72.3	Southern Europe	59.0	43.7

[a] Total meat includes red (pork, beef, veal, lamb/mutton, unclassified), poultry (chicken, turkey, goose, duck) and others (game, rabbit, horse, goat, offal), meat and processed meat (ham, bacon, processed meat cuts, processed minced meat, sausages).

[b] Adapted from Linseisen J, et al. 2002. *Publ Health Nutr.* 5:1243–1258.

[c] Adapted from Norat T, Lukanova A, Ferrari P, Riboli E. 2002. *Int J Cancer.* 98:241–256.

Meat consumption is influenced by various kinds of factors, among them are those associated with health implications. Over the last few years, the expectation of healthiness has increasingly become a quality criterion in consumer choice of a particular food. However, the healthy image of meats has gradually been eroded by a number of factors. From a nutritional standpoint, there are negative perceptions founded on associations between the consumption of some meat constituents (e.g., fat, saturated fatty acids (SFAs), cholesterol, sodium, etc.) and the risk of major society chronic diseases (e.g., ischemic heart disease, cancer, hypertension, and obesity). Several studies report these associations and the importance of meat and meat products in consumer health (Hulshof and others 1999; Higgs 2000; Linseisen and others 2002; Norat and others 2002; Biesalski 2005; Williamson and others 2005; WCRF 2007; Demeyer and others 2008; Ferguson 2010), but the findings of these studies are not always consistent (McAfee and others 2010), although there is a general consensus in recommending moderate meat consumption (smaller portions and less frequent) as part of a balanced diet. The negative publicity is also a consequence of some nonnutritional issues such as meat safety crises relating to bovine

TABLE 11.2

Composition of Selected Meats and Meat Products (Amount in 100 g of Edible Portion)

Product	**Protein (g)**	**Fat (g)**	**SFA (g)**	**MUFA (g)**	**PUFA (g)**	**Calories (kcal)**	**Cholesterol (mg)**	**Sodium (mg)**	**Fe (mg)**	**Se (μg)**
Pork (loin)	21.43	5.66	1.95	2.56	0.61	143	59	52	0.84	36.1
Beef (top round)	23.13	3.37	1.15	1.42	0.14	129	46	64	1.97	31.9
Lamb (leg)	20.52	4.19	1.50	1.69	0.38	125	64	61	1.82	23.4
Chicken (breast)	23.09	1.24	0.33	0.30	0.28	110	58	65	0.72	17.8
Frankfurter	11.53	27.64	10.77	13.67	2.73	305	50	1120	1.15	13.8
Frankfurter (low fat)	11.00	10.00	3.69	4.69	0.93	154	44	1257	1.20	15.1
Bologna	15.20	24.59	9.67	10.52	1.12	304	60	736	1.21	26.1
Pork sausage	15.09	26.53	8.79	11.81	3.55	304	72	636	1.11	0.0
Salami	20.70	33.30	12.04	16.75	2.71	388	102	1826	1.86	—
Bacon	29.10	44.20	16.00	21.90	4.30	517	102	1420	1.86	26.1
Cooked ham	17.93	2.37	0.79	1.67	0.31	122	22	900	0.39	10.4

Source: Adapted from USDA. 2010. National Nutrient Database for Standard Reference. (Accessed May 5, 2010) Available at http://www.nal.usda.gov/fnic/foodcomp/Data/

Note: MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; SFAs, saturated fatty acids.

TABLE 11.3

Nutritional Profile of the Average Spanish Diet and Its Relationship with Consumption of Meat and Meat Derivatives

Nutrients	Total Intake/Day	Nutrients from Meat	Contribution (%) of Meat to the Intake of Each Nutrient
Calories (kcal)	2.761	442	16
Protein (g)	93.5	26.2	28
Carbohydrates[a] (g)	294	0.35	0.1
Fat (g)	126	35.3	28
Cholesterol[a] (mg)	440	175	39.8
Iron (mg)	13.6	2.3	17
Zinc (mg)	11.1	2.9	26
Magnesium (mg)	313	28.2	9
Sodium (mg)	2.995	808	27
Potassium (mg)	3.507	456	13
Phosphorus (mg)	1.534	245	16
Selenium (μg)	108	10.8	10
Thiamine (mg)	1.4	0.51	37
Riboflavine (mg)	1.8	0.32	18
Eq de niacina (mg)	33.7	10.8	32
Vitamin B_6 (mg)	1.7	0.44	26
Vitamin B_{12} (μg)	10.3	1.96	19
Vitamin A:Eq. retinol	1.427	499	35
Retinol (μg)	672	356	53
Folic acid (μg)	207	12.4	6
Vitamin E (mg)	14.2	0.14	1

Source: Adapted from MARM 2008. Valoración de la dieta española de acuerdo al panel de consumo alimentario. *Ministerio de Medio Ambiente y Medio Rural y Marino. Secretaria General Técnica*. Madrid, Spain: Centro de Publicaciones.

[a] Varela G, Moreiras O, Carbajal A, Campo M. 1996. Household budget survey Spain 1990–91. *National Study on Nutrition and Food Consumption*, 1991, Vol. I. Instituto Nacional de Estadística. Madrid, Spain.

TABLE 11.4

Contribution (%) of Meat and Meat Products to the Intake of Total Fat, SFAs, MUFAs, PUFAs, and TFAs in the Diet of Consumers in Several Countries

Countries	Total Fat	SFAs	MUFAs	PUFAs	TFAs
Belgium	26.7	25.1	32.2	19.4	20.7
Finland	23.0	19.5	28.5	23.0	<0.1
France	23.4	20.2	30.0	19.1	11.4
Germany	28.9	18.2	43.0	21.9	5.3
Greece	11.4	13.2	10.9	8.9	14.6
Italy	13.3	15.3	12.6	6.9	13.4
Netherlands	21.2	20.1	29.5	12.9	12.3
Spain	23.7	29.0	24.2	15.8	29.8
UK	18.5	17.1	19.2	12.2	10.3

Source: Adapted from Hulshof KFAM et al. 1999. *Eur J Clin Nutr.* 53:143–157.

spongiform encephalitis, avian flu, and others. Recently consumer perceptions of meat quality have been affected by extrinsic cues related to environmental aspects and production practices, which often advocate the need to reduce levels of meat consumption in industrialized societies. However, it would appear that reducing meat consumption does pose serious nutritional challenges for some key nutrients (Millward and Garnett 2010).

Changes in consumer demand and growing market competition have prompted a need to improve the quality and image of meat, not only to prevent the loss of market share attendant on a negative perception of meats, but also to create new market niches, for instance through the development of products with health-beneficial properties. This opportunity, which addresses the needs of consumers as well as updating nutrient dietary goals, has lent growing impetus in the meat industry to the modification of meat production and processing systems.

11.2 Functional Foods

During the last few decades, the link between nutrition and human health has been strongly established (WHO 2003), and based on this new knowledge an improved appreciation of the potential beneficial or adverse effects of nutrients and other components in the diet has led to the realization that it is possible to design new and healthier food items with specific characteristics that are capable of influencing the body functions over and above meeting basic nutrition needs. These foods have come to be known as "functional." Functional foods have made a strong impression on global markets over the last decade and have rapidly gained market share as value-added products. There are currently various different definitions for functional foods (Table 11.5). The term functional foods was first introduced in Japan in the 1980s, and from there it spread to the United States and Europe. The concept of foods for specific health use (FOSHU) was established by the Japanese Ministry of Health and Welfare in 1991 (Table 11.5). Foods are considered functional because of their effects (scientifically demonstrated) and not their origin, and therefore the category of functional foods may include both natural (unmodified) foods and foods that have been transformed by means of technological or biotechnological processes (Figure 11.1) (Roberfroid 2000; Ashwell 2002; Howlett 2008). Changes in composition are not enough for a food to be considered functional; it must also be satisfactorily shown that consumption has a beneficial effect on certain population groups. In Europe, a consensus was arrived at on the scientific criteria that need to be followed for purposes of assessment or design of foods for which healthy properties are claimed (Aggett and others 2005).

11.3 Functional Meat Products: Identifying and Improving the Content of Physiologically Active Compounds

As previously reported, meat is a highly nutritious food, providing valuable amounts of protein, fatty acids, vitamins, minerals, and energy (Tables 11.2 and 11.3), although it also contains some components which can have negative health implications depending on a variety of factors. In the same way, as the health risks of meat components must be considered, there are many healthy components which are equally important in establishing meat health implications (Williamson and others 2005; McAfee and others 2010). Meats contain compounds thought to be physiologically functional, so meat could therefore be considered a functional food without any additional processing (Arihara 2004; Hasler and others 2004; Ferguson 2010). In order to promote more meaningful public health messages in relation to meat consumption and in order to develop functional meat products, the fullest possible understanding of both positive and negative health implications is required. Only through such an understanding, we can devise suitable strategies to achieve effective control and modulate their composition and other characteristics as needed (Jiménez-Colmenero 2007a).

Different strategies can be effectively used to improve (increase or reduce) the presence of bioactive compounds in meat and meat products and thus develop meat-based functional foods. Most research into

TABLE 11.5

Selected Definitions of "Functional Food"

Japanese Ministry of Health and Welfare: FOSHU are foods which, based on knowledge concerning the relationship between foods or food components and health, are expected to have certain health benefits and have been licensed to bear labeling claiming that a person using them may expect to obtain that health use through the consumption of these foods (Arihara, 2004).
FUFOSE: A functional food is satisfactorily demonstrated to affect beneficially one or more target functions in the body, beyond adequate nutritional effects, in a way that is relevant to either an improved stage of health and well-being and/or reduction of risk of disease. A functional food must remain food and it must demonstrate its effects in amounts that can normally be expected to be consumed in the diet: it is not a pill or a capsule, but part of the normal food pattern (Diplock and others 1999).
Institute of Food Technologists: Foods and food components that provide a health benefit beyond basic nutrition (for the intended population), including conventional foods, fortified, enriched, or enhanced foods, and dietary supplements. They provide essential nutrients often beyond quantities necessary for normal maintenance, growth, and development, and/or other biologically active components that impart health benefits or desirable physiological effects.
American Dietetic Association: Functional foods are foods that have health benefits beyond the nutrients they contain.
International Food Information Council Foundation: Any food or food component that may provide a health benefit beyond basic nutrition.
Health Canada: A functional food is similar in appearance to, or may be, a conventional food, which is consumed as part of a usual diet and is demonstrated to have physiological benefits and/or reduce the risk of chronic disease beyond basic nutritional functions.
Food and Nutrition Board of the National Academy of Science (U.S.): "potentially healthful products that may include any modified food or food ingredient that may provide a health benefit beyond the traditional nutrients it contains."
Korea: Functional foods are defined as dietary supplements whose purpose is to supplement the normal diet and have to be marketed in measured doses, such as in pills, tablets (Kim and others 2006).
Food and Agriculture Organization: Functional foods should be foods similar in appearance to conventional foods (beverage, food matrix), consumed as part of the usual diet which contains biologically active components with demonstrated physiological benefits and offers the potential of reducing the risk of chronic disease beyond basic nutritional functions (FAO 2004).
China: Functional foods are foods with a special health function, suitable for consumption by defined special groups of people. These foods exert effects on specific body functions and should not be confused with drugs, which are used for therapeutic purposes. Functional food (or "health food") products also include nonfood forms such as extracts or tablets. The main differences compared to regular foods include a specific functional effect, a narrowly specified user group, and a recommended daily dosage.

Source: Adapted from Kotilainen L, et al. 2006. Health enhancing foods. Opportunities for strengthening the sector in developing countries. Agriculture and Rural Development. The International Bank for Reconstruction and Development. The World Bank. Discussion Paper 30. [Accessed 2010 April 14]. Available from http://web.worldbank.org, modified.

meat-based functional foods has been founded on animal production (genetic and nutritional) and/or technological strategies aimed at influencing the presence of certain compounds with health implications (Jiménez-Colmenero and others 2001; Arihara 2006; Jiménez-Colmenero 2007a). However, other factors must also be considered, such as food distribution and storage and the conditions in which they are consumed (Jiménez-Colmenero 2007a).

Following is an overview of the endogenous (naturally present in meat) or exogenous (mainly from vegetable and marine sources) bioactive compounds that are considered in approaches to the design of healthier meat and meat products, their implications for human health, and the strategies employed to improve the contents of them (Table 11.6). The role of these substances is analyzed individually, but in many cases the optimization process affects more than one of them simultaneously.

11.3.1 Improving Fat Content

Dietary fat is needed as a metabolic energy source and supplier of essential nutrients (fat-soluble vitamins and fatty acids), but it must be consumed in moderation for reasons of human health. There is growing evidence of the association between dietary fat (total, SFAs, and cholesterol) and chronic disorders

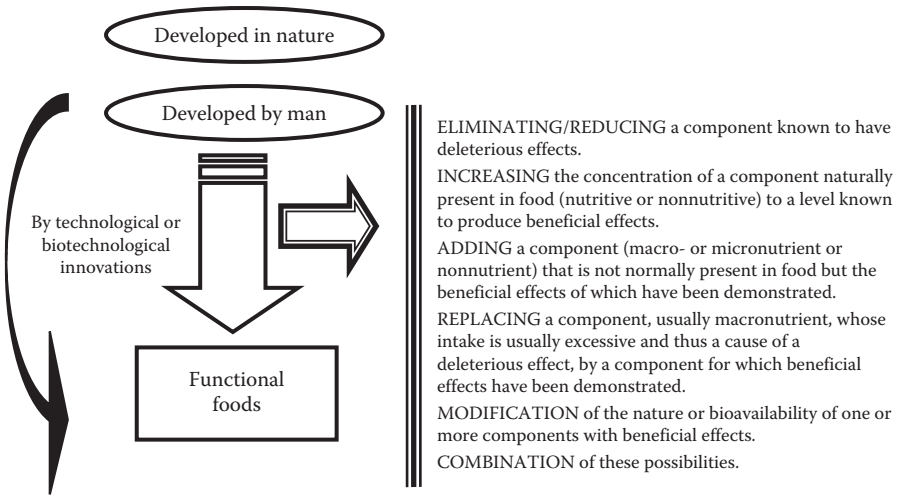

FIGURE 11.1 Approaches for development of functional foods. (Adapted from Jiménez-Colmenero, F. 2007a. In: Hui YH, Associate editors, Chandan and others editors. *Handbook of Food Products Manufacturing*. New Jersey: John Wiley & Sons, Inc. pp. 989–1015.)

such as ischemic heart disease, some types of cancer, and obesity (Hulshof and others 1999; WHO 2003; Williamson and others 2005; McAfee and others 2010; Ferguson 2010). Recommendations for optimal intake of total and unsaturated fatty acids have been proposed (WHO 2003). Dietary fat intake should ideally account for between 15% and 30% of total diet energy. According to dietary recommendations for the intake of specific fatty acids as a proportion of total diet energy, no more than 10% of calorie intake should be from SFAs, 6–10% should be from polyunsaturated fatty acids (PUFAs) (n-6, 5–8%; n-3, 1–2%), around 10–15% should be from monounsaturated fatty acids (MUFAs), and <1% should be from *trans*-fatty acids (TFAs). It is also recommended to limit cholesterol intake to 300 mg/day.

Although dietary recommendations refer to the overall diet, meat and meat products are some of the most important sources of dietary fat (Tables 11.3 and 11.4). Changes in the amounts and the lipid profiles of such products could help improve the nutritional quality of the diet, and so lipids are among the functional ingredients that have received most attention, particularly (in quantitative and qualitative terms) with respect to the development of healthier meat products (Jiménez-Colmenero 2007b).

Three main goals have been identified for improvement of fat content in meats: reduction of total fat (caloric), reduction of cholesterol, and reduction and modification of fatty acid profiles.

11.3.1.1 Reduction of Fat Content

Meat has often been assumed to contribute to an increased likelihood of obesity and increased risk of cancer (Ferguson 2010) and heart disease (Williamson and others 2005; McAfee and others 2010) because of its relatively high contribution to fat intake. In most European countries, meat and meat products are the first or second source of total fat intake (between 11.4% and 28.9%) (Table 11.4); for the average U.S. consumer, the proportion is around 26% (Schweitzer 1995). In industrialized countries ~36–40% of the total calories in the food supply come from fat, nearly half of which is from meat intake (Byers and others 1993; Sheard and others 1998).

Although lean meat contains little fat, the same cannot be said of many meat products which contain high percentages of fat (Table 11.2) and high energy density. There are various strategies that can be used to reduce the fat content of meat and meat products.

Reducing fat in animal tissue (raw meat). Advances in livestock breeding practice have been used to condition animal tissues, and hence the composition of carcass meat and commercial cuts. Fat contents

TABLE 11.6

Strategies and Possibilities for Improving the Concentrations of Physiologically Active Compounds in Meat and Meat Products

Modification of carcass composition (animal tissue) by genetic and nutritional strategies
Reduction of fat content
Improving fatty acid profile: reduction of SFAs, increase of MUFAs, PUFAs, CLA, and improvement of PUFA/SFA and n-6 /n-3 PUFA ratios
Reduction of cholesterol content
Increasing of mineral contents (selenium, iron, . . .)
Increasing antioxidants (vitamin E, vitamin C, flavonoids, . . .)
Meat product reformulation
Reduction of fat content and calories
Improvement of fatty acid profile (replacement of animal fat by vegetal and/or marine oils): reduction of SFAs and TFAs contents and increase of MUFAs, PUFAs (ALA, EPA, DHA), CLA, and improvement of PUFA/SFA and n-6/n-3 PUFA ratios
Reduction of cholesterol content
Improvement of amino acid quality (nonmeat proteins)
Incorporation of prebiotics, probiotics, symbiotics
Enrichment with minerals (selenium, calcium, . . .)
Incorporation of vitamins and antioxidants (folic acid, tocopherols, carotenoids, . . .)
Reduction of sodium, nitrite, and phosphate contents
Elimination of allergens
Incorporation of other healthy ingredients (plant sterol, lecithin, . . .)
Meat processing, storage, and consumption conditions
Minimizing the impact of these steps in terms of loss of bioactive compound contents and bioavailability
Promotion of formation of healthy compounds (bioactive peptides, CLA)
Minimizing the formation of unhealthy compounds (PAHs, HCAs, BAs, nitrosamines, and lipid oxidation products)

Note: ALA, α-linolenic acid; BAs, biogenic amine; CLA, conjugated linoleic acids; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HCAs, heterocyclic amine; MUFAs, monounsaturated fatty acids; PAH, polycyclic aromatic hydrocarbon; PUFAs, polyunsaturated fatty acids; SFAs, saturated fatty acids.

of carcasses have been reduced by about 6–15% in beef, 15–30% in pork, and 10% in lamb in the last decades (Goutefongea and Dumont 1990). This achievement has been due to mainly selective breeding and management practices. More recently, genetic advances have been opening up new possibilities for genetic improvement selection programs to manipulate carcass composition. Genetic markers have become strategic tools for identification of loci underlying the expression of quantitative traits (quantitative-trait-loci (QTL)) that have been found to affect carcass characteristics related to fat content (fatness, backfat, or intramuscular fat) in pigs or fat depth or marbling in beef cattle (Navajas and Simm 2004). Nutritional strategies, easier to apply in monogastric animals than in ruminants, affect carcass composition (fatness), which is determined largely by the composition of diets and by the levels of feeding, particularly the energy and protein intake (Hays and Preston 1994; Dikeman 1997). The reduction of fat content in farm animals by production practices has been extensively reviewed (Bass and others 1990; Hays and Preston 1994; Dikeman 1997; Moloney 2006).

Postslaughtering activities such as trimming the external fat from carcasses and cuts can reduce the fat content. Additionally, defatting can be completed on retail cuts (Kirton and others 1997) and at home by the consumer after purchase, through preparation (trimming of fat) and cooking methods. Fat can be reduced by more than 35% in these ways (BIC 2009).

Meat product fat reduction. Fat reduction in meat products is usually based on two main criteria: the utilization of leaner meat raw materials and the reduction of fat density (dilution) by adding water and other ingredients with little or no caloric content. Among other functions, these ingredients (proteins, carbohydrates, lipids) should help modulate the product's water-binding capacity (water must be retained in the meat matrix), texture, and sensory properties. "Fat replacers," "fat substitutes," "fat analogs," "fat

mimetics," "fat extenders," or "fat barrier compounds" are used (individually or in combinations) to replace animal fat, generally depending on their composition, properties, and the functions that they perform in the meat matrix. In the development of low-fat products, in addition to factors associated with meat raw materials and nonmeat ingredients, reformulation strategies should take manufacturing and preparation procedures into account (Keeton 1994; Jiménez-Colmenero 1996; Kerry and Kerry 2006).

11.3.1.2 Modification of Fatty Acid Profile

Since each fatty acid affects the plasmatic lipids differently, the fatty acid composition of meats is important for the diet/health relationship. In view of this, various different strategies (Table 11.6) have been used to induce qualitative and/or quantitative changes in the lipids present in muscle and fatty tissues and hence in meat and meat derivatives, in order to endow them with desirable (technological and nutritional) properties, especially those conducive to a fatty acid profile more in line with health recommendations—that is, with smaller proportions of SFAs and larger proportions of MUFAs, n-3 PUFAs (especially long-chain), or conjugated linoleic acids (CLA), better n-6/n-3 PUFA and PUFA/SFA ratios, and if possible reduced cholesterol content.

11.3.1.2.1 SFA Reduction

Meat lipids usually contain <50% SFAs and up to 70% unsaturated fatty acids (Table 11.2). Of SFAs, those with <18-carbon atom chain lengths (around 25–35% corresponding to myristic and mainly to palmitic acids) raise levels of low-density lipoprotein cholesterol in blood, which increases the risk of atherosclerosis, while stearic acid may be neutral in its effect on cholesterol level. Dietary recommendations to reduce the risk of cardiovascular disease (CVD) stress the need for a reduction of total fat, and particularly the amount of SFA in the diet (Williamson and others 2005). Recently, however, a meta-analysis of prospective epidemiologic studies showed that there is no significant evidence for concluding that dietary saturated fat is associated with an increased risk of coronary heart disease or CVD (Siri-Tarino and others 2010). Saturated fat intake from meats ranges from 13% to 29% in European consumers (Table 11.4) and close to 28% in U.S. consumers (Schweitzer 1995).

In meat products, SFA contents decline as the total fat contents are reduced and/or the relative proportion of unsaturated fatty acids is increased. Also, meat fatness affects the relative proportion of fatty acids: SFA and MUFA contents increase faster with increasing fatness than do PUFA contents (Raes and others 2004).

11.3.1.2.2 Improving Unsaturated Fatty Acid Content

Meat fat contains ~44–50% MUFAs and over 40% oleic acid. Meat and meat products can supply a considerable proportion (between 11% and 43%) of total MUFA intake, although this varies from country to country (Table 11.4). Diets rich in monounsaturated fats have been associated with positive health benefits (López-Miranda and others 2006), and it is therefore recommended that most fatty acids be derived from monounsaturates (Simopoulos 2002). Meat fat also contains a high concentration of PUFAs (Table 11.2), mainly α-linolenic acid (ALA), with useful quantities of long-chain n-3 PUFAs in which docosapentaenoic acid (DPA C22:5) predominates. PUFA dietary intake from meat and meat products ranges between 7% and 23% in European diets (Table 11.4). There is abundant evidence to suggest that regular consumption of and/or dietary supplementation with long-chain n-3 PUFAs (eicosapentaenoic-EPA, 20:5, DPA and docosahexaenoic-DHA C22:6 acids) confers a number of health benefits (Simopoulos 2002; Garg and others 2006). Based on health implications, the recommended PUFA/SFA ratio is between 0.4 and 1.0, and the n-6/n-3 PUFA ratio should not exceed 4 (Wood and others 2008). Western diets are deficient in n-3 PUFAs (especially long-chain) and contain excessive amounts of n-6 PUFAs, with a n-6/n-3 PUFA ratio of 15–20, which is higher than recommended (Simopoulos 2002). Moreover, consumption trends for food containing n-3 PUFAs are currently static or declining (Lee and others 2006). Therefore, in order to improve the health status of the population, recent advice is to increase the consumption of food rich in n-3 PUFAs (especially for certain sectors of the population) as a means of promoting a reduction in the n-6/n-3 PUFA ratio. PUFA/SFA and n-6/n-3 PUFA ratios in fatty meat often naturally differ widely from the recommended values. Genetic and dietary approaches combined with

processing strategies have been reported as means to increase unsaturated fatty acid contents of meats (Table 11.6).

Genetic strategies. Biotechnology offers interesting possibilities for improving the fat content of meat. Recently, a QTL has been detected that affects fatty acid composition, specifically palmitic, palmitoleic, myristic, linoleic acid (LNA), and ALA acid contents in pork (Clop and others 2003). Transgenic farm animals are a more novel approach to improving fatty acid profiles. Livestock cannot convert n-6 fatty acids into n-3 fatty acids because they lack an n-3 fatty acid desaturase gene. Transgenic pigs have been produced that express a plant gene, *spichad desaturase* (Saeki and others 2004), or *Caenorhabditis elegans*, encoding an n-3 fatty acid desaturase (Lai and others 2006) for synthesis of the essential PUFAs, LNA, and ALA, or produce high levels of n-3 PUFA and reduce n-6/n-3 PUFA ratios.

Feeding strategies. Lipid deposition in animal tissues can be endogenous—that is, synthesized *de novo*—or exogenous—supplied by the feed (direct deposition). Dietary fatty acid composition is an extremely important part of the fatty acid profiles of monogastric animals and less important in ruminants. Numerous animal feeding trials have been carried out in the attempt to increase the unsaturated fatty acid content in animal tissues. MUFA content in meat can be enhanced by increasing the MUFAs in the animal diet. Feeding strategies involving plant (vegetable oils, n-3-rich plants, forages) and marine sources (fish oil or fish meal) have been successfully used to significantly increase PUFAs (Raes and others 2004; Scollan and other 2006; Wood and others 2008). Diets rich in long-chain n-3 PUFAs (from fish) are generally the only way to increase the presence of DHA in both ruminant and monogastric species. Diets rich in short-chain n-3 PUFAs (e.g., linseed) increase the ALA level, but this is only converted into long-chain n-3 PUFAs (EPA and DHA) to a limited extent, so that the increase of EPA and DHA in the fat is only a small one. The effect on the PUFA/SFA ratio is less than on the n-6/n-3 PUFA ratio.

Reformulation process. Meat product fatty acid composition can be modified using the reformulation approach, by means of the ingredients employed—meat raw material and nonmeat ingredients. Healthier lipid formulations based on processing strategies generally entail replacement of the animal fat normally present in the product with another fat whose characteristics are more in line with health recommendations. The procedures used to incorporate natural or processed plant and marine lipids in meat products range from direct addition in the form of liquid oils or solids (including interesterified oils) to incorporation in encapsulated or preemulsified form or as part of plant ingredients (Jiménez-Colmenero 2007b). As a source of MUFAs, olive is the vegetable oil that has received most attention in meat product reformulation. Olive oil is the most monounsaturated vegetable oil and has a high biological value, attributed to a favorable mix of MUFAs and naturally occurring antioxidants (vitamin E, vitamin K, carotenoids, and various polyphenols: hydroxytyrosol, tyrosol, and oleuropein). Partial substitution of pork backfat by olive oil and high-oleic acid sunflower oil has been tried in meat products such as fermented sausages, beef patties, and frankfurters (Jiménez-Colmenero, 2007b; Koutsopoulos and others 2008; Hur and others 2008; López-López and others 2009b; López-López and others 2010). Olive oil increases MUFAs in meat products without significantly altering the n-6/n-3 PUFA ratio (Ansorena and Astiasaran 2004).

Animal fat has been partially replaced with various vegetable oils (cottonseed, corn, soybean, peanut, etc.) to increase PUFA levels, improve PUFA/SFA ratios, and reduce cholesterol contents of different meat products (Jiménez-Colmenero 2007b). The effect on the fatty acid profiles of meat products depends on the fatty acid composition of the vegetable oil (specifically LNA and ALA contents). Other plant materials have been used as ingredients in meat products, some PUFA-rich, like walnuts (Jiménez-Colmenero and others 2010). Fish and algal oils have been used in various forms and levels as sources of long-chain n-3 PUFAs (EPA and DHA) or to enrich meat products (Jiménez-Colmenero 2007b; Cáceres and others 2008a; López-López and others 2009b).

11.3.1.2.3 Reducing TFAs

The double bonds in fatty acids normally appear naturally in the *cis* form. Natural TFAs, nonconjugated and conjugated, are present in small amounts in ruminant meat, produced through the natural biological hydrogenation of *cis*-fatty acids (by means of bacterial action) in the intestine of the animals. Nonconjugated TFAs can also be formed by industrial processing of *cis*-fatty acids (from partially hydrogenated vegetable oils). It has been recognized that TFAs are a major risk factor for CVD (Williamson and others 2005).

While industrial TFAs are the major source of total TFA consumption in humans (Park 2009), meat fats also supply varying proportions. For instance, meats contribute about 5–30% of total TFA intake in the diets of European countries (Table 11.4), although the natural *trans* fat found in meat from ruminants may not have an adverse effect on blood lipid concentration (Williamson and others 2005). This may be related to the presence of both nonconjugated (mainly *trans*-vaccenic acid) and CLA TFAs (Park 2009; McAfee and others 2010). Some meat industries have developed a line of *trans*-fat-free fried products (poultry and turkey meat) using TFA-free oil during cooking.

11.3.1.2.4 Improving CLA Content

CLA, a mixture of geometric (*cis, cis; cis, trans; trans, cis*; and *trans, trans*) and positional (c8, c10; c9, c11; c10, c12, and c11, c13) isomers of LNA (C18:2), is formed through the microbial isomerization of dietary LNA in the digestive tracts of ruminant animals. The increased interest in CLA, mainly *cis*-9, *trans*-11, is due to possible human health benefits in regard to anticarcinogenicity, cardiovascular health, immune modulation, and lean body mass/fat reduction benefits (Rainer and Heiss 2004; Park 2009). Meat containing CLA has been described as a functional food in the light of these beneficial health properties (Hasler and others 2004), but optimal dietary intake remains to be established. Meat and meat products contribute about 25–30% of the total human CLA intake in Western populations, which is estimated to range between 95 and 440 mg (Schmid and others 2006).

CLA concentrations range from 4.3 to 19.0 mg/g lipid in lamb, 1.2 to 10.0 mg/g of lipid in beef, and 2.0 to 2.5 mg/g of lipid in turkey and is usually lower than 1 mg/g of lipid in pork or chicken (Schmid and others 2006). Since CLA content in meat is affected by feed composition, animal diet modulation has been used to enhance chicken, pork, beef, and lamb with CLA (Lynch and Kerry 2000; Raes and others 2004; Zhang and others 2010). An increase of CLA in ruminants can be achieved to some extent by feeding strategies (with high concentrations of grass, LNA or ALA-rich sources, and fish oil). In monogastric animals, only supplementation with CLA as such or its precursor *trans*-vaccenic acid is effective in raising CLA contents (Schmid and others 2006). The CLA content of meat products is comparable to that of the raw material and seems in general not to be influenced by the processing method (Schmid and others 2006). Cooked pork sausages have been manufactured with lean and fat from pigs fed with CLA-rich oil, to produce sausages with up to 12 mg of CLA/g (Marco and others 2009).

Most of the lean tissue meat fractions from animals, with or without animal diet modulation to heighten tissue CLA, would not provide significant enough amounts of CLA to impact human health (Baublits and others 2007). This is even more so when fat reduction strategies (which of course reduce the amount of CLA) are used and when meat is consumed in smaller portions and less frequently following some human health recommendations (Millward and Garnett 2010). Therefore, greater concentrations of CLA than currently available in meat tissues are required to achieve high enough levels to produce health benefits when consumed in smaller portions. Commercially produced CLA isomers have been added to whole muscle (Baublits and others 2007) and meat products (Jiménez-Colmenero 2007b; Martín and others 2008) to achieve this. However, the commercial CLA supplements used do not reflect the natural isomer composition in foods. Whether or not natural CLA sources (meat and milk from ruminants) have a similar impact on human health warrants further research (Schmid and others 2006).

11.3.1.3 Reduction of Cholesterol Content

For most individuals, dietary cholesterol has little or no effect on blood cholesterol levels because reduced synthesis in the body compensates for increased dietary intake (Kerry and Kerry 2006). Thus, any effort to reduce the cholesterol content in meat may have little impact on the health of the general public (Clarke 1997). However, there are individuals who are sensitive to dietary cholesterol, and most authorities recommend a general limit on cholesterol intake for the total population (WHO 2003).

The amount of cholesterol in meat and meat products depends on numerous factors, but in general it is <75 mg/100 g (Table 11.2), except in the case of some kinds of edible offal (heart, kidney, brains, etc.) where the concentrations are much higher. It has been estimated that from one-third to one-half of the daily cholesterol intake is supplied by meat (Schweitzer 1995; Chizzolini and others 1999) (Table 11.3).

There are several possible approaches to cholesterol reduction. Methods include modifying animal production practices, altering the composition of meat products, or direct removal of cholesterol by physicochemical procedures (Clarke 1997). Although variations can be observed among species, muscles, or certain breeds, between sexes or in relation to certain feeding regimes, their magnitude generally appears to be too low for them to be of real use in the diet-related reduction of cholesterol intake (Chizzolini and others 1999).

The amount of fat in meats is not always directly related to the cholesterol level (Table 11.2). Therefore, reduction of the percentage of fat does not seem to be a suitable method of reducing cholesterol in meat products (Jiménez-Colmenero and others 2001). Using processing strategies, cholesterol reduction in different meat products (ground beef, frankfurters, pork patties, etc.) has been achieved by replacing fat and lean meat raw materials (since cholesterol is strictly linked to animal cells) with vegetable oils (olive, corn, sunflower, soybean, etc.) and by incorporating different plant-based proteins (soy, corn, oat, wild rice, wheat gluten, etc.), and other fat replacers (Clarke 1997). This dilution method has made it possible to significantly reduce cholesterol contents with respect to conventional meat products, for example, around 20% in low-fat ground beef and pork sausages (Sandrou and Arvanitoyannis 2000), or 35–60% in frankfurters (Marquez and others 1989; Paneras and others 1998). It has been suggested that cholesterol-reducing bacteria (*Eubacterium coprostanoligenes*) can be used as a starter culture in fermented meat products (Madden and others 1999).

11.3.2 Proteins, Peptides, and Amino Acids

Dietary protein is necessary for growth, maintenance, and repair of the body, and it can also supply energy. The protein content of meat and meat products varies widely (between 10% and 35%) and can supply all essential amino acids, depending on numerous factors. Dietary meat will also improve overall dietary protein quality in terms of digestibility (Millward and Garnett 2010). In most developed countries, average protein intake is in excess of the minimum protein requirement for good health (Williamson and others 2005). Meats contribute about 28% to the average diet in Spain (Table 11.3), 36% in the United Kingdom, 31% in France, 33% in New Zealand (Williamson and others 2005), and 22% in the United States (Schweitzer 1995).

Meat protein has a satiety-inducing effect in humans, with positive effects on lean muscle. Protein intakes from red meat as part of a controlled diet can contribute to weight loss and weight maintenance, as well as helping reduce blood pressure without increasing blood lipids (McAfee and others 2010). Supplying sufficient amino acids to maintain the body's protein reserves is an important factor in antibody synthesis, thus promoting acquired immunity to disease (Romans and others 1994). Essential nutrients can be considered functional components if in addition to the requisite nutritional effect they provide benefits for the human body (Arihara 2004). A number of amino acids from meat have beneficial effects on nervous systems (Gaull 1990). Meat is a rich source (77 and 110 mg/100 g in beef and lamb respectively) of the amino acid taurine (2-aminoethane sulfonic acid), a compound associated with many biological actions including some linked to eye health and heart disease (Purchas and others 2004); this is essential in newborn infants as they are unable to synthesize enough of these amino acids (derived from cysteine and methionine) (Higgs 2000). Glutamine is implicated in metabolic processes and has potential preventive effects for certain diseases (Neu and others 1996).

Carnosine (β-alanyl-l-histidine) and anserine (*N*-β-alanyl-1-methyl-l-histidine) are antioxidative histidyl dipeptides and the most abundant antioxidants in meat, particularly beef and lamb. These peptides have been reported to play a role in wound healing, recovery from fatigue, and prevention of stress-related diseases (Arihara 2006; Arihara and Ohata 2008). Dietary carnosine is absorbed into human plasma after meat consumption (Park and others 2005). Glutathione (γ-Glu-Cys-Gly) is another endogenous peptide found in the skeletal muscle of most vertebrates, known to have antioxidant activity (Chan and Decker 1994). l-Carnitine (a derivative of the amino acid lysine), which is naturally abundant in skeletal muscle, especially in beef and lamb, assists the human body in producing energy, in lowering cholesterol levels and in absorbing calcium. l-Carnitine also blocks apotosis and prevents skeletal muscle myopathy in heart failure (Arihara and Ohata 2008). At least 80% of the l-carnitine found in the body comes from dietary intake (Rigault and others 2008).

11.3.2.1 Bioactive Peptides

The information available on meat protein-derived bioactive peptides is limited, but they constitute a group of promising meat-derived bioactive substances. Bioactive peptides are fragments that are inactive within the precursor protein, but once released during food processing (meat aging or meat fermentation, protease treatment) or during digestion (gastrointestinal proteolysis), they can perform various different physiological functions in the organism. Several bioactive peptides (antihypertensive, antioxidant, or prebiotic peptides) generated from meat proteins have been found in meat and meat products (Arihara 2006; Arihara and Ohata 2008). Among the bioactive peptides derived from meat protein, angiotensin I-converting enzyme (ACE)-inhibitory peptides have been studied extensively. ACE-inhibitory peptides and/or ACE-inhibitory and antihypertensive activities experimentally generated from porcine skeletal muscle protein (Arihara and Ohata 2008) and from extracts of several fermented meat products (Arihara and Ohata 2008; Sentandreu and Toldrá 2007) have been reported. Some bioactive peptides isolated from the hydrolysate of blood hemoglobin exhibit opioid, bacterial growth stimulating, ACE inhibiting, and antioxidant activities (Chang and others 2007). At all events, it is important to note that the dietary levels of many of the bioactive compounds cited in this and other sections that would be required to be beneficial to human health have never been clearly defined.

11.3.2.2 Nonmeat Proteins

Nonmeat proteins from plant (soy, wheat, corn, oat, sunflower, etc.) and animal (collagen, blood and milk proteins, etc.) sources have traditionally been used as nonmeat ingredients in meat products for technological purposes and to reduce costs. They also provide nutritional benefits by lowering caloric and cholesterol contents (when used as fat replacers), raising protein levels or balancing amino acid profiles and incorporating certain health-enhancing components. Vegetable proteins have been used in meat products for their nutritional quality and healthy properties. Some proteins from vegetal sources (sunflower, walnut, etc.) contain high proportions of arginine and have low lysine/arginine ratios, both characteristics that have beneficial effects for heart failure, blood pressure, and stroke; walnut protein has been added as a strategy to reduce lysine/arginine ratios in meat products (Jiménez-Colmenero and others 2010). Soy protein products are typical of proteins with health-enhancing activity and are implicated in reduced risk of CVD, prevention of cancer and osteoporosis and reduced menopausal symptoms. Soy protein has been used as a functional ingredient in various FOSHU meat products, such as low-fat sausage. It is claimed that an acceptable blood cholesterol level can be maintained by consumption of these products (Arihara 2004).

11.3.3 Probiotics

Probiotics are live microorganisms present in food which, when ingested in sufficient quantities, exert health benefits on the consumer (Ashwell 2002). In general, these health benefits depend essentially on the presence of selected viable strains of lactic acid bacteria (LAB) and are related to combating of gastrointestinal infections, antimicrobial activity, improvement in lactose metabolism, lowering of blood serum cholesterol levels, immune system stimulation, antimutagenic properties, anticarcinogenic activities, antidiarrheal properties, improvement in inflammatory bowel disease, and so on (De Vuyst and others 2008; Rivera-Espinoza and Gallardo-Navarro 2010).

Although the food matrices most typically used for probiotic bacteria are fermented dairy products, other matrices—including meat products—can serve to obtain probiotic foods. Probiotics could be a promising market for the meat industry, opening up new fields of consumer choice. Meat products processed by fermentation without heating are an excellent vehicle for probiotics. Bacteria from meat environments and commercial meat starter cultures have been considered for the development of probiotic meat products because of their probiotic properties. There are various factors affecting the choice of appropriate microorganisms for use as probiotic strains in a fermented matrix. Among the most crucial characteristics are resistance to technological processing (presence of nitrite and sodium chloride, pH, water activity, mechanical and thermal treatments, etc.) and the environment in the gastrointestinal tract

(Ammor and Mayo 2007; De Vuyst and others 2008). Alginate-microencapsulation has been used to increase survival in the formulation of fermented meat products (Muthukumarasamy and Holley 2006).

Raw fermented sausages contain large numbers of LAB, but these are not regarded as probiotics (Leroy and others 2006). In recent years, however, there have been a number of specific studies on the addition of probiotic bacteria to fermented meats (Table 11.7). Most research concerning this strategy focuses on the survival of the added species in the meat matrix and its influence on the technological and sensory characteristics of the final product. Another aspect that has been addressed is pathogen inactivation, as probiotic strains with additional food safety assets could confer added value to healthy fermented meat products. Human contact studies using probiotic fermented meats have been very scarce up to now (De Vuyst and others 2008). It has been reported that consumption of 50 g/day of a probiotic sausage (*Lactobacillus paracasei*) modulated various aspects of the immunity of healthy volunteers (Jahreis and others 2002). There have been a number of reviews on probiotics in fermented meat products (Työppönen and others 2003; Arihara 2006; Ammor and Mayo 2007; De Vuyst and others 2008; Rivera-Espinoza and Gallardo-Navarro 2010). Commercial probiotic functional fermented meat products (salami in Germany and meat spread in Japan) have been reported to contain human intestinal LAB such as *L. acidophilus, L. casei, Bifidobacterium* spp., and *L. rhamnosus* (Arihara 2006).

11.3.4 Prebiotics and Dietary Fiber

A prebiotic is a "non-digestible food ingredients that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health" (Gibson and Roberfroid 1995) (pp. 1405–1406). Recently, however, it has been proposed to extrapolate the changes in the colonic ecosystem to gastrointestinal microflora (Gibson and others 2004). Prebiotics have great potential as agents to improve or maintain a balanced intestinal microflora and so enhance health and well-being. Prebiotic effects have been attributed to many food components, including dietary fiber (complex vegetable carbohydrates that are not digestible in the human organism), without due consideration of the criteria required. Some prebiotics (e.g., inulin, a mixture of oligomers and polymers of fructose obtained from chicory) can be fibers, but not all dietary carbohydrates are prebiotics. The recommended dietary fiber intake is more than 25 g/day (WHO 2003), but current intake levels are lower, and therefore fiber-enrichment of frequently consumed foods like meat products seems a possible means of attaining the recommended amount (Jiménez-Colmenero 2007a).

Various different dietary fibers (prebiotic and otherwise), alone or combined, have been incorporated in meat products for their known technological effects (water- and fat-binding properties, texture, etc.) and physiological properties (reducing diabetes risk, favoring blood lipid regulation, preventing CVD, colon cancer and constipation, and regulating intestinal transit). In recent years, there has been an

TABLE 11.7

Examples of Study of Specific Probiotic Bacteria in Meat Products

Product	Probiotic Microorganisms	Reference
Fermented meat	*L. acidophilus, B. crispatus, L. amylovorus, B. gallinarum, L. gasseri, L. johnsonii*	Arihara and others (1998)
Fermented dry sausage	*L. casei; Bifidobacterium lactis*	Andersen (1998)
Fermented sausage	*L. rhamnosus; L. paracasei*	Sameshima and others (1998)
Fermented dry sausage	*L. rhamnosus*	Erkkilä and others (2001)
Probiotic sausage	*L. paracasei* LTH 2579	Jahreis and others (2002)
Greek dry-fermented sausage	*L. plantarum; L. curvatus*	Papamanoli and others (2003)
Scandinavian-type fermented sausage	*L. plantarum, L. pentosus*	Klingberg and others (2005)
Fermented sausage	*L. plantarum*	Pennacchia and others (2006)
Dry fermented sausage	*Lactobacillus reuteri*	Muthukumarasamy and Holley (2006)
Dry fermented sausage	*L. casei/paracasei*	Rebucci and others (2007)

extraordinary increase in the number of studies on the addition of different concentrations (1.5–3%) of dietary fiber from highly diverse sources—oats, wheat, peach, sugar beet, soy, rice, apple, pea, lemon, orange, and so on—in the formulation of fresh (ground and sausage), cooked, and fermented meat products (Jiménez-Colmenero and others 2006). The use of antioxidant fibers in meat products is of particular interest in this respect (Bastida and others 2009; Sáyago-Ayerdi and others 2009).

11.3.5 Minerals

Meats are an important dietary source of many minerals (Fe, Se, Ca, Zn, etc.) with different effects on the organism. Various strategies (Table 11.6) have been tried to modulate concentrations of these (endogenous or exogenous) in foods.

11.3.5.1 Iron

Meat contains large quantities of highly bioavailable iron (Table 11.3), which moreover assists the absorption of nonheme iron from other foods and contributes about 14–22% of total iron intake (Schweitzer 1995; Higgs 2000; Williamson and others 2005). Iron deficiency produces anemia, which is one of the principal public health problems, affecting a quarter of the world's population in both industrialized and developing countries. It occurs in all socioeconomic status and age groups and particularly affects young women and children (Williamson and others 2005; McAfee and others 2010). Dietary iron intake in Spain is a little <14 mg/day, which is no more than 80% of the recommended level for women aged 20–39 (MARM 2008). Surveys carried out in France and North America have reported iron deficiency in nearly 20% of women (O'Sullivan and others 2002). Therefore, including meat in the diet has a positive effect on intakes of dietary iron (Williamson and others 2005), and although reduced meat intake has been recommended for health and environmental reasons (Millward and Garnett 2010), such a reduction may also increase the risk of low iron status (McAfee and others 2010), especially in certain populations. At the same time, it has also been suggested that heme iron could be a promoter of carcinogenesis (Ferguson 2010).

11.3.5.2 Zinc

Meat and meat products, the main sources of zinc (2–5 mg/100 g), supply ~20–40% of total intake (Higgs 2000; Williamson and others 2005; Millward and Garnett 2010) (Table 11.3). Furthermore, more zinc is absorbed from a diet rich in animal protein than from plant foods. Zn is involved in the regulation of gene expression, protein synthesis, normal cell growth, and cell differentiation. Zn deficiency is part of the etiology of stunting of growth, hypogonadism, impaired immune function, skin disorders, and so on. In developing countries, Zn deficiency has been estimated to account for 4.4% of child deaths (Millward and Garnett 2010) and is recognized as a problem among adolescents in the United Kingdom (Williamson and others 2005) and among persons aged between 20 and 39 in Spain (MARM 2008). Since meat contributes more to total Zn intake than to total Fe intake, reducing meat consumption (without replacement by Zn-containing foods) could cause more serious problems than arise from reduced Fe intake, since it would produce a greater increase in the percentage of the population falling short of the recommended zinc intake level (Millward and Garnett 2010).

11.3.5.3 Selenium

Although highly dependent on animal dietary intake, meat is a good source of selenium (Table 11.2), providing about 10% of dietary Se in Spain (Table 11.3) and 17% in the United States (Biesalski 2005), with levels of 20–25% of the recommended daily allowance (55 μg/day). As an essential component of glutathione peroxidase, dietary intake of selenium favors cognitive and immune functions, has antiviral effects, reduces the risk of coronary heart disease, or cancer, and so on (Rayma 2008). Recommended Se intakes are not currently achieved in most European countries (Rayma 2008). Selenium-enriched

chicken, pork, and beef can be produced by using organic selenium in the diet of poultry and farm animals (Fisinin and others 2009). Selenium has also been added directly to raise the concentration in meat products (Garcia-Iñiguez and others 2010).

11.3.5.4 Calcium

Calcium is one of the most important nutrients in the human diet. Growing concern about bone health in people of all ages has prompted the food industry to respond by adding calcium to foods and beverages. Since meat is a rather poor source of calcium (around 10 mg/100 g), technological strategies have been used to achieve calcium-enriched meat products (Arihara 2004; Cáceres and others 2006). It is also the case that the addition of some nonmeat ingredients (walnut, seaweeds, etc.) to processed meat can increase the levels of a number of minerals (Cu, Mg, Mn, K, etc.) (López-López and other 2009a; Jiménez-Colmenero and others 2010).

11.3.6 Vitamins and Antioxidants

Meats are excellent sources of the B group vitamins thiamine (B_1), riboflavin (B_2), niacin, pantothenic acid, vitamin B_6, and vitamin B_{12}. They do not contain significant amounts of vitamins A, C, D, E, or K, although vitamin A is abundant in certain organs (liver, kidney). However, there are strategies whereby concentrations can be modulated (animal feeding or addition during product manufacture). Potential implications for human health (unrelated to the vitamin function) vary depending on the type of vitamin.

Vitamins E, A, and C possess considerable antioxidant potential. It has been demonstrated that dietary antioxidants increase plasma concentrations of these compounds and are associated with reduced mortality from some chronic disorders and the risk of several diseases, in particular CVD, some cancers, Alzheimer's disease, and vision impairment (cataracts). The most prominent dietary antioxidants are the tocols (including tocopherols, e.g., vitamin E and tocotrienols), vitamin C, carotenoids (e.g., β-carotene—a precursor of vitamin A—lycopene or lutein), flavonoids, and simple phenolic compounds (Holm 2003).

Meat generally contains small amounts of vitamin E, essentially located in the fatty tissues. However, vitamin E concentrations have been increasing in recent years as a result of changes in animal diets, to the extent that meat is now a moderate source. Farm animal dietary supplementation with vitamin E (α-tocopherol) significantly in excess of physiological levels (the accumulation depends on species, muscle characteristics, levels of supplementation and duration) reduces lipid oxidation as well as myoglobin oxidation in meats (Lynch and Kerry 2000) and in meat products in which these meat raw materials are employed: reformed and restructured cured turkey products (Walsh and others 1998), cooked ham (Santos and others 2004), or dry fermented sausage (Hoz and others 2004). Vitamins E and C have both been added during manufacturing, either as a specific preparation or as a component of nonmeat ingredients (Jiménez-Colmenero 2007a).

Folates and folic acid have been found to lower the plasma homocysteine level in humans, reducing the risk of CVD and some types of cancers. Folate supply deficiencies have been reported in some societies or specific consumer groups. For example, dietary folic acid in Spain among men and women aged 20–39 is only 52% of the recommended intake (MARM 2008); in European countries, the dietary folate intakes of more than 90% of women of childbearing age are below optimal level (Biesalski 2005). Folic acid enrichment (6, 12, and 24 ppm) has been tried in cooked sausages (Cáceres and others 2008b) and hamburgers (Galán and others 2010).

Carotenoids have been associated with antioxidant activity and reduction of the risk of CVD, cancer, age-related macular degeneration, and cataracts. Humans are not capable of synthesizing carotenoids *de novo* and their presence in human tissues is therefore entirely of dietary origin. Animal diets enriched with various carotenoids (β-carotene, lycopene, zeaxanthin, lutein, etc.) have been employed to incorporate carotenoids into intact muscle, but the best way to augment concentrations in meat products is as exogenous antioxidant additives. To that end, a variety of vegetables have been used as nonmeat ingredients in various processed meats (beef patties, restructured beef steak, frankfurters, etc.). Instances include lycopene-rich tomato derivatives (pulp, dried peel, or juice), carrot and sweet potato (rich in

provitamin A), and spinach (rich in lutein and zeaxanthin) (Jiménez-Colmenero 2007a; Garcia-Iñiguez and others 2009). Recently extracts of the carotenoid lutein (Csapo and others 2006; Hayes and others 2009; Daly and others 2010; Granado-Lorencio and others 2010) have also been used in meat product reformulation and in bioaccessibility or bioactivity studies on lutein-enriched meat products (Daly and others 2010; Granado-Lorencio and others 2010).

Some plant-derivatives rich in natural antioxidants (flavonoids and phenolic compounds) have been used to enhance the oxidative stability of meat and meat products. In addition, some of these compounds also have anti-inflammatory and anticancer activities and antimicrobial abilities (Zhang and others 2010). They have been incorporated in the animal tissue by feeding (Lynch and Kerry 2000; Descalzo and Sancho 2008), while in meat products they have been added (as extracts or as part of plant ingredients) during manufacture. Antioxidant activity has been reported for extracts of fruits (grape, licorine root, horsetail, arbustus-berries, etc.) (Nissen and others 2004; Ganhão and others 2010), herbs and spices (clove, rosemary, nutmeg, cardamom, oregano, green tea, etc.) (Sánchez-Escalante and others 2001; Valencia and others 2008; Haak and others 2009; Hernández-Hernández and others 2009; Kong and others 2010), and other sources. Some of these antioxidants can inhibit the formation of unhealthy compounds (other than oxidation products) such as heterocyclic amines (HCA) or *N*-nitroso compounds (NOC) (Jiménez-Colmenero 2007a; Gibis and Weiss 2010).

Several endogenous compounds (including ubiquinone, glutathione, lipoic acid, and spermine in addition to some of the peptides already mentioned) form part of endogenous antioxidant systems in skeletal muscle (Decker and Xu 1998). Coenzyme Q_{10}, present in meat (0.9–1.5 mg/100 g) (Purchas and others 2006), has antioxidant properties (Decker and Xu 1998). Inclusion of some compounds with potential antioxidant activity (ubiquinone, plant phenolics, glutathione, phytoestrogens, carnosine, carnitine, etc.) in animal diets has been reported as a means of increasing the oxidative stability of muscle food (Decker and Xu 1998; Lynch and Kerry 2000).

11.3.7 Reduction of Unhealthy Components

Notwithstanding earlier notes regarding fat components, some ingredients and additives used in the manufacture of meat products can, either as such or due to one of their components (when consumed in excessive quantities), produce health negative effects. Strategies have been assayed to optimize the presence of several of these sodium, phosphate, nitrite or allergens, *inter alia* (Table 11.6).

11.3.7.1 Sodium and Phosphate

There is strong evidence that salt (sodium) consumption is currently the major factor increasing blood pressure and thereby CVD. Moreover, salt intake has direct harmful effects in that it increases the risk of stroke, left ventricular hypertrophy, and kidney disease, and it can also contribute to obesity through soft drink consumption; it is associated with kidney stones and osteoporosis and is probably a major cause of stomach cancer (He and MacGregor 2009). In industrialized countries, sodium intake is estimated at 3–5 g/day, well above the recommended intake of <2 g/day (WHO 2003). Reduction in population salt intake worldwide would produce a major improvement in public health, and therefore a gradual and sustained reduction in the amount of salt added to food by the food industry is needed (He and MacGregor 2009). This challenge is especially important for the meat industry inasmuch as meat products comprise one of the major sources (over 20%) of dietary sodium intake. Existing approaches to salt reduction in meat products are based on the following strategies: selection of meat raw material, modification of processing techniques, use of salt substitutes and flavor enhancers and masking agents, and optimization of the physical form of salt (Desmond 2006). A large number of reduced-salt (even salt-free) meat products have been marketed in the last few years.

A diet rich in inorganic phosphate (added to many processed foods including meat products) has been reported to increase the incidence of lung tumors, so that careful regulation of dietary inorganic phosphate may be critical for lung cancer prevention (Jin and others 2009). There are now a number of meat products without added phosphate on the market.

11.3.7.2 Nitrite

There is a general tendency to use less nitrates and nitrites in meat products. By the reaction of nitrite and nitrogen oxides with secondary amines and *N*-alkylamides, NOC (potent carcinogens) may be formed either in processed meats or else endogenously after consumption. The probability of NOC formation is affected by other dietary components, such as heme iron, which may catalyze the formation of NOC from natural precursors in the gut (Ferguson 2010). Both the presence of heme iron and endogenous NOC formation seem to be potential factors in the contribution of processed meats to colorectal cancer (Demeyer and others 2008). The procedures that have been proposed to inhibit damaging nitros(yl)ation upon consumption of red meat or heme iron-containing processed meats include the use of ingredients (dietary components such as: pre- and probiotics, resistant starch, vitamins C and E, etc.) in meat processing (Demeyer and others 2008).

11.3.7.3 Allergens

Some of the ingredients used in the preparation of meat products (vegetable, egg, or milk proteins) contain substances that cause allergic reactions in some consumers. Meat products which do not contain these are described as "allergen-free," and some have now been approved as FOSHU in Japan (Arihara 2004).

11.3.8 Other Compounds

In addition, to the ones already mentioned, meat products may contain a wide variety of substances of varying origin (added as they are, as part of the ingredients used in their preparation, or formed during storage and/or consumption), some with health implications. Some of these are described below.

Creatine and its phosphorylated derivative, creatine phosphate, play an important role in muscle energy metabolism, and there is extensive evidence that under some circumstances creatine supplements can enhance muscle performance. In human beings, creatine from exogenous sources such as meat (e.g., beef, which contains around 350 mg/100 g of creatine and 205 mg/100 g of creatinine) (Purchas and others 2006) is reported to be absorbed from the gut (Pegg and others 2006).

Meat intake has been significantly associated with an increased risk of colon cancer. The general consensus is that the carcinogenicity of meat depends on the type of meat consumed (red or processed), the method of cooking, the quantity consumed, and the individual genetic risk. The most widely accepted mechanism is that formation of mutagenic compounds, including HCA and polycyclic aromatic hydrocarbons (PAH), which form in meat cooked at high temperatures to a well-done state, are responsible for carcinogenesis (McAfee and others 2010). Procedures have been proposed to reduce the formation and/or mutagenicity of HCA and PAH (Ferguson 2010).

Biogenic amines (BAs) are biologically active low-molecular-weight basic nitrogenous compounds which are present in the great majority of foods. BAs, produced by enzymatic decarboxylation of certain free amino acids, are present in meat and meat products in widely varying concentrations. Some of these amines play important roles in many human and animal physiological functions. They are necessary for normal cell growth and play an important role in nucleic acid regulation and protein synthesis, and possibly also in the stabilization of membranes. The intake of foods containing high concentrations of BAs can present a health hazard through the direct toxic effect of these compounds and their interaction with some medicaments (Ruiz-Capillas and Jiménez-Colmenero 2004).

Plant sterols, bioactive components of vegetables, have been shown to have a hypocholesterolemic effect without affecting high-density lipoprotein cholesterol concentration. Plant sterol-enriched products thus open up a new avenue for dietary management of high-serum cholesterol concentrations. The introduction of meat products (frankfurters and cold cuts) enriched with nonesterified tall oil-derived plant sterols and minerals (potassium, calcium, and magnesium) to the habitual Finnish diet reduced the serum total cholesterol concentration in hypercholesterolemic subjects when the intake of plant sterols was 2.1 g/day (Tapola and others 2004). Some plant ingredients used in meat processing contain phytosterols such as β-sitosterol, campesterol, and stigmasterol, which pass into meat products.

11.4 Final Considerations

Functional foods have progressed very fast over the last few years and are contributing to the development of new foods, including meats. Functional foods can offer a means of providing some health or well-being benefits in a way that demands smaller behavioral changes from the individual consumer. Improved products may also supply dietary components that would otherwise be difficult to obtain in sufficient amounts from normal diets. Consumer interest in health maintenance and awareness of the role of functional foods in this are key factors generating demand. The development of meat-based functional foods offers enormous opportunities to the meat industry.

Development of meat-based functional foods depends on bioactive components, whose presence and activity have to be taken into account in the various stages of the process, from conception through design, formulation, characterization, and study to the final clinical or quality-of-life outcome. In this respect, various strategies have been proposed to optimize the composition of meat and meat products, in which the amount of a bioactive component that is to be added depends on how and how often the food is expected to be consumed. It is important to bear in mind also that the bioavailability of these components is influenced by a variety of factors arising from characteristics of the host, the diet as a whole, and the food itself. The food (meat) matrix, including processing, storage, or culinary preparation, can significantly influence the activity or release of the key component. In this sense, there are few points that can be applied generally to all foods and food components. These and other factors are essential for scientific demonstration of the beneficial effect of a functional food, since simple changes do not assure the functional effect. Such demonstration is vital if nutrition and health claims are to be made to highlight a beneficial effect of a food category, a food, or one of its constituents due to its nutritional properties or to its effect on health. Foods promoted with such claims may be perceived by consumers as having an overall nutritional, physiological, or other health advantage over similar or other products; it is therefore necessary to avoid situations where nutrition or health claims mask the overall nutritional status of a food product, which could mislead consumers trying to make healthy choices in the context of a balanced diet. Such distortions can result from aggressive marketing of bioactive compounds (functional foods) even in situations where they might be consumed in excess or where intake might not be appropriate, even to the extent of producing undesirable alterations of consumer behavior.

Regulation of nutrition and health claims is therefore essential in order to achieve a high level of protection of human health, to create a level playing-field for the food industry and to promote the protection of consumer interests.

ACKNOWLEDGMENTS

The authors acknowledge Projects AGL2007-61038/ALI, AGL2008-04892-CO3-01, and Consolider-Ingenio 2010:CARNISENUSA (CSD2007-00016) for funding the research.

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12

Meat Species Identification

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12.1 Introduction

Food authenticity is a major necessity to meet the demands of conscious consumers and to reassure them regarding the origin and content of their food, and also to fulfill the needs of manufacturers to enable them to comply with government regulations and labeling obligations for their products. The determination of food authenticity, particularly meat species identification, has great importance in food quality control and safety. Fraudulent substitution or undeclared adulteration of meat species in comminuted and highly processed meat products is a widespread problem in retail markets. Undesired practices in general include replacement of cheaper or undesired meats with higher commercial value meat species and the nondeclaring of proportion of meat species used in the production. These cases have led to the suggestion of some analytical techniques for the qualitative and quantitative determination of such replacements in meat mixtures. These methods include different protein-based techniques and related methods such as electrophoretic techniques, enzyme-linked immunosorbent assays (ELISA), and isoelectric focusing (IEF). However, these methods have been reported to be sometimes inadequate or less sensitive for the species identification of thermally processed foods due to the denaturation of proteins. DNA-based

techniques could offer a potential to identify the animal species because of the higher stability of DNA molecules in thermal processing conditions compared to proteins and their high specificity to all types of cells. Polymerase chain reaction (PCR) is a fast, sensitive, and highly specific technique, and it could be an alternative to the protein-based methods. However, when a conventional PCR technique is used, it only allows qualitative identification of the species, not quantitative identification. Nowadays, real-time PCR technique is a very popular tool for the species identification and quantification because of its high sensitivity and specificity, larger dynamic detection range, and less carryover contamination. In this respect, real-time PCR method now appears to be the most effective and robust technique for the identification and quantification of trace quantities of different animal species, even in complex foods. Consequently, advances in analytical techniques constantly offer new possibilities for meat species identification. However, the recognition of such techniques by food control laboratories is influenced by several factors such as their sensitivity, specificity, cost, rapidity, reliability, etc. In this chapter, we will describe a wide range of techniques, some of which are currently in practice.

12.2 Spectroscopic Techniques

Vibrational spectroscopy techniques have been used for many years to investigate the molecular structure of organic materials. For decades, several methods based on ultraviolet (UV), Near-infrared (NIR), mid-infrared (MIR), or Raman spectroscopy have been proposed for the assessment of a food product's authenticity.

NIR spectroscopy is based on electromagnetic absorption in the near-infrared region. Near-infrared spectra generally consist of overlapping vibrational bands that may appear nonspecific and poorly resolved. Therefore, a variety of multivariate analysis techniques, such as multiple regression analysis, principal component analysis, and canonical variate analysis, are often employed to eliminate or reduce the complexity of the NIR spectra (Downey 1996). Ding and Xu (1999) developed a visible/near-infrared spectroscopic method with canonical discriminant analysis and stepwise multiple linear regression to discriminate between kangaroo meat and beef. The same researchers also reported that visible/near-infrared spectroscopy can be used to detect beef hamburgers adulterated with 5–25% mutton, pork, skimmed milk powder, or wheat flour with a detection accuracy of up to 92.7% (Ding and Xu 2000). Rannou and Downey (1997) assessed the potential of visible, near-, and mid-infrared spectroscopy techniques alone and in combination to identify raw chicken, pork, and turkey meats and obtained optimum classification by using combined spectral data in the wavelength ranges of 400–750 plus 5000–12,500 nm.

The performance of separate and combined visible–near-infrared reflectance spectra was compared for lamb-in-beef mixtures by McElhinney and others (1999). Their results showed that the best predictive model was obtained using the 1100–2498 nm wavelength range with a second derivative pretreatment, and lamb content was predicted with a standard error of prediction of 4.1% (w/w). Similarly, in another study, visible and near-infrared reflectance spectroscopy (NIRS) was used to identify beef, lamb, pork, and chicken meat samples, and the discriminant analysis of raw meat species indicated that the main components of muscle such as moisture, fat, and pigments provided good information for identification purposes. In addition, intramuscular fat, fatty acids, and other characteristics (e.g., muscle structure, type of muscle fibers) provided additional information for the discrimination between different meat species (Cozzolino and Murray 2004).

The application of MIR spectroscopic methods, particularly fourier transform-middle infrared (FT-MIR) spectroscopy, for the evaluation of food quality has recently increased. Al-Jowder and others (1997) reported that FT-MIR had the potential for the authentication and quality control of meat products. They showed that it was possible to identify minced chicken, pork, and turkey meats by using their infrared spectra, and for each meat species, good discrimination between fresh and frozen–thawed samples was also observed.

Recently, chemometric mid-fourier transform infrared spectroscopy (MID-FTIR) models have been shown to be an attractive option for meat quality screening without sample pretreatments, which can identify adulteration and quantify the percentage of adulterant as well as the chemical composition of the

sample. Using FTIR-multivariate analysis and soft independent modeling of class analogies (SIMCA), it was possible to successfully detect and quantify adulterants (horse meat, fat beef trimmings, and soy protein) in minced beef samples (Meza-Márquez and others 2010).

Although IR spectroscopy has certain limitations, the capabilities of IR spectroscopy instrumentation are being continually improved to maximize the performance of this technology. IR and Raman spectroscopy are nondestructive vibrational techniques that have been employed in qualitative structural analysis and to quantify food components in a complex food matrix. Raman spectroscopy is based on discrete vibrational transitions that take place in the ground electronic state of molecules. Ellis and others (2005) examined the potential of Raman spectroscopy for the identification of different meat species. Although small spectral differences were obtained, it was possible to discriminate between two closely related poultry species (e.g., chicken and turkey).

12.3 Chromatographic Techniques

Chromatographic methods such as high-performance liquid chromatography (HPLC) and gas chromatography (GC) have been commonly used in the analysis of food samples to separate and/or detect food components, additives, and contaminants in a variety of food systems. Chromatographic analysis of minor and specific compounds or groups of meats has been reported for the identification of meat species.

For instance, the imidazole dipeptide carnosine (CAR) and its methylated analogs anserine (ANS) and balenine (BAL) are histidine-containing dipeptides present in high concentrations in the skeletal muscle of many mammals. The relative concentrations of the three dipeptides are characteristic for each species (Carnegie and others 1983) and can be used for the identification of meat species. Tinbergen and Slump (1976) found a distinctive difference between the ratio of anserine and carnosine contents (ANS/CAR ratio) in beef and pork and of that in chicken meat, and they proved that the high ANS/CAR ratio of chicken meat is a suitable parameter for the detection of these species in meat products. The ratio of ANS/CAR was also found to be different in sheep, cattle, horse, and kangaroo, and it could be used to monitor adulteration by using the HPLC method (Carnegie and others 1985). Similarly, Plowman and Close (1988) indicated that the presence of significant amounts of balenine in red deer meat allowed for the differentiation of red deer meat from meats of other species in processed products.

Moreover, the reversed-phase or ion-exchange chromatographic separation of histidine dipeptides has been successfully applied for the detection of animal-origin proteins in feeds for ruminants (Schönherr 2002; Aristoy and others 2004; Aristoy and Toldrá 2004). In contrast to reversed-phase liquid chromatography, the use of harsh chemicals in the mobile phase, which can denature proteins, size-exclusion chromatography, or ion-exchange chromatography provides more gentle conditions for the extracted proteins. Therefore, the possibility to differentiate meat species through different peak pattern of hemoglobins with cation-exchange chromatography separation after the extraction of meat was examined and the different peak patterns for the extracted hemoglobins of cow, lamb, or pork meat were obtained (Wissiack and others 2003). Recently, Chou and others (2007) described a reliable method based on HPLC with electrochemical detection using copper nanoparticle-plated electrodes for the rapid differentiation of meats from 15 animal species. In the results, each species exhibited unique electrochemical profiles, and it was claimed that a clear differentiation of meat species was possible by utilizing the characteristics of 2–4 major peaks in each chromatogram.

Another approach used for the detection of meat species in meat products is by determining the characteristic composition of fatty acids of the species studied. Ashoor and Osman (1988) described a simple and rapid liquid chromatographic (LC) method to quantitate chicken and turkey in unheated chicken–turkey mixtures. This sensitive method detects as little as 1% chicken or turkey but cannot be applied to heat-treated poultry meats. In another study, Ashoor and others (1988) reported an LC method for the qualitative and quantitative detection of raw beef, pork, veal, lamb, chicken, turkey, and duck species in unheated meat blends. Likewise, Saeed and others (1989) described a different HPLC method for the detection of pork and lard adulteration in fresh and heat-processed meat mixtures by utilizing the increased ratio of the triglyceride containing saturated fatty acid and the triglyceride containing unsaturated fatty acid, and they detected the presence of pork at levels of 1% and 3% in beef and mutton, respectively.

In addition, gas chromatographic analysis of fatty acid composition has been reported for the identification of meat of bovine, ovine, and porcine species (Hubbard and Pocklington 1968), and the detection of adulterated meat mixtures with 15% horse meat or 33% kangaroo meat was possible (Payne 1971). Likewise, another differentiation between pork and beef fat was reported by using linoleic acid levels (Matter 1992).

Chromatographic analysis of fatty acid composition is applicable not only for fresh meat, but also for processed meat since fat is not significantly affected during processing (Saeed and others 1989). However, the fatty acid content of animal tissue is influenced by some other factors such as sex, breed, diet, and age, and fat profiling methods are also not suitable for chemically modified fats such as hydrogenated fats. On the other hand, chromatographic methods may be successful in identifying and differentiating individual species, but there may be difficulties in understanding the complex chromatographic data sets observed from meat mixtures including target adulterants, and more time is usually required for sample preparation and derivatization steps.

12.4 Electrophoretic Techniques

Protein electrophoresis is a particularly well-known separation technique, applied to control the authenticity of meat products. The electrophoresis of sarcoplasmic and myofibrillar proteins has been evaluated as a reliable technique for meat species identification. The principle of these methods is based on the presupposition that each animal species has a specific protein composition that is uniform within the species. During the 1970s and 1980s, the suitability of electrophoretic methods was extensively tested for meat species identification studies. For instance, conventional electrophoretical methods such as polyacrylamide gel electrophoresis (PAGE), sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), and IEF techniques have proved to be highly reliable methods, with some additional advantages, for example, they are cheaper and faster and demand less sophisticated equipment and less specialized personnel. However, the major drawback of electrophoretic methods is that the results obtained are dependent on various influencing factors. Also, the interpretation of electropherograms in relation to species identification requires great care (Hofmann 1987). In this area, more recently, more sensitive and automated capillary electrophoresis (CE) systems have been used to identify the meat species.

12.4.1 PAGE and SDS-PAGE

PAGE and SDS-PAGE are widely used techniques to separate proteins according to their electrophoretical mobility. Unlike PAGE, in the SDS-PAGE technique, negatively charged denatured proteins are separated based on molecular weight by running through a polyacrylamide gel in an electric field. Since SDS-PAGE provides high resolution, good repeatability, and molecular weight-based discrimination, it was accepted as a valuable technique for the detection and determination of foreign meat proteins in a protein mixture (Höyem and Thorson 1970; Parisi and Agulari 1985; Parisi and others 1985; Zerifi and others 1991). However, various studies have shown that this method produces satisfactory results only for the detection of raw muscles of different species (Heinert and Klinger 1978, 1980; Kaiser and others 1980).

For the successful detection of meat species, the protein patterns of the different species must be sufficiently distinct from each other, and these differences should be greater than those that may occur between different muscle types of one and the same animal species. But it was reported that these differences might not be always species-specific, and they may be sometimes originating from various factors such as age, nutritional stage of animals, effects of stress, and/or other individual differences (Hofmann 1987).

12.4.2 Isoelectric Focusing

IEF is an electrophoretic method in which proteins are separated on the basis of their isoelectric points, in a stabilized pH gradient. The obtained protein profiles can be visualized by following an appropriate staining step. The most commonly used dyes for the species identification include Coomassie Blue, silver salts, or enzymatic staining (Hofmann 1997). For instance, the silver-staining technique has been proved to be a

useful method for the visualization of small amounts of protein in the electrophoretic gels (Rabilloud 1992). Polyacrylamide gel isoelectric focusing (PAGIF) has a higher resolution capability than that of conventional electrophoresis; thus, it has been extensively employed in meat speciation studies (Bauer and Hofmann 1987; Hofmann and Bauer 1987; Jemmi and Schlosser 1993; Waiblinger and others 1998; Ozgen Arun and Ugur 2000; Barbieri and Forni 2000). However, only a few heat-stable proteins such as parvalbumins (Rehbein 1992) and some myofibrillar proteins (myosin light chains and tropomyosin) may be suitable for species identification in heat-treated samples (Etienne and others 1999).

Additionally, the results of PAGIF are influenced by many factors, such as age, sex, gender of the animals, or different metabolic state of the muscles in the same animal (red and white muscles, warm and cold blooded). However, it was demonstrated that the myoglobin band profile is characteristic and many meat species (cattle, horses, pigs, sheep, roe deer, kangaroos, camels, brown bears, rabbits, hares, chickens, ducks, and ostriches) can be identified on the basis of myoglobin patterns of the muscles (Hofmann 1997). By staining some specific protein or enzyme of the animals, species identification may also be possible. For example, one of them is the pseudoperoxidase-staining method in which only myoglobin bands are stained. Myoglobin is a highly resistant protein to heat treatment up to a certain degree of temperature. If the sample is heated to <100°C, the pseudoperoxidase staining of myoglobin might be sufficient. On the other hand, when the applied temperature exceeds 100°C, employing the sensitive silver staining and the use of gels with a narrow pH gradient (6–9) may be sufficient to allow the species identification for the highly heated meat samples (Hofmann and Blüchel 1992; Hofmann 1997; Montowska and Pospiech 2007). However, the PAGIF method, based on pseudoperoxidase staining of myoglobin bands, has some drawbacks. Hofmann and Bauer (1989) reported that the pseudoperoxidase-staining method could only be applied to muscle types (horse, cattle, etc.) containing a high amount of myoglobin. Similarly, Ozgen Arun and Ugur (1999) could not detect any myoglobin bands in the electropherograms of pork sausages because of the low concentration of myoglobin in pork. In addition, since the myoglobin content of muscles can vary greatly as a function of metabolic activity, there are noticeable differences in myoglobin band intensity both between different muscles from the same animal and between the same or comparable muscles from different species (Hofmann 1987).

12.4.3 Capillary Electrophoresis

CE is an alternative technique to gel electrophoresis, and it has been recently employed for the analysis of food protein. Proteins are separated within a small-diameter capillary and detected with various detectors, combined with CE, including fluorescence, refractive index, UV absorbance, and mass spectrometers.

Sodium dodecyl sulfate polymer-filled capillary gel electrophoresis (CE-SDS) has been employed for the detection of qualitative and quantitative differences in sarcoplasmic protein in raw beef, pork, and turkey meats (Cota-Rivas and Vallejo-Cordoba 1997) and bovine and ostrich meat (Vallejo-Cordoba and others 2010). The CE-SDS approach is applied to separate both sarcoplasmic and myofibrillar meat proteins but the CE-SDS profiles of sarcoplasmic proteins were found to be more specific for each species, and thus, more suitable in differentiation and identification studies (Cota-Rivas and Vallejo-Cordoba 1997). Linear discriminant analysis (LDA) is used as a useful pattern recognition statistical model in the interpretation of CE-SDS protein data, for the differentiation of meat species (Vallejo-Cordoba and Cota-Rivas 1998).

The capillary format of IEF, capillary isoelectric focusing (CIEF), has both the high resolution of slab gel IEF and the advantages of a column-based separation technology that include automation and quantification. CIEF is performed for the characterization of different meat extracts by using poly (vinyl alcohol)-coated capillaries to eliminate the undesired interactions of protein and the capillary wall (Jegle and others 1997). In another study, a CIEF method was established for the differentiation of beef, ostrich, horse, and pork meat by analyzing the sarcoplasmic proteins in a polyacrylamide-coated capillary by Vallejo-Cordoba and others (2005).

In the past decade, CE became a powerful separation technique for the analysis of DNA molecules. PCR-based techniques combined with CE separations are becoming an attractive alternative technique for meat authentication studies, as reviewed by Vallejo-Cordoba and others (2005).

Consequently, CE separation has been demonstrated as a powerful analytical method for the detection of meat species in the mixtures. In addition to the protein-based methods, CE has also demonstrated great potential when combined with PCR amplification-based techniques. Despite the multiple applications of CE in the species identification, there are still some problems, such as low sensitivity and reproducibility and some other difficulties, in analyzing the complex matrices or heat-treated samples that have to be addressed.

12.5 Immunoassays

Immunoassays are the biochemical tests that are based on the specific binding between an antigen and its antibody in order to identify and quantify a target substance in a sample with a complex mixture. Commercially, many immunoassay test kits are available for widespread use in food analysis. Among the immunoassay-based techniques, the ELISA is the most widely used technique for meat species identification because of its specificity, simplicity, sensitivity, and suitability for routine controls of the foods (Hsieh 2005).

Several ELISA methods using both polyclonal antibodies (PAbs) and monoclonal antibodies (MAbs) have been developed to detect the species of origin of the meat products, and evaluate their practicability for routine analysis. Most of the early studies were based on the usage of PAbs, raised against either blood proteins (Whittaker and others 1983; Patterson and Jones 1990) or muscle soluble proteins (Martin and others 1988a, b). The species identification of cooked meats is more difficult than that of raw samples because heat treatment leads to the denaturation of most immunogenic proteins. For the detection of denatured proteins, PAbs are often the preferred choice because they target multiple epitopes, and so they generally provide more robust detection and tolerance to small changes in the nature of the antigen they recognize. On the other hand, PAbs have several disadvantages such as variability in the antibody spectra during immunological response, causing reproducibility problems and extensive purification procedures required to eliminate the nonspecific reactivities dependent on the multiple specificity of PAbs (Harlow and Lane 1999). In contrast to PAbs, MAbs target a single epitope and usually have very high specificity and reproducibility. However, the development of MAbs requires high-level technology, higher costs, and longer time than the development of PAbs.

MAbs have been applied to ELISAs for the identification of raw meat (Martín and others 1991; Garcia and others 1994; Morales and others 1994; Billett and others 1996), cooked mammalian and poultry meats (Hsieh and others 1998; Sheu and Hsieh 1998; Djurdjevic and others 2005), cooked pork proteins (Chen and Hsieh 2000; Chen and others 2002; Liu and others 2006), and rendered ruminant muscles (Chen and others 2002, 2004). Chen and others (1998) reported the development of four MAbs (5H9, 5H8, 2F2, and 8A4) specific to a thermal-stable muscle protein for the detection of pork in heat-processed meat products. The ELISA method specifically detected porcine skeletal muscle without cross-reaction to blood, milk, and gelatin (Chen and Hsieh 2000). The potential application of MAbs against troponin I (TnI), a regulatory myofibrillar protein, as a suitable thermostable marker protein for the identification of the origin of meats in severely heated products has been demonstrated by both an indirect ELISA procedure (Chen and Hsieh 2001a, b) and sandwich ELISA (Chen and others 2004). Recently, Liu and others (2006) paired MAb 5H9 with a mammalian TnI-specific MAb 8F10 to develop a user-friendly and sensitive sandwich ELISA capable of detecting low levels of porcine content in both heat-treated and untreated meat products.

Besides qualitative identification, several studies have also been reported for the quantitative detection of meat species in heat-processed meat products by using heat-stable antigens. For example, Andrews and others (1992) reported that different meat species were detected up to 0.13% in cooked meat samples by using the PAb-based double sandwich ELISA procedure. Using the same technique, Berger and others (1988) detected 1% chicken meat or pork in sterilized beef frankfurters. Different meat species in adulterated hamburger samples were identified at concentrations as low as 0.6% by a dot-ELISA using PAbs produced against bovine, chicken, swine, and horse albumin with a notably high specificity (Macedo-Silva and others 2000). Also, a PAbs-based indirect competitive ELISA (IC-ELISA) method was used for the specific identification of heat-processed poultry, horse, kangaroo, and rat

muscular tissue, with a sensitivity of 1–5% (Rencova and others 2000). Using the MAb-based sandwich ELISA, Martin and others (1991) quantified chicken meat at 1–100% in raw meat mixtures. Chen and others (1998) developed the MAb-based indirect ELISA and detected 1% of cooked pork in chicken meat mixtures.

Several commercial companies such as Tepnel, Neogen Corporation, Strategic Diagnostics Inc., Eurofins, ELISA Technologies Inc., and many others have developed a variety of ELISA test kits to detect low contents of animal species, even in highly processed meat and meat products as well as animal feeds. The ELISA techniques have been known to have several advantages like being robust, rapid, inexpensive, reliable, and easy to perform. But limits on the detection in processed products depend on various parameters, such as the fat content, the severity of heat processing, the origin of muscles, and the maturation state of the meat (Giovannacci and others 2004). Currently, some of these ELISA test kits are still being used by many regulatory authorities to detect meat species adulteration and/or to enforce laws and regulations around the world.

Consequently, as an analytical tool, ELISA is known to be highly sensitive and selective, robust, rapid, cost-effective, and easy to perform. The use of MAbs, with the careful selection of target antigen, and suitable ELISA format has greatly increased their usage for the species identification in foods. However, the disadvantages of the ELISA include initial difficulty in producing an antibody specific to a particular target, heat lability, and the instability of antibodies at extreme pH levels or with high concentrations of salt and/or solvent. On the other hand, it is expected that the recombinant antibody technology recently introduced to this area will bring effective solutions for these types of problems (Catala and Puchades 2008).

12.6 DNA-Based Methods

In the past three decades, powerful new methods based on the analysis of specific DNA sequence have been developed in meat species identification for authenticating animal meat products in parallel with developments in the field of molecular biology. In species identification studies, protein-based methods are being gradually replaced by techniques based on species-specific DNA analysis for several reasons; DNA is a more stable molecule compared to proteins, it shows the same features in all cells and tissues, and it gives more information about the individual.

12.6.1 DNA Hybridization

DNA hybridization techniques were widely used in early studies in which DNA was used for the detection of meat species. In this technique, target nuclear material can be detected and quantified by using labeled probes without the need for amplification.

DNA hybridization assay has been successfully applied for the quantitation of pork by using a ^{32}P-labeled probe made from genomic porcine DNA. In one study, the detection limit was determined to be ~0.5% pork in beef for heat-treated samples (Ebbehøj and Thomsen 1991a). However, although this technique gives very sensitive results in meat species detection in heat-treated products, it is reported to be unsuccessful in the distinction of closely related species due to cross-hybridization, also requiring radioactivity that is disadvantageous in terms of marking probes, difficulties in usage, and pollution (Chikuni and others 1990). It was shown that the nonspecific binding of probes to DNA sequences from closely related species was reduced by using unlabeled DNA from the cross-hybridizing species, and that closely related species, such as sheep and goat, could only to be differentiated at the level of 10% (Ebbehøj and Thomsen 1991b).

By using satellite-specific oligonucleotide probes, clear species discrimination was observed even between closely related species such as chicken and turkey. Also, the probes allowed the detection of target species present in both raw and autoclaved meat samples (Buntjer and others 1995; Hunt and others 1997). The strength of the signal obtained in a DNA hybridization assay depends on the amount, as well as on the degradation degree, of the target DNA. Although a substantial reduction in the hybridization signal obtained from freezing and thawing meat samples did not occur, the heating of meat at 100°C

or 120°C caused signal reduction because of DNA degradation. It was concluded that DNA hybridization assays are not suitable for the quantification of admixtures as the quantitative hybridization signal is influenced by factors such as tissue origin and sample processing (Buntjer and others 1999). Moreover, since DNA hybridization is expensive and its application is time consuming, it is not suitable for the routine determination of species composition in meat admixtures.

12.6.2 PCR-Based Techniques

The PCR is a rapid and powerful technique based on *in vitro* amplification of DNA (Mullis and others 1986). PCR-based methods have frequently been employed for the species identification of meat products. All the problems that were encountered in the detection of meat species were overcome to a great extent by using the PCR method, which enables fast amplification of specific DNA sequences. The PCR method is based on amplification by undergoing a series of replications by means of oligonucleotide primers that are hybridized to the flanking regions of one or many target DNA sequences. The main purpose of DNA amplification under *in vitro* conditions is to obtain a target DNA fragment in large quantities with the aim of using it in molecular analyses.

The DNA fragment that is to be amplified by PCR is chosen according to the differences displayed for an individual, population, species, or family detection in accordance with the aim of the detection. The nucleotide sequences of the target DNA fragment that is to be used in species detection should show the most difference among species and the least difference among individuals and populations within the species (Mackie and others 1999).

PCR-based species detection in meat and meat products concentrate mostly on the amplification of mitochondrial DNA (mtDNA). The mammalian mitochondrial DNA (mtDNA) contains 37 genes, which encode 22 tRNA, 2 rRNA (12S rRNA and 16S rRNA), and 13 protein-encoding genes, responsible for the synthesis of some proteins belonging to the electron transport chain in mitochondria.

Mitochondrial DNA has many advantages for species identification studies in meat products when compared to nuclear DNA. For example, the fact that mtDNA has many more copies (Alberts and others 1990) in the cell, compared to nuclear DNA, increases the sensitivity of PCR analysis. Due to the maternal inheritance of mtDNA, the genes usually found only in one allele, which analyzes problems because of heterozygosity, can be excluded (Lansman and others 1983; Hayashi and Walle 1985). Furthermore, mitochondrial DNA is more resistant to heat treatment than nuclear DNA since it has a circular structure. The higher mutation rates of mitochondrial genes are sufficient enough to enable the differentiation of closely related species (Borgo and others 1996). Since the cytochrome *b* (cyt *b*) gene is the first gene used for the determination of the phylogenetic relations of vertebrates, the nucleotide sequence of this gene is available in databases for many animal species. Therefore, the mitochondrial cyt *b* gene was used as a molecular marker in early studies, carried out for the identification of meat species. In addition to the cyt *b* gene, the other mitochondrial genes, the 12S and 16S ribosomal RNA subunits, and the displacement loop region (*D-loop*) are also commonly used markers in the development of DNA methods for meat authentication studies.

Besides mitochondrial gene targets, nuclear markers such as the growth hormone gene, actin gene, phosphodiesterase gene, and the ryanodin receptor gene are also used for meat species identification by exploiting the existence of introns of different sizes that allow the amplification of species-specific DNA fragments. In addition to the above genes, satellite DNA, tandemly repeated segments of nDNA, are commonly used for the differentiation of meat species because they have a high degree of polymorphism.

PCR-based molecular methods have been accepted as useful tools for the detection of meat species, thanks to their high specificity and sensitivity as well as their rapid processing time and low cost. However, these methods also have some shortcomings that limit their effectiveness in many cases. For instance, the presence of inhibitors in meat products can diminish amplification efficiency. Also, particularly under low-stringency conditions, nonspecific amplification of nontarget species, hairpin loop, and primer dimer formations result in the generation of an undesired fragment that can lead to false-positive results.

In heat-processed meat products, PCR methods in fact reach precision and sensitivity levels that are difficult to achieve by applying protein-based assays because of protein instability in heat-treated

materials. However, some researchers have stated that as the time and degree of heat treatment applied to meat products increase, DNA degradation increases and the detection limit decreases. For example, by using the species-specific PCR method, Guoli and others (1999) reported detection limits of 33.6 fg DNA in raw bovine meat, 0.334 pg DNA in cooked bovine meat, and 322 pg DNA in autoclaved bovine meat.

A number of PCR-based methods have been developed for species detection in meat products. These studies are summarized as follows.

12.6.2.1 Sequencing of PCR Products

Sequencing is the most straightforward way of acquiring information from PCR products. Bartlett and Davidson (1991a) developed a procedure called FINS (forensically informative nucleotide sequencing) consisting of four steps: the first step is the isolation of the DNA from a wide range of biological samples, including processed foods; the second is the amplification of a specific segment of DNA using PCR; the third is the determination of the nucleotide sequence of the amplified segment of DNA; and the fourth step is the phylogenetic analysis of the nucleotide sequence using a database to identify the most related species. The nucleotide sequence of the DNA segment targeted for sequence analysis should be accessible from the GenBank nucleotide sequence database for a large number of animal species. Therefore, although variable parts of the cyt *b* gene were initially targeted for sequence analysis, other mtDNA genes were used for the same purpose in subsequent studies. For example, cattle, pig, sheep, chicken, turkey (Bartlett and Davidson 1991a), buffalo, emu, and crocodile meats (Forrest and Carnegie 1994), and tuna and tuna-related species (Bartlett and Davidson 1991b; Unseld and others 1995) were detected with the sequence analysis of cyt *b* gene amplification products. Chikuni and others (1994) were able to distinguish 92% homolog PCR products of sheep and goat species through the sequence analysis of a 374-bp fragment of satellite I DNA. Matsunaga and others (1998) targeted the 18S ribosomal RNA gene for the detection of kangaroo, cattle, crocodile, turkey, frog, and Alaska pollack species. Recently, in another study, the sequence analysis of mitochondrial 12S rRNA was applied for the identification of closely related species, such as cattle and buffalo, and sheep and goat, and each species could be differentiated decisively (Girish and others 2004). Similarly in another study, it was indicated that the sequencing of a partial 450-bp 12S rRNA gene fragment, amplified from cooked meat, could be used for buffalo, cow, goat, and pig species identification (Rastogi and others 2004). Although sequence analysis is a method that identifies individual meat species in the most accurate and precise way, it cannot be applied to detect adulterants in admixed/adulterated meats because the evaluation of the sequence data from a mixture is impossible. Therefore, this method is generally used for the verification of results that are obtained through the species-specific PCR and real-time PCR. In addition, the need for expensive instruments has restricted its use for regulatory purposes.

12.6.2.2 Species-Specific PCR

Once the nucleotide sequence of a selected DNA segment is determined, this information can be used to design faster, simpler, and better secondary methods for routine analyses when compared to sequence analysis; it also can be used directly in the determination of species (Lockley and Bardsley 2000). Since sequence information is readily available in the GenBank nucleotide sequence database for many animal and plant species, it is possible to design species-specific primers allowed species identification without the need for any subsequent method such as "sequencing" or "digestion of PCR products with restriction enzymes."

Species-specific primer design can be utilized simultaneously with sophisticated software capable of handling multiple sequences, database cross-homology identification, and primer thermodynamic considerations. For a specific detection, primer specificity that occurs on the primer's 3′ end is essential because terminal mismatches prevent the elongation of the Taq polymerase, thus impeding any nonspecific amplification (Huang and others 1992).

Under optimized amplification conditions, species-specific primers can produce a specific amplicon as a complementer only to the DNA of the target species within a heterogeneous DNA pool obtained from

a food product. If the complete sequence information of an amplified fragment is present, identification can be verified according to the amplicon size determined electrophoretically (Lookley and Bradsley 2000). False-positives because of cross-homology are a persistent problem in species-specific identification assays. Thus, the specificity of designed primers should be checked to determine whether or not it displays a cross-reaction with nontarget species.

Specific primers for many animal species were designed in previous studies on mitochondrial genes, such as the cyt *b* gene (Colombo and others 2000; Brodmann and others 2001; Pascoal and others 2005), the $tRNA^{Lys}$ and ATPase 8- and 6-subunit complex (Tartaglia and others 1998; Krcmár and Rencová 2003), D-loop (Monteil-Sosa and others 2000) and 12S ribosomal DNA (Rodríguez and others 2004; Martín and others 2007; Che Man and others 2007), satellite DNA (Guoli and others 1999), and actin genes (Fairbrother and others 1998; Hopwood and others 1999; Rodríguez and others 2003); these genes have been successfully used in species detection in comminuted and highly processed meat products. Numerous species-specific PCR methods have been developed to determine the presence of pork DNA in fresh or processed meat mixtures since frequent adulteration occurs with the addition of pork (*Sus scrofa*) to beef (*Bos taurus*) products, generally for economical reasons. For example, Meyer and others (1994) detected pork levels of <2% in heated meat mixtures (at 121°C for 10 min) using specific primers that produce a 108 bp fragment from porcine growth hormone. Likewise, Monteil-Sosa and others (2000) detected a level of 5% pork in processed or heat-treated meat mixtures by using species-specific primers targeting the mitochondrial D-loop region. Calvo and others (2001) detected up to 0.005% pork in both heated and unheated (at 120°C for 30 min) meat mixtures by amplifying a specific fragment of porcine DNA. Kesmen and others (2007) detected pork in cooked sausages at a level of 0.1% by using pork-specific primers for the amplification of the mitochondrial ND 5 (NADH dehydrogenase subunit 5) gene. As can be seen from the above, different detection limits have been reported for the same meat species in scientific studies. This situation might be due to the differences in a number of factors employed in each research, such as features of the meat products (e.g., raw or heat-treated), the DNA isolation method, amplified target DNA segments, designed PCR primers, PCR composition, and thermal cycler conditions.

Species-specific PCR methods are considered to be the most appropriate method for the detection of adulteration in meat mixtures consisting of different meat species. In addition, band intensity in the gel images is proportional with DNA concentration, and therefore this method can be used as a semiquantitative method in meat species detection studies (Meyer and others 1995; Matsunaga and others 1999).

12.6.2.3 Species-Specific Multiplex PCR

Multiplex PCR is a variant of PCR, enabling simultaneous amplification of many targets of interest in one reaction by using more than one pair of primers. The simultaneous detection of several meat species with a multiplex PCR approach is relatively rapid and cost-effective. Multiplex PCR studies have generally been designed using a sense primer common to many mammal species and species-specific antisense primers to minimize the number of diverse primers. In primer design, the most important point to be considered is variation in the length of the amplicon that is produced by these primers to analyze different species. The length of each fragment can be predicted if the complete sequence is known, and a given species can be identified by gel-based visualization of an amplicon of appropriate size (Lockley and Bradsley 2000).

In multiplex PCR, all targets are obviously amplified under the same solution and temperature cycling conditions, so there is no possibility of individual optimizations. Therefore, the design of primers is very important in multiplex PCR techniques because physical parameters like cycle number, cycle duration, and annealing temperature should be identical for the amplification of all target sequences (Kämpke 2007).

Several studies have been carried out on the simultaneous detection of different meat species in meat products by using multiplex PCR assay. Fei and others (1996) designed multiplex PCR primers based on mitochondrial D-loop DNA sequences for the simultaneous identification of cattle, pig, and chicken. Matsunaga and others (1999) developed a quick and simple multiplex PCR method for the identification of six different meat species (cattle, pork, chicken, sheep, goat, and horse) in raw and cooked meats.

Multiplex detection was observed by mixing in appropriate ratios of a common forward primer targeting the conservative sequence of the cyt *b* mitochondrial DNA and six reverse primers on the species-specific DNA sequence for each species. In another study, Dalmasso and others (2004) successfully utilized each species-specific forward and reverse primer set targeting the different regions of mitochondrial DNA (12S rRNA, $tRNA^{Vae}$, and 16S rRNA) using the multiplex PCR method for the identification of poultry, ruminant, fish, and pork materials in feedstuffs. Primers generated specific fragments for each species analyzed, enabling the detection of fish and ruminants in a feed mix at levels of 0.004% and 0.002%, respectively. Rodríguez and others (2001) described a dublex PCR assay for the differentiation of goose from mule, and duck and foie gras, based on the amplification of specific fragments of the 5S rDNA gene in each species. Since the basic unit of the 5S rDNA gene is tandemly repeated on the chromosome, the amplification of the 5S rDNA gene resulted in more than one DNA fragment, making it difficult for the semiquantitative detection of duck in goose/duck foie gras products. Similarly, a dublex PCR-based assay was described for the detection of pork meat in fresh horse sausages and it was also used to evaluate the presence of fraudulently added pork meat (Di Pinto and others 2005). Recently, a common single primer multiplex polymerase chain reaction (CSP-M-PCR) method was developed to simplify the PCR reaction system and eliminate inconsistencies in amplification efficiency from different primers. A CSP was designed as a common adapter at the 5′-end of species-specific reverse primers and employed for the identification of five different meat species (chicken, cattle, sheep, pig, and horse) in minced meat products (Xu and others 2008).

12.6.2.4 PCR-Restriction Fragment Length Polymorphism

PCR-restriction fragment length polymorphism (PCR-RFLP) is one of the most common methods used in meat species identification studies. PCR-RFLP analysis is based on the generation of a species-specific pattern of the restriction fragments by the digestion of PCR amplicons with one or more appropriate restriction enzyme that recognizes specific DNA sequences.

Several PCR-RFLP methods developed targeted both nuclear and mitochondrial genes for the identification of meat species. Among the widely used mitochondrial genes, the cytochrome *b* gene (Meyer and others 1995; Matsunaga and others 1998; Zimmermann and others 1998; Wolf and others 1999; Partis and others 2000; Abdulmawjood and Buelte 2002; Pascoal and others 2004; Shin and others 2008; Murugaiah and others 2009), D-loop (Montiel-Sosa and others 2000; Krkoska and others 2003; Malisa and others 2006), 12S rRNA gene (Abdulmawjood and Buelte 2001; Girish and others 2005, 2007; Fajardo and others 2006, 2007; Gupta and others 2008), and the 16S rRNA gene (Borgo and others 1996) and satellite I (Chikuni and others 1994) have been used for species identification in raw and heat-treated meat samples.

Recently, a microchip-based CE technology that replaced the gel electrophoresis step by an automated Lab-Chip electrophoretic system has been developed. It was reported that the miniaturized analytical system increased the sensitivity, speed, reliability, and reproducibility of the analysis compared to the traditional PCR-RFLP (Dooley and others 2005a,b). By using the PCR-RFLP technique, closely related species can be separated without the need for a sequence analysis. However, a major drawback of the PCR-RFLP method is the existence of previously undetected intraspecies variations. Therefore, the lack of intraspecies polymorphisms at the target sites should be confirmed by the analysis of numerous individuals from the same species.

In addition, although this technique is suitable for the identification of raw and heat-treated pure species, the analysis of meat mixtures is difficult since the results may not be representative of the target species present in the mixture (Partis and others 2000).

12.6.2.5 PCR-Random Amplified Polymorphic DNA

It is possible to detect the meat species using short PCR primers of ~10 bases, which are designed randomly without requiring any prior knowledge of the DNA sequence of the species to be analyzed. Under proper PCR conditions, these primers produce fragments with different sizes by amplifying some DNA regions. These fragments compose species-specific fingerprint patterns by moving at different speeds in

the gel (Lockley and Bradsley 2000). This technique, which is also called PCR-random amplified polymorphic DNA (PCR-RAPD), has been applied successfully in many meat and fish identification studies. For example, Koh and others (1998) assessed the activity and specificity of 29 primers of 10 nucleotides with their guanine–cytosine content varying between 50% and 80% for 10 meat species (cattle, porcine, kangaroo, deer, wild boar, horse, buffalo, rabbit, dog, and cat), and they reported that some primers produced fingerprint patterns specific to the meat species that were examined. Likewise, Arslan and others (2004) obtained characteristic RAPD profiles that will enable the identification of many species (cattle, goat, sheep, camel, porcine, wild boar, donkey, cat, dog, rabbit, and bear) by using a 10-base primer (ACGACCCACG). In another study, the PCR-RAPD method was employed for the simultaneous identification of five animal species (pork, beef, lamb, chicken, and turkey) in meat products, such as hamburgers, raw sausages, dry fermented sausage, and cooked meat products, and obtained specific amplicon profiles for each analyzed species (Saez and others 2004). However, the main drawbacks of the PCR-RAPD technique are considered to be the following: the interpretation of gel results is generally difficult, the results of the analysis vary depending on intraspecific polymorphisms and PCR conditions, and it is not suitable for the species identification of meat mixtures.

12.6.2.6 PCR-Single-Strand Conformation Polymorphism

For all the PCR methods described above, the nucleotide sequence of both the primers and amplified fragment should be known. However, some methods that do not require any sequence information to some extent have been used in species identification. PCR-single-strand conformation polymorphism (PCR-SSCP), which is one of these methods, is in fact a quick way of determining the point of mutation and other DNA polymorphisms (Rehbein and others 1997). PCR-SSCP is a simple and reliable method containing sequentially PCR amplification, denaturation of PCR product, and the analysis of denatured fragments by electrophoresis. Under proper conditions, denaturated products with different secondary structures move at different speeds and produce species-specific profiles (Lockley and Bradsley 2000). This technique has been applied successfully to distinguish domestic and wild porcine species (Rea and others 1996) and to identify many fish species (Rehbein and others 1995, 1997; Weder and others 2004). On the other hand, the disadvantages of this technique are the necessity to perform under carefully controlled conditions and to run the references and samples side by side on the same gel to obtain reproducible results.

12.6.3 Real-Time PCR

PCR methods offer a great potential for the precise detection of animal species in meat products that have been subjected to even intensive heat processing and in products with a complex composition. Conventional PCR techniques allow qualitative detection of different animal species in a mixture, but they may not be appropriate to make quantification of the animal tissue in the product. However, the need for methods that give quantitative results has increased after the introduction of labeling obligations requested by authorized food control agencies, especially within the past decade. Moreover, the quantification of undesirable material is also necessary to detect the intentional adulteration of meat products or accidental contamination in the production line. The most recent reports showed that quantification studies have focused on the use of real-time PCR for meat species identification and quantification (Lahiff and others 2002; Laube and others 2003; Sawyer and others 2003). In addition to meeting the need for quantitative determination of meat species, this technique also has other advantages such as higher sensitivity and specificity, a larger dynamic range of detection, and a less carryover contamination risk.

In the quantitative real-time PCR (qPCR) technique, amplification of the target gene is monitored by an increased fluorescent signal that enables direct assessment of the results after PCR application without additional detection steps (Holland and others 1991). The fluorescent signal increases in direct proportion to the amount of PCR product in a reaction and is monitored in the real-time PCR system. The amount of fluorescent emission at each cycle, recorded and displayed as relative fluorescence units (RFU) by the data analysis software, enables real-time calculation and plotting. Quantification of target sequences is typically achieved by determining the number of amplification cycles required for

the fluorescent signal to cross the threshold (i.e., exceeds background noise level). The number of cycles required to reach the threshold is called the threshold cycle (Ct) value. Thus, the Ct value is a quantitative measurement of the copies of the target present in any sample (Heid and others 1996; Gibson and others 1996; Livak 1997, 2001). A standard curve obtained by plotting Ct values against log-transformed concentrations of serial 10-fold dilutions of the target nucleic acid is a very useful tool for determining the qualities of an assay. The standard curve using a defined template should result in a slope, coefficient of determination (r^2), and y-intercept that demonstrate good efficiency, accuracy, and sensitivity. Assay efficiency, which is a function of the slope, is primarily an indication of how well the PCR reaction has proceeded. This value is calculated by the formula: efficiency = [10(–1/slope)]–1.

Several approaches have been employed to obtain a fluorescent signal from PCR products. The first approach uses fluorescent dyes, which bind to the double-stranded PCR product nonspecifically. The others are based on the specific binding of hydrolysis of hybridization probes to the target sequence. In the quantification of DNA sequences, each of the detection chemistries has a different principle and performance characteristics such as specificity, sensitivity, repeatability, dynamic range, robustness, and cost efficiency. The most widely used detection chemistries are briefly reviewed below.

12.6.3.1 Intercalating Dyes

DNA-intercalating dyes are the simplest real-time PCR reporter systems. SYBR Green I is currently the most frequently used intercalating fluorescent dye in real-time PCR studies. When SYBR Green I binds to the minor groove of double-helical DNA, the intensity of the fluorescent emissions increases. Thus, the fluorescent signal emitting from the DNA–dye complex is used to detect the accumulation of any double-stranded PCR product (Higuchi and others 1993). The SYBR Green I-based detection system provides convenient and cost-effective real-time chemistry to screen many samples and targets. The primary disadvantage of using intercalating dyes is their interaction with any and all dsDNA in the reaction, including primer dimers and other nonspecific PCR products, which results in an overestimation of the target concentration (Wittwer and others 1997; Morrison and others 1998). However, this problem can easily be overcome by running a PCR melting curve analysis to determine the melting temperature, which allows for the differentiation of different PCR products based upon the G + C% content and length of the amplicon (Rasmussen and others 1998).

Several SYBR Green I-based PCR methods have been proposed for the identification and quantification of animal species in food and feed products. For instance, Walker and others (2003) developed three species-specific intra-SINE-based PCR assays for the identification and quantitation of bovine, porcine, and chicken DNA and a multispecies ruminant-specific intra-SINE-based PCR assay for the sensitive detection of common ruminant species. This nuclear-based PCR assay takes advantage of multiple target amplification sites in the genome of interest, like mitochondrial-based systems. Using SYBR Green-based detection, trace quantities of species-specific DNA were detected ranging from 0.0005% (0.05 pg) to 0.05% (5 pg) in a mixture of bovine, porcine, and chicken DNA templates. Also, no significant difference in assay sensitivity was observed with TaqMan-based detection compared to SYBR Green. In another study, the same researchers designed and evaluated a series of class-specific (Aves), order-specific (Rodentia), and species-specific (equine, canine, feline, rat, hamster, guinea pig, and rabbit) quantitative PCR assays based on the amplification of genome-specific short and long interspersed elements. Using SYBR Green-based detection, the minimum effective quantitation levels of the assays ranged from 0.1 ng to 0.1 pg of starting DNA template (Walker and others 2004).

Real-time PCR uniplex and duplex SYBR Green I/melting curve methods have been developed for the identification and quantification of bovine, porcine, horse, and wallaroo DNA in food products. Detection limits in uniplex reactions were 0.04 pg for porcine and wallaroo DNA and 0.4 pg for cattle and horse DNA. Accordingly, the results of the melting curves of PCR products suggested that the real-time SYBR Green I format can yield accurate results when either mixed-DNA templates in uniplex reactions or duplex PCR assays on single-DNA templates are used, but the combination of multiplex reactions with mixed-species DNA may produce a sensitivity loss for some DNA combinations (López-Andreo and others 2006).

Recently, a real-time PCR SYBR Green method using primers targeting the porcine-specific mitochondrial 12S rRNA gene was developed for the detection and quantification of porcine DNA in mixtures containing <0.1% porcine tissue in a heat-treated (133°C for 20 min at a pressure of 300 kPa) material (Martín and others 2009). Although the SYBR Green real-time PCR system is a more convenient and cheaper alternative to approaches where specific amplicons are detected by fluorogenic probes, this chemical is not suitable for the multiple detection of target species.

12.6.3.2 Oligoprobe-Based Chemistries

Probe-based chemistries use fluorogenic probes that interact with their complementary sequence in target PCR products during PCR cycles, allowing the specific detection of target sequences. Probe chemistries, including hybridization and hydrolysis probes, use the so-called fluorescence resonance energy transfer (FRET) principle. FRET is based on the transfer of energy between two molecules that can interact with one another, at least one of which must be capable of fluorescence. The first is the donor dye and is usually called the reporter. The fluorescent signal from the reporter dyes is monitored during the reaction. While several common fluorescent dyes can be used for the purpose of labeling, such as 6-FAM, ROX™, VIC™, HEX™, JOE™, TET™, Yakima Yellow™, Cy3™, and Cy5™, the most widely used reporter dye is 6-FAM. The second, the acceptor or quencher molecule, can be a fluorescent dye or a nonfluorescent molecule that absorbs the emission of the fluorescent reporter when in close vicinity. The most commonly used quenchers include 6-carboxy-tetra-methyl-rhodamine (TAMRA), 4-(dimethylamino) azobenzene-4′-carboxylic acid (DABCYL), and black hole quencher (BHQ).

Many fluorogenic probe-based real-time PCR methods have been developed for the detection and quantification of meat species in contaminated food and feedstuffs (Table 12.1).

Hydrolysis probe-based chemistry is commonly based on the use of a TaqMan fluorogenic probe system. TaqMan probes are dual-fluorophore-labeled oligonucleotides, with reporter dye at the 5′ end and a quencher dye that inhibits fluorescence at the 3′ end. During the extension phase of the PCR cycle, the probe is cleaved by the 5′–3′ nucleolytic activity of the DNA polymerase and releases the reporter dye from the probe. The resulting relative increase in reporter fluorescent dye emission indicates the probe-specific PCR product has been generated and the accumulation of PCR products is monitored by a real-time PCR instrument (Lee and others 1993; Livak and others 1995; Heid and others 1996).

TaqMan chemistry is the most popular choice in real-time PCR detection systems because of its improved specificity, higher signal-to-noise ratio, and ability to perform multiplex reactions. However, the most frequently reported disadvantages of this technique are that the initial cost of the probe may be high and a unique probe design might be tedious.

A modification of the hydrolysis oligoprobe strategy resulted in a novel class of DNA probes that are characterized by having a conjugated minor groove binder (MGB) ligand, which forms extremely stable duplexes with complementary single-stranded DNA targets. The hydrophobic binding of the MGB ligand to the DNA helix increases the melting temperature, which allows the use of shorter probes with high specificity. This chemistry replaces the standard TAMRA quencher with a 3′ nonfluorescent quencher (NFQ), which offers the advantage of lower background signal resulting in better precision in quantitation (Kutyavin and others 2000; Afonina and others 2002).

Scorpion is another fluorescence detection system containing a primer sequence at the 3′ end and a hairpin-loop structure at the 5′ end. Similar to beacon, the loop contains a sequence complementary to the target and the hairpin brings the reporter and quencher into close proximity so that fluorescence is quenched. After the extension of the primer during PCR amplification, the specific probe sequence is able to bind to its complement within the same strand of DNA. This conformational reorganization opens the hairpin loop so that fluorescence is no longer quenched and an increase in signal is observed (Whitcombe and others 1999).

An alternative hybridization probe system, also known as HybProbes, optimized for the use with LightCycler™ technology (Roche Molecular Biochemicals), consists of two sequence-specific hybridization probes labeled with a single fluorescent dye that are designed in close proximity to one another, only 1–3 bases apart on a single DNA strand. When the probes anneal to target sequences, the fluorescent dye is excited by the light source in the instrument and transfers its energy to the second dye (a rhodamine

TABLE 12.1

Identification and Differentiation of Meat Species by Different Real-Time PCR Methods

Detection Chemistry	Target Species	Target Gene	Amplicon Length (bp)	Sensitivity	References
SYBR Green	Cattle		98	0.005% (0.5 pg) DNA	Walker and others (2003)
	Porcine	SINE	134	0.0005% (0.05 pg) DNA	
	Chicken		169	0.05% (5 pg) DNA	
	Equine	SINE	105	0.1 pg	
	Canine		83	1 pg (0.01%)	Walker and others (2004)
	Feline		98	1 ng (10%)	
	Rabbit		160	0.1 ng (1%)	
	Mouse		118	0.1 ng (1%)	
	Hamster		132	0.1 ng (1%)	
	Guinea pig		71	1 pg (0.01%)	
	Rat	LINE	161	0.1 ng (1%)	
	Avian		197	0.01 ng (0.1%)	
	Water bird		174	0.01 ng	
	Cattle	cyt *b*	92	0.4 pg DNA	López-Andreo and others (2006)
	Horse		250		
	Porcine		100	0.04 pg DNA	
	Wallaroo		205		
	Porcine	12S rRNA	75	0.1 pg porcine raw and heat-treated material	Martín and others (2009)
TaqMan™	Cattle	ATPase 8–ATPase 6	271	0.001%	Lahiff and others (2002)
	Cattle	Phosphodiesterase	104	0.1% in meat mixture	Laube and others (2003)
	Swine	Ryanodin	108		
	Beef	Growth hormone	76	0.01% in DNA dilutions	Brodmann and Moor (2003)
	Beef	cyt *b*	116	0.5% of target species in raw meat admixtures	Dooley and others (2004)
	Lamb		133		
	Pork		149		
	Chicken		133		
	Turkey		86		

(*continued*)

TABLE 12.1 (continued)

Identification and Differentiation of Meat Species by Different Real-Time PCR Methods

Detection Chemistry	Target Species	Target Gene	Amplicon Length (bp)	Sensitivity	References
	Pig	cyt *b*	66-bp		Hird and others (2004)
	Duck	12S rRNA	96	1% in duck/goose foie gras binary mixtures	Rodríguez and others (2004)
	Pork	12S rRNA	411	0.5% in binary pork/beef muscle mixtures	Rodríguez and others (2005)
	Cattle	tRNALys/ATP8	68	0.1% heavily treated MBM samples	Fumière and others (2006)
	Pig		68		
	Pig	Ryanodine receptor	108	0.1% in unprocessed products and ultrahigh heat-treated cans	Laube and others (2007)
	Cattle	Cyclic GMP phosphodiesterase	104		
	Lamb		97		
	Goat		96		
	Chicken	Interleukin-2 precursor	95		
	Turkey		86		
	Duck		212		
	Cattle	Satellite IV DNA	76	0.1% in meat mixtures	Jonker and others (2008)
	Pork	cyt *b*	67		
	Horse		78	0.05% in meat mixtures	
	Mutton		86		
	Chicken		82		
	Turkey		68		
	Donkey	ND2	83	0.0001% in raw and cooked meat mixtures	Kesmen and others (2009)
	Pork	ND5	115		
	Horse	ATP 6-8	147		
TaqMan MGB probe	Horse	cyt *b*	69	25 pg DNA in water	Chisholm and others (2005)
	Donkey		119	1 pg DNA in water	

	Pheasant	cyt *b*	78	10 pg DNA in water	Chisholm and others (2008)
	Quail		67	2 pg DNA in water	
	Pork	cyt *b*	131	100 fg/μL DNA in 10 ng/μL wheat DNA matrix	Tanabe and others (2007)
	Chicken		106		
	Beef		120		
	Mutton		95		
	Horse		75		
	Cattle		92	0.03 pg DNA	López-Andreo and others (2005)
	Pork		100	0.07 pg DNA	
	Lamb	mt t-Glu-cyt *b*	119	0.07 pg DNA	
	Chicken	ND5-cyt *b*	117	0.006 pg DNA	
	Turkey		146	0.80 pg DNA	
	Ostrich		120	0.03 pg DNA	
	Ruminant (cattle, sheep, and goat)	16S rRNA	117	—	Chiappini and others (2005)
Multiplex real-time PCR	Cattle	β-Actin	96	0.32% (0.32 ng) DNA	Köppel and others (2008)
	Pork		80		
	Chicken	TF-GB3	76		
	Turkey	Prolactin receptor	83		
	Cattle	β-Actin	96		
	Pork		80	2% at minimum for all seven species	Köppel and others (2009)
	Chicken	TF-GB3	76		
	Turkey	Prolactin receptor	83		
	Horse	cyt *b*	85		
	Sheep		101		
	Goat		140		

(*continued*)

TABLE 12.1 (continued)

Identification and Differentiation of Meat Species by Different Real-Time PCR Methods

Detection Chemistry	Target Species	Target Gene	Amplicon Length (bp)	Sensitivity	References
Light-cycler PCR	Cattle	cyt *b*	370	0.05% w/w Bovine MBM	Rensen and others (2006)
	Ovine			0.1% w/w Lamb MBM	
	Cattle	16S rRNA	112	0.05 ng DNA	Frezza and others (2008)
	Ovine		104	0.01 ng DNA	
	Swine	cyt *b*	114	0.5 ng DNA	
	Chicken	ATPase 8	98		
Scorpion	Cattle	D-loop	237	0.1% beef DNA in lamb DNA	Sawyer and others (2003)

Note: SINE, short interspersed elements; LINE, long interspersed elements; MBM, meat and bone meal; TF-GB3, transforming growth factor beta 3 gene.

dye) by FRET. It is the emitted signal from the acceptor dye that is detected by the real-time instrument and recorded (Wittwer and others 1997).

The ability for the simultaneous amplification of two or more DNA targets in a single reaction tube (multiplex PCR) by probe color and melting temperature (Tm), greatly expands the power of real-time analysis. Intercalating dyes like SYBR Green I multiplexed by Tm can be used to distinguish different products amplified at the same time (Wittwer and others 2001). On the other hand, color multiplexing is possible with sequence-specific oligonucleotide probes (TaqMan or Hyb probes) that are labeled with fluorophores that emit light at different wavelengths. This feature of real-time PCR is used for cost-effective and time-consuming detection and allows quantification of multiple meat species simultaneously (Köppel and others 2008, 2009).

The real-time PCR technique has become a very powerful tool in species identification because of its specificity and sensitivity. The specificity of the method is due to the specificity of the first degree designed primer and probe. For this purpose, two complementary strategies were considered to confer specificity on a real-time PCR assay; the first by placing the 3′ end of the primers on a point of sequence heterogeneity and the second by truncating the primers at the 5′ end to lower the calculated melting temperature. For instance, Chisholm and others (2005) developed a TaqMan probe method that targeted the mitochondrial cyt *b* gene to identify the horse and donkey species. The researchers eliminated the cross-amplification problem caused by a high degree of sequence homology between these two species by truncating the primers at the 5′ end. Although, it is well known that truncating the primers at the 5′ end increases specificity, it causes a decrease in efficiency. For this reason, sequence heterogeneity is essential for specific and sensitive detection. On the other hand, the length of the target must be minimized to address the DNA degradation that occurs in highly processed meat products.

In probe-based assays, the probe bound to an amplicon is cleaved by the 5′ endonuclease activity of the polymerase; this slows the extension of the amplicon. It should be kept in mind that probe-based assays are not recommended to exceed 150 bp, and amplicons of <100 bp are recommended (Durham and Chinnery 2006). Restriction on the lengths of the target amplicon have the advantage of quantifying small-size products amplified from highly degraded sample material, such as rendered meat and bone meal (MBM).

In real-time PCR, the applications where samples may give negative results for all measured parameters, it must be able to differentiate a true-negative result from a false-negative one caused by amplification inhibitors. False-negative results can be excluded by using an amplification control. Coamplification of an internal positive control (IPC) increases the reliability of the results because the amplification control should result in a positive signal in PCR even when the targeted species is not detected. In order to detect the IPC and the species-specific target in the same reaction tube, each of the primer–probe systems are labeled different fluorescent probes (Laube and others 2007). In practice, different internal control systems can be used. One approach is to detect an endogenous gene that occurs naturally in the samples. The use of an endogenous control, which amplifies any mammalian DNA from the sample, is a valuable tool in the detection of some factors affecting the amplification of the target DNA. By a comparison of the species-specific versus endogenous control signal obtained from the samples, it is possible to get a clear result as to whether amplification variations found with the species-specific primers were due to differences in target species content or to some other factors such as DNA degradation, inhibition, or differences in the amount and quality of the DNA obtained from the sample (Rodríguez and others 2004, 2005).

12.7 Conclusion

Meat species identification is one of the subsubjects in food authenticity studies, which is an important issue for producers, processors, distributors, consumers, and regulatory agencies. Many methods have been developed thus far in order to detect fraudulent substitutions and adulterations by following the progress in analytical techniques made in recent years. Recently, DNA-based techniques have been and will be the popular approach for species identification studies, for both raw and severely processed meat mixtures. The feasibility of biosensor-based techniques, such as electronic nose and microarray-based techniques, could not be evaluated in this chapter but they are also potential detection systems in this area. In recent years, commercial kit-based detection systems have started to be used extensively for the

detection of species adulteration in the routine control laboratories of many regulatory agencies. Consequently, it could be speculated that the challenge for food regulatory agencies and food scientists is to develop or constantly improve new technologies to have better insight into the complex nature of the food systems, in order to identify one or more possible indicator components for food authentication considerations.

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13

Sources and Control of Microbial Contamination on Red Meat

John Mills

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13.1 Introduction

The potential for the consumption of raw flesh-foods to be a route for transmission of disease was recognized early in the development of human civilization. Both Biblical and Islamic scriptures contain prohibitions and rules concerning the slaughter of animals for food, which included prescriptions against consuming meat derived from sick or dead animals. Similarly, Greek and Roman scholars acknowledged the connection between food consumed and human health, the Romans regulating hygiene, adulteration and trading practices in civil law, and appointing "market police" to carry out inspections and supervising the disposal of condemned meat into the Tiber River (Bell 1993).

While meat production has been regulated by many other societies throughout history, there is considerable evidence that supervision was largely perfunctory until the beginning of the twentieth century. British health reports (1860) noted that up to 20% of meat sold in England was derived from diseased stock (cited in Howarth 1918), while in America, the publication of *The Jungle* (Sinclair 1906) illustrated vividly the consequences on nonenforcement of regulations and culminated in the enactment of the Meat Inspection Act of 1906, later replaced by the Wholesome Meat Act in 1967, and finally the contemporary regulations discussed elsewhere in this volume.

Considerable advances were also made in determining the etiology of food-borne disease during this period. Although the parasites *Trichinella spiralis* and *Taenia solium* had been discovered in meat in 1835 and 1850, respectively, the connection between bacteria and food poisoning did not emerge until around 1880, and was subsequently confirmed by Gaertner's demonstration of gastroenteritis due to the transmission of the bacterium he named *Bacillus enteritidis* (*Salmonella enteritidis*) from the flesh of a cow, to the humans that ate it in 1888 (Hardy 1999). Many similar bacteria were isolated thereafter, which stimulated the authorities to incorporate food microbiology into the field of preventative medicine, separate from the legislation that existed relating to chemical adulteration.

Science developments during the twentieth century have enabled the detection of an increasing number of microorganisms on meat, as well as providing insights into the microbial ecology at the meat surface. Around 40 genera of bacteria, eight genera of yeasts, and 16 genera of molds are currently described as being commonly found on fresh meats, with a smaller number found on processed and packaged meats (Marshall and Bal'a 2001; Koutsoumanis and Sofos 2004). Only a small number of these organisms ("pathogens") pose the risk of significant disease if they are consumed by humans, while some other genera will cause the meat to spoil if the storage conditions are not appropriate to retard their growth. Another group, while causing neither disease nor spoilage in their own right, nevertheless behave similarly and are easier to isolate and characterize, and so are used as "indicators" of the potential presence of these organisms for sanitary purposes. Most of the remaining organisms are of environmental origin and not well adapted to compete on the meat matrix, and being transient in nature, are consequently of lesser interest to the meat microbiologist.

13.2 Sources and Prevention of Contamination

The microorganisms present on meat have their origins either from infection of the living animal prior to conversion of muscle into meat, that is, endogenous contamination, or by contamination of the carcass postmortem, that is, exogenous contamination (Lawrie 1998). Contemporary methods to control the microbial contamination of meat during production must consider the microbiological characteristics of each of these, along with the process whereby the meat becomes contaminated, such that critical controls can be established whereby this can be reduced to the absolute minimum. This information therefore forms the basis of hygienic meat production, whether it be managed and verified at the premise level by good manufacturing (hygienic) practice and Hazard Analysis Critical Control Point (HACCP) plans (Pennington 2000), or by the regulator through risk analysis and legislation. A full description of HACCP is provided elsewhere in this volume.

13.2.1 The Role of Meat Inspection

The primary purpose of veterinary inspection of each animal and the resulting carcass is to detect signs of disease, thereby preventing endogenously contaminated meat from entering the marketplace (Anon 1985; Wilson 2005). Some diseases (e.g., rabies, listeriosis, salmonellosis, and bovine spongiform encephalopathy (BSE)) have distinct clinical symptoms that can be detected during antemortem inspection and the animal can therefore be prevented from entering the slaughterhouse. Postmortem inspection typically includes the examination of lymph nodes, internal organs, and the exposed portions of the carcass for abnormalities that indicate the presence of disease, and any signs of visible contamination (e.g., feces, ingesta, dust, and hair), and excising the affected areas or, depending on the nature of the abnormality, condemning the entire carcass. These measures are essentially generic in that they identify visible signs of disease or contamination rather than target-specific food-borne microorganisms, but they are nevertheless effective in reducing meat-borne transmission to humans from parasitic infestations such as *Taenia* and *Trichinella*, and some bacterial diseases, for example, bovine tuberculosis, Johne's disease, listeriosis, and yersiniosis. Where an effective meat inspection program is in place, it is probable that the majority of pathogenic and spoilage microorganisms found on retailed meat are derived from exogenous sources, the majority of which are undetectable by traditional methods of meat inspection (Edwards and others 1997).

13.2.2 Contamination from the Hide/Fleece

For red meat, the initial and possibly most significant potential source of exogenous contamination is from the hide/fleece of the animal (Sheridan 1998; Koohmaraie and others 2005). A large proportion of this contamination has been shown to be of fecal origin, but environmental organisms from soil and mud at the stock supply farm are also present (Broda and others 2009). To date, no means has been found to completely prevent contamination with microorganisms when cuts are made from the outer to inner surfaces of the hide (Gill and others 1998). Bacteria are transferred to the meat from the blades of knives

while cutting through the skin, which is only rarely accompanied by obvious visible defects that can be trimmed. Contamination can also occur by direct contact of the outer hide surface and the meat due to "roll-back" of the skin onto the meat, from equipment or the hands of workers, during the stunning procedure (if using captive bolt) or from particles or water droplets shaken from the hide (or that of an adjacent animal) during processing (Nottingham 1982). Dressing methods that minimize the number and extent of opening cuts through the skin, such as the inverted dressing of sheep and lamb, have been shown to significantly reduce the levels of bacterial contamination compared to conventional systems (Bell and Hathaway 1996).

For bovine, ovine, caprine, and cervid animals, the skins are removed from the animal before dressing of the carcass commences. For porcine, the carcass is traditionally dressed skin-on, however, the skin is scalded and dehaired, a process that has been shown to leave the surface contaminated with both mesophilic enteric pathogens and psychrotrophic pathogens and spoilage bacteria (Gill and Bryant 1993).

13.2.3 Dressing and Eviscerating the Carcass

There are several phases of the carcass dressing process where organisms can be transferred from the previously live animal to the meat, often without obvious signs of visible contamination (Gill 2005b). During work to remove the head of the carcass, enteric bacteria can be transferred via saliva and mucus, as well as direct transfer of material from the tonsils and maxillary lymph nodes. While the esophagus is commonly clipped during this phase, some outflow of ingesta may still occur. Similarly, while it is a common practice to cut around and enclose the anus in a bag prior to evisceration, release of fecal material occurs sporadically. Accidental release of stomach and intestinal contents, as well as bile (which may contain *Salmonellae*) can also occur during evisceration if the premise HACCP plan is not strictly adhered to. For larger species, evisceration is usually followed by splitting of the carcass into two parts by sawing through the vertebrae. In cattle, the spinal cord is classified as a specified risk material due to the potential for transmission of BSE, and so must be removed from the carcass at the slaughter premise (Anon 2009b).

13.2.4 Preharvest Interventions

With the live animals established as a primary source of carcass contamination, management of the animals preslaughter to reduce the shedding of pathogens and subsequent transfer via hide or visceral contents is the first important step toward prevention of carcass contamination. For beef slaughter in the United States, the Food Safety and Inspection Service (FSIS) recommend that beef producers implement one or more documented preharvest management practices to reduce the fecal shedding (FSIS 2010a). The management controls and interventions recommended are

- Providing cattle with treated (chlorinated, electrolyzed, or ozonated) drinking water. This has been shown to reduce the herd transmission but does not directly affect the shedding.
- Changing the diet (i.e., between hay, grain, distiller grains, and forage) has been shown to alter rates of shedding, but results are inconsistent and therefore require verification if used.
- Using water or feed additives to reduce the carriage and shedding. Compounds recommended by FSIS include Tasco-14 (a seaweed extract), Ractopamine (a β-agonist shown to have antimicrobial properties), antibiotic feed additives, for example, Neomycin (research is inconclusive), and probiotics (e.g., *Lactobacillus acidophilus* and colicin-producing *Escherichia coli*).
- Sound management practices, including proper sanitation measures and pest control, have been shown to reduce the levels of *E. coli* O157:H7 and other pathogens in the cattle's environment.
- Live animal treatments such as bacteriophages (viruses that kill bacteria) and non-O157 *E. coli* strains from cattle that could displace established *E. coli* O157 in cattle are still in the development phase.
- Vaccination with bacterial extract vaccines. A number of trials have now been conducted with one such vaccine (Econiche™) that show favorable results (Smith and others 2009).

13.2.5 Contamination during Carcass Breaking

Carcass breaking (also known as butchering, cutting, boning, or fabrication) occurs in further processing facilities that are separate from the slaughter and dressing floor and operate at chilled temperatures (typically 7–12°C). The risks of cross-contamination between carcasses, and secondary contamination from the processing equipment and environment, exist during this process even when hygienic practices are scrupulously maintained (Gill and McGinnis 2000; Giovannacci and others 2001). It has been shown that routine cleaning may not completely remove accumulations of organic material from these areas, and that visibly clean surfaces can harbor films of protein and fat (Brightwell and others 2006). While the cold temperatures ensure that the microbiota of these areas is dominated by saprophytic organisms, including those associated with spoilage of chilled meats, there is also the potential for proliferation of psychrotrophic pathogens such as *Listeria monocytogenes* and *Yersinia enterocolitica*, as well as concentration of mesophilic pathogens in areas where detritus gathers, for example, saw guides and chutes (Gill 2005b). Contamination of meat from equipment and surfaces in these areas is potentially high risk because the bacteria may have proliferated to transfer numbers far in excess of those from animal sources, and the organisms will be adapted to survival at colder temperatures.

13.2.6 Contamination from Workers

During carcass dressing, inspection, and breaking, there are processes where contact between the meat and workers hands and/or personal protective equipment is unavoidable (Gill and McGinnis 2003). It is therefore important that workers do not process meat while suffering from any form of infectious disease, including open sores. While the manual operations performed on the carcass do have the potential for cross-contamination and redistribution of contamination, this can be controlled by good hygienic discipline. Worker education and the use of communication tools such as "infosheets" in the workplace have been shown to significantly reduce the risk of contamination by food handlers associated with both raw and ready-to-eat (RTE) products (Chapman and others 2010).

13.2.7 Processed Meats

The processing of RTE meat and poultry products involves the application of biologically lethal treatments such as thermal, fermentation/drying, or high pressure to eliminate pathogenic microorganisms (Sofos and Georgnas 2010). Such products are likely to be consumed without further lethal treatments. The potential sources of microorganisms on such products include the raw ingredients, the processing equipment and environment, and the hands of the workers. Consequently, to prevent recontamination and subsequent proliferation with microorganisms it is critical that the processing procedures are appropriate, and that there is adequate control of the hygiene and sanitation of the food handlers, that no cross-contamination can occur between raw and fresh RTE foods, and that there is no temperature abuse during storage (Todd and others 2007).

A source of particular concern in RTE manufacture is the formation of biofilms in the processing environment that become ongoing sources of cross-contamination. Biofilms of concern to the meat industry are formed primarily by bacteria on food contact and peripheral surfaces within the processing environment, such as conveyor belts, cutting boards, dead spaces, walls, pipes, and drains. Bacteria adhere initially to these surfaces, usually in the proximity of organic residues, as a consequence of hydrophobic or electrostatic attachment of planktonic cells. Extracellular polymeric substances are then secreted forming a matrix that envelops the cells into a sessile state, forming a structure that allows the transport of oxygen, nutrients, and waste (Sofos and Georgnas 2010). From this point, biofilm structures continually shed organisms back into the environment. Such structures are highly resistant to heat, desiccation, pH changes, high salt concentrations, and the action of antimicrobials and food preservatives (Xu and others 2011), and physical cleaning is usually required to facilitate their removal (Krysinski and others 1992). Bacteria that form biofilms include pathogens such as *Listeria*, *Salmonella*, *Campylobacter*, and *E. coli*, and spoilage organisms such as *Pseudomonas*, *Serratia*, and lactic acid bacteria.

13.3 Use of Antimicrobial Interventions Postslaughter

Two divergent philosophies exist with respect to control of food safety during meat production (Bolton and others 2001):

Nonintervention HACCP relies on inspection and trimming on completion of dressing to identify and remove the contamination. This system is in place in the European Union and is based primarily on controlling carcass hygiene by strict control of good manufacturing practice (GMP). The philosophy is heavily dependent on the presence of a postproduction lethal control point, usually cooking, prior to consumption of the meat to prevent the transmission of disease due to invisible microbial contaminants (Midgley and Small 2006).

Defendants of this system object to the use of antimicrobial interventions on several grounds. First, that washing may not remove the contamination, but instead redistribute it, and at higher pressures could drive organisms deeper into the meat where thermal processes are less effective. Second, organisms that are not removed by washing or eliminated by chemicals may become dormant, recovering to proliferate later in the production chain, potentially propagating antimicrobial resistance (Anon 2009a). There are also environmental concerns around the use of biocides, and the formation of persistent, bioaccumulable, or carcinogenic compounds. Finally, there is concern that using interventions encourages unhygienic practices with poor adherence to GMP, as workers rely on the intervention to clean the carcass.

Intervention HACCP is in use in the United States and uses a combination of hygienic practices and antimicrobial interventions strategically positioned along the production line to decrease the levels of microbial contamination to as close to zero as is practically feasible. Advocates of this system argue that as GMP remains an important prerequisite, there exists a further level of control, which lessens the reliance on the consumer administering a final lethal control point to prevent food-borne disease. This philosophy has arisen largely in response to numerous outbreaks of meat-borne disease in the United States, particularly those associated with enterohemorrhagic *E. coli*, which occurred despite the food having been cooked (albeit suboptimally) prior to consumption (Tuttle and others 1999; Edwards and Fung 2006). Gill (2005a) noted that for intervention HACCP systems to be effective, the protocol needed to be expanded so that different stages of production were investigated separately but sequentially. Standard operating procedures and microbiological characteristics could then be established for each operation, together with actions to improve hygienic performance, including decontamination technologies, and finally the development of microbiological criteria to assess the performance of these processes.

The decontamination technologies that are approved for use postslaughter by the meat industry in the United States can generally be subdivided into physical and chemical methods.

Physical decontamination methods include trimming of the carcass to remove visible contamination (which may be aided by ultraviolet detection of chlorophyll), water spray washing, hot water spray or deluge washing, steam-vacuum (steam and hot-water directed onto the meat from a hand-piece, which contains a central vacuum nozzle to remove the residue), and whole carcass steam pasteurization (Bacon 2005).

Chemical interventions are typically applied as sprays; antimicrobial compounds approved for spraying onto red meat carcasses at the time of writing include organic acids (lactic acid, acetic acid, citric acid, and peroxy-acids), various forms of chlorine (hypochlorites, chlorine dioxide, and acidified sodium chlorite) ozonated or electrolyzed water, 1,3-Dibromo-5,5-dimethylhydantoin (DBDMH), and lactoferrin (Acuff 2005; FSIS 2010b).

The majority of these interventions are applied generically (i.e., to the whole carcass), the notable exceptions being trimming, and steam vacuuming.

All of these treatments have been shown to be effective individually under experimental conditions; however, given the array of technologies available, determining their efficacy under commercial processing conditions is of greater value (Sheridan 2004). It is important to note here that no decontamination technology evaluated to date has been shown to be able to sterilize meat; rather, each will reduce the microbial population by a greater or lesser extent. There has therefore been a tendency to implement a series of decontaminating treatments in the belief that each will be additive. Unfortunately, this is not the case; as while some improvement can be demonstrated deploying two or more interventions in sequence,

there remains an upper threshold of effectiveness for each system, which rarely achieves a reduction of >3 $\log_{10}$cfu of the target organism (Graves-Delmore and others 1998). Similarly, a study conducted under commercial conditions showed that some treatments used during the dressing process were relatively ineffective, while the hot water or steam pasteurization intervention on completion of dressing achieved the highest level of reduction (Gill and Landers 2003).

The difference between experimental results and commercial practice has been attributed to a number of factors; which include the potential for recontamination and redistribution at each processing stage, differences between the number and physiological state of the inoculating organism, and the meat substrate upon which the study is conducted. These factors are also likely to explain differences in results observed when the same interventions were trialed at different premises (Gill 2009).

Koohmaraie and others (2007) concluded that the interventions that are used in beef processing plants have the capacity to control only a given level of *E. coli* O157:H7, and that so long as the load on the incoming cattle is within this capacity, then the organism should not be detectable in the final product. As a consequence, the washing of cattle hides postslaughter was developed specifically to reduce the incoming bacterial load and its subsequent transfer to the carcass to a level that could then be reduced to extinction by the postslaughter carcass interventions (Arthur and others 2007).

The levels of contamination on carcass hides are highly variable, which in the case of *E. coli* O157:H7 on beef animals may be attributable to the presence of "super shedders" that excrete high levels of contaminants (>10^4 cfu/g) in feces. Equally, the prevention of hide-to-carcass transfer has been shown to processing plant-specific components, which can vary depending on levels of staffing and training (Arthur and others 2010). The potential therefore remains for the incoming bacterial load to overwhelm the intervention capacity of a premise from time to time, and consequently, a process known as *test-and-hold* is utilized. Each lot (typically 1 h production for US premises) of raw grinding material or finished ground beef is sampled and tested for *E. coli* O157:H7, to identify such occurrences and redirect the lot to be cooked or rendered (Koohmaraie and others 2007).

13.4 Risk Analysis

Risk analysis provides a systematic approach to assessing and controlling the risk posed by a particular pathogen in a particular food commodity. While such assessments have undertaken for many years, they have not been performed in a consistent or systematic fashion until relatively recently (Hathaway and Cook 1997). The principles and guidelines for conducting microbiological risk assessment are now defined within the framework of Codex Alimentarius (Codex 1999). At present, such assessment frameworks have largely been developed for use by governments or expert bodies within the context of Codex to estimate and manage the impact of hazards in foods to human health, rather than by the food industries themselves, whose food safety assessment models are targeted more toward the effect of control options on hazards in foods (van Schothorst 2002).

There are four analytical stages to a risk assessment:

1. Hazard identification
2. Hazard characterization—including dose/response
3. Exposure characterization
4. Risk characterization

The identification of microbiological hazards is based on the data published in the scientific literature as to the correlation some pathogens have with particular foods. Some typical examples are the identification of ground beef as being one of the primary vectors of *E. coli* O157:H7, poultry, eggs, and pork being associated with *Salmonella* spp., *Campylobacter* with poultry, *Y. enterocolitica* with pork, and *L. monocytogenes* with chilled ready to eat foods such as cooked sausages (Roberts and others 1996).

Characterizing these hazards relates the level of exposure with the probable morbidity or mortality, which also involves estimating the infectious dose, typically established using mathematical models based on epidemiological studies, clinical trials, and *in vitro* studies using cell lines and biomarkers (Duffy 2005).

Exposure assessment involves determining the amount of the contaminant to which a consumer is likely to be exposed, relating the amount of contaminant to the amount of food consumed in a single serving. To fully assess the risk posed by a particular food, the prevalence and quantity of a particular pathogen should be established longitudinally throughout the production and supply chain. Again, mathematical models and predictive microbiology are used to determine the dynamics of microbiological growth, survival, and death in the food matrix under study (Ross and McMeekin 2003).

In the final risk characterization stage, the results of exposure assessment and hazard characterization (dose response) are combined, producing an estimate of the risk to a population as a consequence of exposure.

After the risk posed by a pathogen in a food has been characterized, a framework must then be established for their management. The principles and guidelines for this are also set out in Codex, who define risk management as the process, distinct from risk assessment, of weighing policy alternatives, in consultation with all interested parties, considering risk assessment and other factors relevant for the health protection of consumers and for the promotion of fair trading practices, and if needed, selecting appropriate prevention and control options (Codex 2007, 2010). Based on the risk characterization, along with study of the best- and worst-case outcomes of the model used, risk managers then make judgments of the actual risks involved, and determine whether these are acceptable or whether changes are required in industry practice.

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14

Application of Proteomics to Understand Meat Quality

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14.1 Introduction

The term "proteome" refers to the set of all proteins expressed in a cell, tissue, or organism. In contrast to the cell's static genome, the proteome is dynamic and changes from time to time, and hence is accurately defined as the set of proteins present in a sample at a specific point of time (Liebler 2002). The systematic and methodological analysis of a proteome for protein identification, quantification, and functional characterization is known as proteomics.

Efficient proteome analysis is based on four major platforms—fast and simple purification methods for proteins from complex mixtures, sensitive methods to generate information about the protein of interest, access to protein databases, and utilization of computer algorithms to generate structural information about the proteins from the data gathered. In biological systems, one-dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was replaced by two-dimensional gel electrophoresis (2-DE) as the choice of technique for protein separation because the latter allows concomitant separation of several hundreds of proteins based on charge as well as mass. The integration of 2-DE and high-performance liquid chromatography (HPLC) with mass spectrometry (MS) and expanding protein databases empowered researchers to separate, detect, and quantify several thousand proteins in a reasonable timeframe.

In the postgenomic era, MS has become an important tool to correlate protein expression with their genes (Yates 1998). The past two decades witnessed emergence of MS from the method for analyses of volatile compounds to applications for characterization of nonvolatile macromolecules, especially proteins. Furthermore, MS proved superior to conventional techniques in protein chemistry and became an indispensable tool for proteomic analyses because of its unique capabilities. MS can rapidly determine the accurate molecular weight of proteins in relatively low abundance in complex mixtures and sequence peptides in a proteolytic digest. The sensitivity and speed of MS-based protein sequence analyses are superior to traditional Edman degradation techniques.

In proteomics, MS is more widely used as a qualitative tool than for quantitative approaches, because of the unpredictable ionization properties of individual peptides. In part, the use of high-throughput MS

to identify and characterize proteins was achieved through rapid improvements in ionization techniques namely, matrix-assisted laser desorption ionization (MALDI) and electrospray ionization (ESI). These ionization technologies allowed creation of gas-phase ions from large biomolecules, especially peptides and proteins, and became indispensable in modern day MS. Fundamental information on the principles of proteomic techniques were delineated by Liebler (2002), and there is no doubt that selection of appropriate proteomic techniques is critical to the success.

14.2 Proteomic Tools to Understand Meat Quality

Applications of proteomics in agricultural sciences represent an important cornerstone for understanding and improving food quality. While some disciplines have extensively exploited the cutting-edge analytical tools in proteomics, the application of proteomics in meat research is in the early stages. Proteomic studies are necessary to advance our understanding on meat quality because skeletal muscle proteome changes significantly antemortem (various stages of animal growth), perimortem (muscle-to-meat conversion), and postmortem (processing and storage). The integration of 2-DE and MS-based proteomic tools for investigating meat quality can be broadly classified into preharvest and postharvest applications. Several reviews may be consulted for extensive coverage on this area (Bendixen 2005; Mullen and others 2006; Hollung and others 2007).

Preharvest applications of proteomics include implications in animal production, such as growth, metabolism, muscle biology, and genetics of food animals. Several investigations were undertaken to explain the variations in meat quality due to breed (Park and others 2007; Xu and others 2009), diet (Lametsch and others 2006), gender (Hakimov and others 2009), genetics (Kwasiborski and others 2008a,b; Laville and others 2009a; Hollung and others 2009), and management (Kwasiborski and others 2008a,b; Shibata and others 2009). In this chapter, emphasis is on application of MS and proteomics in postharvest aspects of meat production, such as muscle-to-meat conversion, meat quality attributes, product characterization, and meat species differentiation, to understand molecular bases of quality of meat derived from livestock.

14.2.1 Muscle-to-Meat Conversion

The biochemistry behind the shift in cellular metabolism in postmortem skeletal muscles and how it affects muscle-to-meat conversion are not completely understood. In postmortem muscle studies, proteomic tools are utilized to understand the biochemical processes responsible for muscle-to-meat conversion and to characterize how proteome changes happens over the course of immediate postmortem.

The robustness of 2-DE as a powerful analytical tool for meat proteome analyses was demonstrated by Lametsch and Bendixen (2001), and this study reported changes in 15 notable proteins over a period of 48 h postmortem in pork using computerized analyses of gel images. Further work (Lametsch and others 2002) investigated postmortem proteome changes in pork Longissimus dorsi muscle and documented that 18 proteins/peptides changed within 48 h of exsanguination. This investigation concluded that the 18 peptides were the result of proteolytic activity in muscle and originated from three structural proteins (actin, myosin heavy chain, and troponin-T) and six metabolic proteins (glycogen phosphorylase, creatine kinase, phosphopyruvate hydratase, myokinase, pyruvate kinase, and dihydrolipoamide succinyltransferase). Aforementioned studies are considered as a starting point, and further investigations attempted to elucidate the complexity of cellular mechanisms governing postmortem muscle metabolism.

Morzel and others (2004) studied the influence of preslaughter transportation and postmortem storage on proteome changes of pork muscles during 72 h aging period. While aging time affected the intensity of 37 protein spots, preslaughter transportation affected only eight proteins. Of specific, seven spots were consistently present in higher quantities in animals subjected to preslaughter transportation, which exhibited rapid pH decline. Relative abundance of several structural proteins was also influenced by aging time. Mitochondrial ATP-ase was observed to be highly expressed in the pigs subjected to transportation immediately before slaughter. Because stress-induced rapid pH decline is considered as a major feature in pale, soft, exudative (PSE) condition in pork, these results suggested the possibility of predicting the quality defects using proteome components.

With the objective to differentiate PSE zones in deeper regions within hams, the proteome of Semimembranosus muscle from normal animals and those demonstrated PSE zones were compared (Laville and others 2005). Analyses of 2-DE gels demonstrated that troponin-T, myosin light chain 1, and α-crystallin were less proteolyzed in PSE zone samples. These proteins, resistant against degradation, are either the constituents of myofibrils (troponin-T, myosin light chain 1) or the ones tightly bound to myofibrils (α-crystallin). Creatine kinase fragments were more abundant in PSE zone samples than in normal pork muscles, indicating enhanced proteolysis of this protein in PSE zones, whereas heat shock protein 27 kDa (HSP27) was totally absent in PSE zone samples. In general, meat from PSE zones and fast pH fall-PSE pork exhibited numerous similarities in protein characteristics.

Changes in cytosolic proteins, comprising primarily of soluble enzymes and metabolic proteins, in beef Longissimus dorsi and Semitendinosus were examined using 2-DE and principal component analyses with the objective to identify how does muscle source influence postmortem proteome changes (Jia and others 2006a). The results of this study indicated that while five proteins (cofilin, lactoylglutathione lyase, SP-22, HSP27, and HSP20) in both muscles changed during the first 24 h postmortem, 15 previously unreported protein changes were observed in either muscle during the same period. Further studies (Jia and others 2006b) analyzed the shifts in energy metabolism at the proteome level in Longissimus thoracis muscles from the preslaughter state (antemortem) to shortly after slaughter (postmortem) of the beef animals. Muscle samples were isolated 4 days before slaughter and 60 min after slaughter. The findings suggested that 24 proteins exhibited significant changes between the time points, and these proteins belonged to the group of either metabolic proteins or heat shock proteins. Furthermore, five of the metabolic proteins were enzymes involved in the glycolytic pathway and the tricarboxylic acid cycle. Several glycolytic enzymes, 3-hydroxyisobutyrate dehydrogenase, and two enzymes in the tricarboxylic acid cycle increased in intensity in postmortem muscles. This finding suggested that the oxidative metabolism pathways were functional at a higher rate in 1 h postmortem muscles than in live animals. The changes in proteome components involved in the tricarboxylic acid cycle indicated an increase in aerobic energy metabolism to replenish the depleting ATP in muscle immediately after harvest.

Changes in energy metabolism in beef Longissimus thoracis during various stages of early postmortem (up to 24 h) were also investigated (Jia and others 2007). Of the total 47 proteins that significantly changed in the first 24 h postmortem, 39 belonged to either metabolic enzymes, defense and stress proteins, structural proteins, and proteolytic enzymes or unclassified proteins. Moreover, the identified metabolic enzymes were associated with ATP- and NADH-generating pathways, which indicated that the level of ATP is maintained in muscles for several hours postmortem and that the energy production was operative during the shift of metabolism from aerobic to anaerobic. Increased abundance of several stress- and defense-related proteins, which may delay cell death and minimize stress, supported the fact that slaughter-induced nutrient and oxygen depletion increase the stress in muscles.

Water-holding capacity is a major quality influencing various sensory attributes, whereas drip loss leads to revenue loss as a result of decreased product yield. Van de Wiel and Zhang (2007) analyzed proteome of pork muscles collected immediately after slaughter to identify the individual proteins that correlate with drip loss and to utilize them as markers for predicting the water-holding capacity of meat. Results of this investigation indicated the potential of three proteins to be utilized as markers for water-holding capacity, namely creatine phosphokinase M-type, desmin, and a transcription activator. Developing markers for these two major quality attributes enables identifying the mechanisms of drip loss and will aid engineering preventive strategies.

Proteomic tools were utilized by Park and others (2007) to examine the effect of fiber type on postmortem proteolysis in Longissimus muscle of Landrace and Korean native black pigs. Application of gel-based proteome analysis revealed that 26 proteins exhibited differences in degradation during 7 day aging between the two breeds investigated. Although myosin regulatory light chain 2, myosin light chain isotype v/sb, fatty acid-binding protein, and albumin were overexpressed in Korean pigs, their relationship to fiber type was inconclusive. Noticeably, actin isoforms exhibited differential degradation pattern over the aging period. This study revealed the existence of breed-dependent proteolytic mechanisms in postmortem pork.

In a recent study, the changes in the insoluble protein fraction of Longissimus thoracis muscle from Norwegian Red young bulls during the first 48 h postmortem were investigated using 2-DE and MALDI-TOF MS-MS (Bjarnadottir and others 2010). These authors observed significant changes in 35 proteins,

of which 26 belonged to one of the three groups, namely metabolic enzymes, cellular defense/stress proteins, and structural proteins. While majority of the identified metabolic enzymes were involved in cellular energy metabolism, the defense/stress proteins were involved in regulation and stabilization of myofibrillar proteins. Two metabolic enzymes (2,3-bisphosphoglycerate mutase and NADH dehydrogenase) and one stress-related protein (HSP70) identified in this study have not been previously identified in the insoluble muscle protein fraction. The authors concluded that the presence of these soluble proteins in the insoluble fraction could be possibly due to protein precipitation or aggregation, which are induced by several physicochemical changes happening in postmortem muscles, leading to protein insolubilization.

14.2.2 Meat Tenderness

Tenderness is the most important quality attribute contributing to the eating satisfaction and consumer acceptance of meat. Meat tenderness originates from structural and biochemical properties of skeletal muscle fibers and intramuscular connective tissue. Tenderness of meat is improved significantly by aging (i.e., postmortem storage), mainly due to the degradation of myofibrils and structural weakening of the collagen connective tissue. Although postmortem degradation of several structural proteins has been studied, the fundamental mechanisms through which these changes are correlated to meat tenderization are not completely understood. In part, this could be attributed to the low resolving power of one-dimensional SDS-PAGE, the predominant analytical technique used to study postmortem proteolysis in meat. With the emergence of 2-DE as a robust technique to profile proteome changes in meat, application of proteomics became popular in the area of meat tenderness, and since early 2000s proteome changes in postmortem skeletal muscles have been investigated to elucidate a correlation between the appearance of specific markers from structural proteins and observed tenderness of meat.

Investigation by Lametsch and others (2003) was one of the first studies reporting the potential of postmortem proteome analysis to elucidate the molecular bases of meat tenderization. Using 2-DE and MALDI-TOF-MS, specific postmortem protein changes in pork muscles were correlated to Warner–Bratzler shear force, the objective measurement of tenderness. The findings indicated that 113 protein spots changed significantly during the first 72 h postmortem period, and the identity of proteins in 27 most pronounced changes were determined. The identified proteins included fragments of actin, myosin heavy chain, titin, myosin light chain I, myosin light II, Cap Z, and cofilin. Statistical analysis revealed significant correlation between myosin light chain II, triose phosphate isomerase I, and several actin and myosin heavy chain fragments to shear force. This study demonstrated that postmortem degradation of actin and myosin heavy chain is correlated to the objective measurement of meat tenderness. Nonetheless, the molecular basis for the relationship between these changes and meat texture was not elucidated.

The potential relationship between proteolytic pattern of myofibrillar protein at 36 h postmortem and tenderness of beef Longissimus dorsi on day 7 was investigated (Sawdy and others 2004) with the objective of predicting beef tenderness. Seven bands in SDS-PAGE gel were found to be correlated with tenderness. Of these, two bands were identified, employing MS, as fragments of myosin heavy chain. These findings offered potential for an accurate method to predict beef tenderness based on molecular marker peptides.

Specific degradation patterns in structural proteins were studied to characterize the role of μ-calpain in tenderness (Lametsch and others 2004). Purified pork myofibrils from longissimus dorsi were incubated with μ-calpain at 4°C for 2 or 4 days (simulated postmortem conditions), and proteolytic changes were analyzed using electrophoresis and MALDI-TOF MS. Peptide mass fingerprinting revealed that μ-calpain degraded several proteins, including desmin, actin, myosin heavy chain, myosin light chain I, troponin-T, tropomyosin α-1, tropomyosin α-4, thioredoxin, and Cap Z. Contrary to the previous reports that actin and myosin heavy chain are resistant to degradation by μ-calpain, the findings of this investigation suggested that these proteins as substrates of μ-calpain. Nonetheless, actin and myosin heavy chain were found to be less favorable substrate targets than desmin.

Investigations by Hwang and others (2005) examined the correlation between proteolytic products and pork quality attributes, mainly shear force and drip loss, utilizing proteomic tools. Of the 133 protein spots identified, 27 demonstrated a correlation with the objective quality traits. These proteins included actin and its relevant peptides, myosin light chain 1, desmin, troponin-T, cofilin 2, F-actin capping subunit, ATP synthase,

carbonate dehydratase, triose phosphate isomerase, peroxiredoxin 2, and HSP27. However, a conclusive relationship between these protein spots and meat quality traits could not be established in this study.

Te Pas and others (2009) studied the global changes in sarcoplasmic proteome during aging in two pig breeds (Yorkshire and Duroc), differing in meat quality parameters, with the objective to monitor and predict meat quality relative to tenderization and water-holding capacity. Proteome analysis by surface-enhanced laser desorption ionization-time of flight (SELDI-TOF)-MS revealed four types of profiles. While several potential protein biomarkers for drip loss and shear force were suggested by association analyses in both breeds, none were identified for cooking loss. These results demonstrated the involvement of nonstructural proteins in textural attributes of meat and supplemented the previous reports on the contribution of structural proteins in meat texture.

Phosphorylation of myosin light chains regulates muscle contraction and is reported to modulate the physicochemical properties of muscle as food. Muroya and others (2007a) investigated phosphorylation of myosin light chains in postmortem beef Longissimus to elucidate its role in the development of rigor mortis. Application of fluorescent differential gel electrophoresis and phosphoprotein-specific staining revealed that myosin light chain 2 was phosphorylated, whereas no phosphorylation was noticed in the myosin light chains 1 and 3. MALDI-TOF-MS analyses of tryptic peptides from phosphorylated myosin light chain 2 identified two peptides that are singly or doubly phosphorylated. Although the exact sites of phosphorylation were not determined, the results of collision induced fragmentation and tandem-MS indicated that the modified residues were located in the peptide AAAEGGSSSVFSMFDQTQIQEFK. In congruence with rigor development, the ratio of the phosphorylated protein to native protein increased at 24 h postmortem. Furthermore, the appearance of the doubly phosphorylated protein after 8 h postmortem coincided with the fast phase of rigor mortis and indicated its potential role in muscle shortening.

Postmortem changes in troponin-T in beef longissimus was examined (Muroya and others 2007b). Evaluation of various fragments using 2-DE and MS revealed that several troponin-T isoforms underwent degradation in 14 days postmortem. Analysis of peptides demonstrated that during postmortem aging all of the isoforms are cleaved exclusively at the glutamic acid-rich amino-terminal region, and a conventional 30 kDa band in gel was identified to be originated from troponin-T. Furthermore, these authors suggested that the troponin-T fragments could be utilized as good markers for monitoring postmortem beef tenderization.

Sarcoplasmic proteome represents one-third of the proteins in skeletal muscles, and it consists of soluble proteins and enzymes involved in the biochemical pathways governing the physicochemical properties of postmortem muscle. Differential abundance of sarcoplasmic proteome in Longissimus muscle of two groups of pigs (tough meat and tender meat) was compared with the aim to elaborate the existing body of knowledge of the role of sarcoplasmic proteins in metabolic processes influencing shear force in cooked meat (Laville and others 2007). Sarcoplasmic proteome was isolated and analyzed using 2-DE and MALDI-TOF-MS. Fourteen protein spots differed quantitatively between the two groups and were identified by peptide mass fingerprinting. The tender meat group exhibited overabundance of eight proteins, which were related to lipid metabolism (adipocyte fatty acid-binding protein, acyl-CoA-binding protein, enoyl-CoA hydratase, aldose reductase, and triose phosphate isomerase) as well as protein folding and polymerization (initiation factor elf-3β, chaperonin subunit 2, and profilin II). On the other hand, tough meat exhibited overabundance of six proteins, including malate dehydrogenase and adenylate kinase. The overabundance of adipocyte-specific proteins in the tender group suggested higher levels of adipocytes in the muscles. These authors concluded that the shear force in pork could be influenced by the muscle's potential for adipogenesis as well as myogenesis.

Proteome of Longissimus thoracis of Blonde d'Aquitaine beef animals, known for highly developed muscles, was examined with the objective to identify early predictors of tenderness (Morzel and others 2008). Although succinate dehydrogenase was suggested as an excellent predictor for initial and overall tenderness, HSP27 and its fragments explained most variations in sensory scores. The results of this study indicated the possibility of HSP27-related cellular mechanism on tenderness development in Blonde d'Aquitaine beef. Based on the close relationship between the protein in fresh skeletal muscles and tenderness after aging, the authors concluded that HSP27 could be employed as a potential marker of beef tenderness.

Calpains are intracellular calcium-dependent cysteine proteases involved in meat tenderization through degradation of key myofibrillar as well as myofibril-associated proteins. The presence of cysteine in the active site makes calpain sensitive to oxidation. Lametsch and others (2008) investigated the effect of oxidative processes on the activity of μ-calpain and utilized liquid chromatography tandem mass spectrometry (LC-MS-MS) to identify oxidation-induced modifications in the enzyme. The results revealed that exposure to hydrogen peroxide decreased the activity of μ-calpain in a reversible manner. MS-MS analyses of tryptic peptides from oxidized enzyme revealed that the residues 105–133 contained a disulfide bond between cysteine at positions 108 and 115, which could be one of the possible oxidation mechanisms of the enzyme.

Hanwoo beef is an expensive, high-quality beef from the native cattle in South Korea. Investigations by Kim and others (2008) focused on identifying differentially expressed proteins in Longissimus dorsi muscle of Hanwoo cattle producing low- and high-quality grade beef and to explore the potential to use these proteins as biomarkers for quality attributes, mainly tenderness and intramuscular fat content. The findings of this study revealed that while the expression of α-actin was greater in high-quality grade beef, the expression of T-complex protein 1 (TCP-1), HSP27, and inositol 1,4,5-triphosphate receptor type1 (IP3R1) was increased in low-quality grade beef. Quantitative expression of HSP27 and IP3R1 correlated with intramuscular fat content, meat tenderness, and free calcium levels, and suggested that these proteins could be utilized as potential biomarkers for quality of Hanwoo beef.

Houbak and others (2008) examined degradation of beef muscle proteins by proteasome and calpains with the aim to characterize the contribution of postmortem proteolysis in tenderness development. When proteasomal chymotrypsin- and trypsin-like activity were inhibited, structural (actin, troponin-T, myosin light chain, and nebulin) as well as several sarcoplasmic proteins were not degraded. On the other hand, inhibition of the ubiquitous calpain resulted in minor changes in the degradation pattern and indicated the involvement of p94 (muscle-specific calpain not inhibited by calpastatin) in postmortem proteolysis. Based on these results, the authors summarized that postmortem proteolysis and subsequent meat tenderization involve a sequential degradation of the structural proteins, where calpain initiates disruption and destabilization of myofibrillar structure, and allowing the proteasome to act on the myofibrillar matrix proteins.

In their recent work, Jia and others (2009) analyzed sarcoplasmic proteome of beef Longissimus thoracis muscle biopsied 4 days before harvest to identify the possible protein markers for tenderness. The beef specimens from 26 animals were divided into two groups—tender and tough, based on shear force; the proteome was isolated by 2-DE, and protein spots were identified using MALDI-TOF-MS. Four proteins, namely stress-70 protein, protein DJ-1, peroxiredoxin-6, and malate dehydrogenase, demonstrated differential abundance between the tender and tough animal groups. Further analyses of proteome (from a different set of 30 animals) isolated from antemortem and postmortem muscles using Western blotting revealed that peroxiredoxin-6, an antioxidant enzyme that protects cells from oxidative damage, demonstrated consistent variation between tender and tough beef. On the other hand, differential abundance of other three proteins varied with animals. These researchers concluded that peroxiredoxin-6 could be utilized as protein marker, detectable in antemortem and postmortem muscles, for meat tenderness.

Variation in tenderness of Longissimus thoracis within Charolais young bulls were examined through analyzing proteome of tough and tender groups over time using 2-DE and MALDI-TOF-MS (Laville and others 2009b). Analyses of soluble and insoluble muscle proteins, by hierarchical classification, revealed presence of greater quantity of actin fragments and proteins from the inner and outer mitochondrial membranes in the tender group on day 0 postmortem. Mitochondrial fragmentation happens in apoptosis, and the findings of this study suggested a possible role of apoptotic process in meat tenderization. The authors concluded that determination of correlation between apoptosis markers and shear force is necessary to verify this possibility.

14.2.3 Meat Color

Meat color is the most important quality attribute influencing the consumers' purchasing decisions at the point of sale. While analysis of whole-muscle proteome or its fractions (myofibrillar proteome or sarcoplasmic proteome) was the main strategy in characterizing the molecular basis of tenderness and

muscle-to-meat conversion, proteomic investigations in meat color research focused mainly on interactions between myoglobin and small biomolecules governing postmortem color stability of skeletal muscles. A major area in such investigations was lipid oxidation-induced myoglobin oxidation and concomitant meat discoloration.

The relationship between lipid oxidation and meat color became evident during investigations on the effect of lipid-soluble vitamin E on meat quality. Incorporation of vitamin E in finishing diets improved color stability and minimized lipid oxidation in beef by preventing generation of reactive lipid oxidation products, which interacts with myoglobin and promotes myoglobin oxidation. The secondary products of lipid oxidation possess more polarity than their parent compounds, and therefore can easily diffuse from the lipid bilayer into sarcoplasm, where they interact with water-soluble myoglobin. Fundamental studies on lipid oxidation-induced meat discoloration used 4-hydroxy-2-nonenal (HNE; an α, β-unsaturated aldehyde) as a model aldehyde because its biochemical interactions with proteins and enzymes have been widely examined in medical sciences.

Using ESI-MS, Faustman and others (1999) investigated interactions of HNE with equine oxymyoglobin and determined that adduction of this reactive product results in metmyoglobin formation. This is considered as one of the first studies in a series of investigations in this area. Further studies confirmed nucleophilic attack and subsequent HNE adduction as the bases of lipid oxidation-induced oxymyoglobin oxidation and discoloration in pork (Lee and others 2003; Suman and others 2006, 2007) and beef (Alderton and others 2003; Suman and others 2006, 2007). Results of tandem-MS analyses of myoglobin peptides indicated that HNE adduction happened via Michael addition, and adduction occurred exclusively at histidine residues in pork and beef myoglobins (Alderton and others 2003; Suman and others 2006, 2007). Cysteine residues are the ones highly susceptible to HNE adduction in several proteins and enzymes. Livestock myoglobins do not contain any cysteine, and thus nucleophilic histidines become the target for HNE adduction at the imidazole ring. Histidine residues at position 64 and 93 of myoglobin are critical to maintaining the stability of heme group, and HNE adduction at these residues compromises the stability of the heme protein leading to oxidation and subsequent discoloration.

MS-based proteomic tools were utilized to understand the molecular basis of the differential effect of vitamin E on lipid oxidation-induced discoloration in pork and beef. Earlier studies indicated that vitamin E improved color and lipid stabilities in beef. In contrast, in pork, while lipid oxidation was minimized by vitamin E supplementation, a color-stabilizing effect was not readily observable. MALDI-TOF-MS analyses of myoglobins incubated with HNE at meat storage conditions indicated that the number of adducts were greater in beef myoglobin than in pork myoglobin. In addition, tandem-MS identified four histidines (positions 36, 81, 88, and 152) adducted in beef myoglobin compared with two histidines (positions 24 and 36) in pork myoglobin (Suman and others 2006). These results, along with the fact that beef myoglobin contains 13 histidines versus 9 in pork myoglobin, indicated that lipid oxidation-induced myoglobin oxidation is more deleterious to beef than pork. Further studies (Suman and others 2007) on mass spectrometric quantitation of HNE-adducted myoglobin peptides reacted with isotope-labeled phenyl isocyanate indicated that histidine 36 was preferentially adducted in pork, whereas histidines 81, 88, and 93 were the predominant sites of HNE adduction in beef. Preferential HNE adduction at the proximal histidine (position 93), observed exclusively in beef, explained, partially, why lipid oxidation-induced myoglobin oxidation appears more extensive in beef than in pork and why vitamin E supplementation has limited effect on pork color.

With the Food and Drug Administration's approval of using low level (0.4%) of carbon monoxide in modified atmosphere packaging for retailing red meats in the United States, carboxymyoglobin became relevant to the meat industry. The possibility of lipid oxidation influencing oxidation of carboxymyoglobin exists because both oxymyoglobin and carboxymyoglobin have the same amino acid sequence. However, until recently, MS-based investigations were not directed to explore this. Investigations by Joseph and others (2009) provided mass spectrometric evidence for adduction of reactive aldehydes with carboxymyoglobin in a pH- and temperature-dependent manner similar to oxymyoglobin.

Lactate is a nonmeat ingredient known to stabilize meat color. Although direct interactions between lactate and amino acids in myoglobin were hypothesized as the reason for lactate-modulated redox stability in myoglobin (Giardina and others 1996), present-day tools in MS were not available more than a decade ago to verify the hypothesis. The possibility of lactate adduction to cherry-red colored

redox forms, oxymyoglobin and carboxymyoglobin, was investigated using MALDI-TOF-MS to elucidate the nature of direct interaction between lactate and myoglobin (Mancini and others 2010). Analyses of mass spectra revealed that lactate did not form covalent adducts with oxymyoglobin and carboxymyoglobin.

While majority of the proteomic work on meat color focused on myoglobin redox chemistry, Sayd and others (2006) attempted to characterize the influence of sarcoplasmic proteome on variation in color stability in pork. Sarcoplasmic proteome was isolated from Semimembranosus of two groups of animals—light- and dark-colored meat, based on extreme L^* values at 36 h postmortem, and was analyzed. Results of 2-DE revealed that 22 protein spots were differentially expressed in these groups. While dark meat had overabundance of mitochondrial enzymes, hemoglobin, and chaperones, light pork demonstrated overabundance of glycolytic enzymes. The authors concluded that the observed differences in sarcoplasmic proteome could affect metabolism pathways in postmortem muscles, and thus influence depletion of ATP, pH decline, and protein denaturation, which are known to induce meat discoloration.

14.2.4 Meat Species Differentiation

Meat adulteration has been a major concern in many countries, primarily due to public health concerns and religious factors. Oftentimes, inferior or less-expensive meats are used to substitute expensive meats, and various methods from sensory evaluation to DNA-based assays have been utilized to differentiate meat species. Protein-based techniques such as isoelectric focusing and enzyme-linked immunosorbent assays could be used for meat species identification, albeit with limited success.

The potential of ESI-MS to measure exact molecular mass of globin polypeptide chain was successfully utilized to differentiate heme pigments from farm animals (pig, cattle, sheep, and horse) for meat species differentiation (Taylor and others 1993). Analyses of purified hemoglobin and myoglobin in ESI-MS revealed the ions corresponding to the molecular mass of the globin portions of the heme proteins. While boiling had limited effect on the intactness of globin chain, autoclaving resulted in partial hydrolysis. Nevertheless, some hydrolytic fragments were useful in establishing the species identify of the hemoglobin. In their further studies, collision-induced dissociation was coupled with ESI-MS to differentiate myoglobin from different meat-producing livestock based on their unique fragmentation pattern and species-specific amino acid sequences (Ponce-Alquicira and Taylor 2000). Recently, MALDI-TOF-MS has been utilized in distinguishing the myoglobins from exotic and minor meat species based on the differences in the molecular mass of intact protein ions (Joseph and others 2010; Suman and others 2010), and the molecular mass of myoglobins from mammalian and avian meat species were significantly different in the MALDI-TOF mass spectra (Figures 14.1 and 14.2).

14.2.5 Dry-Cured Meats

Dry-cured meats are popular in different parts of the world and are characterized by extensive protein degradation, which is, in part, responsible for the unique quality attributes of these products. Proteolytic changes and generation of peptides in dry-cured hams have been investigated using proteomic tools to characterize the product-specific quality attributes and to identify suitable molecular markers. Luccia and others (2005) analyzed changes in the myofibrillar proteome in dry-cured hams over ripening times and observed progressive disappearance of actin, tropomyosin, and myosin light chains. In addition, these authors observed that most myofibrillar proteins were completely hydrolyzed in 12-month-old dry-cured ham and several sarcoplasmic proteins markedly decreased or disappeared during the ripening process. The presence of two spots of tropomyosin chains in the water-soluble fraction suggested that some myofibrillar proteins can disappear from the salt-soluble fraction as a result of salt solubilization. These results indicated that proteomic tools could be employed to identify the fingerprints characteristic of the ripened meat products.

As an alternate method for 2-DE, Picariello and others (2006) utilized acetic acid–urea–triton polyacrylamide gel in the first dimension and SDS-PAGE in the second dimension to examine the changes in water-soluble muscle proteins in dry-cured ham (a nonfermented meat), Naples-type salami (a fermented meat), and Coppa (a semifermented product). Electrophoretically separated proteins were

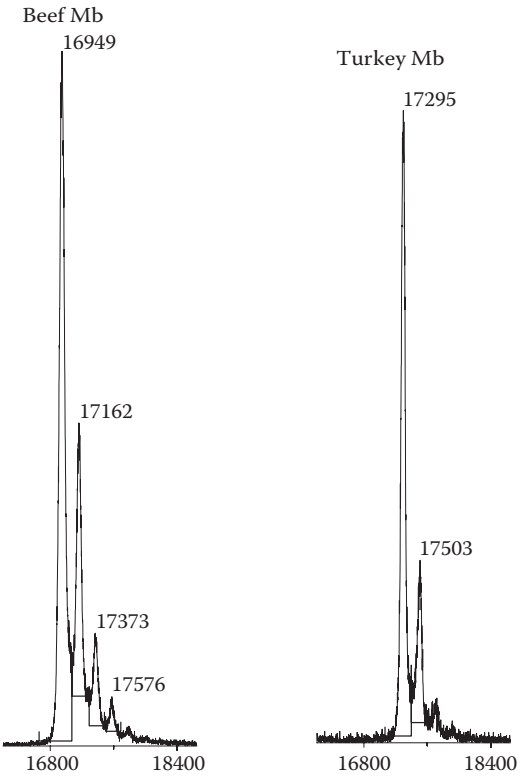

FIGURE 14.1 MALDI-TOF mass spectra of turkey and beef myoglobins. (From Joseph P, Suman SP, Li S, Beach CM, Claus JR. 2010. *LWT-Food Sci Technol* 43:273–8. With permission.)

identified by peptide mass fingerprinting using MALDI-TOF-MS. Electrophoretically separated proteins were identified by peptide mass fingerprinting using MALDI-TOF-MS. In Coppa, proteolytic events are characterized by the disappearance of glycogen phosphorylase and appearance of a polypeptide fragment of 14 kDa, whereas in dry-cured ham the conspicuous features were the hydrolysis of phosphoglycerate kinase 1, creatine kinase M, lactate dehydrogenase muscle isoform,

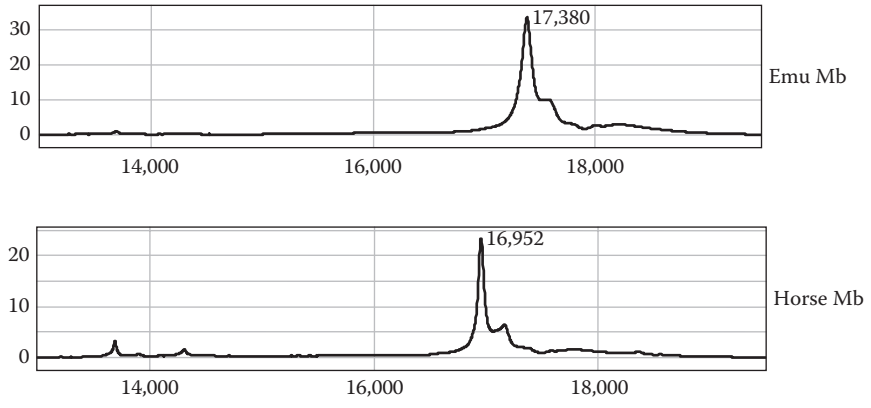

FIGURE 14.2 MALDI-TOF mass spectra of emu and horse myoglobins. The *X*-axis indicates the mass (*m*/*z*) of the singly charged protein ions, whereas the *Y*-axis indicates % intensity of the ions. (From Suman SP, Joseph P, Li S, Beach CM, Steinke L, Fontaine M. 2010. *Meat Sci* 86:623–8. With permission.)

glyceraldehyde-3-phosphate dehydrogenase, and glycogen phosphorylase muscle form, along with the appearance of spots with 14, 37, and 38 kDa molecular mass. On the other hand, Naples-type salami demonstrated hydrolysis of enolase B and the same proteins as in dry-cured ham, but lacked the spots at 37 and 38 kDa. The findings of this study revealed that each ripened product exhibits unique pattern in proteolysis.

Utilizing LC-MS-MS Sentandreu and others (2007) attempted to identify the peptides generated in Spanish dry-cured hams during the ripening process. Collision-induced dissociation of peptides separated in reverse-phase HPLC identified four oligopeptides: (1) TKQEYDEAGPSIVHR, (2) ITKQEYDEAGPSIVHRK, (3) DSGDGVTHNVPIYE, and (4) DSGDGVTHNVPIYEG in deproteinized ham extracts. Sequence analysis of these peptides revealed that they originated from actin, and these results suggested extensive hydrolysis of actin during dry-curing. Some cleavage sites leading to formation of these fragments were similar to the ones generated when actin was incubated with cathepsin D, indicative of the role of this enzyme in myofibrillar proteolysis during ripening of Spanish dry-cured ham.

Mora and others (2009a) evaluated proteolysis in Serrano ham, which is one of the popular dry-cured meat products in Spain, to identify the origin of the peptides and to understand the mechanisms responsible for the development of quality attributes of this product. MS analyses identified 14 peptide fragments derived from myosin light chain I and titin. Of these, nine peptides originated from myosin light chain I. The loss of dipeptides at the amino-terminal position observed in some myosin peptides suggested the involvement of dipeptidyl peptidases in generation of these dipeptides, which contribute to the characteristic taste of Serrano ham. The other five identified peptides came from titin's PEVK region, which is believed to confer elasticity to the sarcomere and contribute to the ability to bind calpains. These results insinuated the action of calpains on the PEVK region of titin during the dry-curing stage in Serrano ham.

The proteolytic changes of creatine kinase enzyme in Spanish dry-cured ham were investigated using LC-MS-MS (Mora and others 2009b) with the aim to identify the peptides derived from this protein; 58 peptides originating from different regions of creatine kinase were identified by tandem-MS. Furthermore, sequence analyses of the peptides revealed that aminopeptidases and carboxypeptidases were responsible for the generation of peptides and the release of free amino acids during ripening. The results of this study indicated extensive degradation of creatine kinase during the processing of dry-cured ham as well as the role played by endo- and exopeptidases in the generation of small peptides and free amino acids, which contribute to the development of the characteristic flavor in Spanish dry-cured ham.

14.3 Conclusions

The last decade witnessed the emergence of proteomics as a powerful, high-throughput, and dependable analytical tool in meat research. Combining 2-DE, HPLC, and MS enabled meat researchers to elucidate the molecular mechanisms governing meat quality, involving proteins. Applications of proteomics in meat quality have grown exponentially, and the resulting publications explained how interactions between proteins and biomolecules influence biochemical and physicochemical properties of skeletal muscle as food. Future research should focus on applications to characterize the relationships/interactions between proteome and genome of skeletal muscles and how they influence meat quality.

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Part IV

Primary Processing

15

Antemortem Handling

Jarumi Aguilar-Guggembuhl

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15.1 Introduction

Meat consumption has increased mainly due to higher income and urbanization, combined with population growth. This resulted in higher and better animal production. Every person involved in the meat production chain, starting with the animal producer to those dealing with transport until workers at the slaughterhouse, is responsible to producing high-quality meat linked to humane handling and sacrifice of animals (Knowles and Warris 2000). For this reason, animal welfare is of most importance. A wide variety of factors affect meat quality; they can be classified into ante- and postmortem (Rosmini 2006). This chapter deals with the main factors affecting meat quality and animal welfare in several meat species during handling prior to slaughtering.

During the last decade, world animal production had the fastest increase of all agricultural produce. The main production of farm animals in developed countries is of cattle, swine, sheep, and poultry, and

to a lesser extent of horses and rabbits. Of minor importance, although with certain contribution to meat supply, are game and ostrich (López and Casp 2004). Global economy forecast for 2010 mentions that world meat production will increase 283 million metric tonnes (MT), that is, 60 million MT (27%) more than the 1998–2000 period; almost 75% of the predicted increase will be in developing countries, with an estimated average increase of 2.2% per year. It was predicted that in the year 2010, developing countries would produce 80% of world domestic ruminants and approximately 70% of poultry and pigs. Therefore, world animal production participation of developing countries represent an increase from 46% in 1992, to 54% in 1998–2000, and 59% in 2010 (European Union 2004).

15.2 Animal Welfare

This term describes how the animal confronts environmental conditions (World Organization for Animal Health 2010). It is assumed that the animal must be guaranteed with health, comfort, adequate feeding, safety, natural behavior expression, and being free from unpleasant sensations (pain, fear, etc.) (Fergusson and others 2007). Welfare Quality, a European Project (European Union 2004), developed a system to objectively evaluate animal health in farms and abattoirs, identified causes of deficiency in welfare, and assess producers to improve their systems. This project also provides information to consumers on animal welfare, obtaining benefits from a value-added market. Animal welfare is affected by a number of factors related to physical and behavioral health (Velarde and Dalmau 2010).

15.3 Factors Affecting Animal Welfare

Once a meat animal reaches the market weight, it is subjected to different handling conditions before slaughtering. All animals feel some degree of stress before being slaughtered, related to the type and duration of the stressing agents and susceptibility of the animal. Stages prior to slaughtering include conditions and handling during transport from the farm to the stunning area, before actual killing. During this period, several stimuli are present such as an increase in human contact and handling, transport, unknown environments, feed and water deprivation, weather changes, and so on. As a result, the animal experiences fear, dehydration, and hunger, as well as physiological unbalances and compensation reactions to adapt to these new situations. An increase in physical activity also occurs, resulting in fatigue and even injuries. In addition, inability to satisfy feed and water requirements causes more stress (Fergusson and others 2007).

15.3.1 Fasting

This is a frequent practice for all meat species prior to slaughtering. It fulfills two roles: decreases vomits during transport and reduces carcass contamination risks by the gastrointestinal content (Morrow 2002). Fasting duration depends on the animal species, distance to be transported, and holding time in the abattoir. The effect of fasting on carcass yield and meat quality has been reported by several authors. When fasting is more than 24 h, carcass yield decreases (Eikelenboom and others 1991; Fergusson and others 2007).

Fasting in poultry considerably decreases glycogen and can affect meat color. However, it is not as stressful as hanging the animals by the feet in the case of poultry. Struggling rapidly decreases pH (Becerril-Herrera and others 2007).

As a general rule, pigs fast 12–24 h before being slaughtered. Mota-Rojas and others (2005a) report the need of 12–14 h with enough water supply; watering, in addition to decreasing the stress caused by hunger, improves bleeding after slaughtering. Warris and Brown (1994) found that fasting decreased mortality during transport (Earley and others 2006).

However, fasting during long time is prohibited by several legislations. The European Union requires that animals must be fed every 24 h (Earley and others 2006); and even every 12 h in some cases. Conversely as in Europe, New Zealand's practice recommend fasting 4–6 h before starting a journey, in order to reduce feces during transport. Feeding withdrawn for 20 h before slaughtering decreases intestine content 1–2 kg more than fasting for 6 h; this represents 2500 kg/day less manure in an abattoir where 2000 animals are slaughtered.

One of the main concerns related to cattle transport is that this operation considerably decreases carcass commercial value (Warriss 1993; Warriss and Brown 1994). There is evidence that long fasting periods result in live weight loss and decrease in meat quality; from the physiological point of view, feed withdrawn affect metabolism markers such as lactate, β-hydroxibutirate, and urea (Warriss and others 1998). However, it has been reported that meat quality can be improved when animals fast before being slaughtered (Velarde and others 2003). Fasting causes a reduction in glycogen reserve; during anaerobic glycolysis, less available glycogen produces less lactic acid, and, therefore, less pH reduction. Meat juiciness can be increased in animals fasted for few hours. Yue and others (2010) studied the effect of transport stress on blood metabolism, glycolytic potential, and meat quality in poultry. The authors concluded that transport during long periods significantly decreased plasma glucose, and increased fiber density.

15.3.2 Noise

Excessive noise is a distraction for cattle as these animals hear frequencies higher than those perceived by humans. Cattle hearing peak sensitivity is at 8000 Hz, whereas in humans it is between 1000 and 3000 Hz. Bovines are easily handled if shouts and other noises are reduced. Metal doors must be covered with any material suitable for reducing the noise when closed (Grandin 1985).

15.3.3 Animals from Different Farms

Commonly, animals from different groups and farms are transported together and remain in the same holding pen (D'Eath and others 2010). According to Geverink and others (1996), pigs do not fight with each other during transport, but establish a social dominancy as they arrive at the holding pens.

15.3.4 Genetic Background

Certain bovine genetic lines are more susceptible to excite, becoming injured when facing new environments (Grandin 1997). Genetic factors and locus halothane also affected production parameters (D'Eath and others 2010). It is well documented that locus halothane has a negative effect on stress and pork quality (Fabregas and others 2001).

15.3.5 Driving

This is important in large, but not in small, animals as they can be easily caught and restrained. Cattle, pigs, and sheep slaughtering are generally carried out in a place distant from the producing area. Therefore, it is necessary to load the animals onto trucks (Grandin 1997). All personnel handling the animals, and in particular during driving, must be familiar with natural animal behavior to avoid accidents (Grandin 1985). Animal driving is related to the flight zone; this is the distance an animal tolerates as not being aggressive in the presence of a foreign object (Grandin 1993). If a person invades the limits of the flight zone, the animal tends to move away; if a person gets closer, the animal runs away or turns against the person. The flight zone size depends on the animal's tameness (Grandin 1985). Rough handling can be more damaging and stressful for certain animals (Day and others 2002). Some specific aspects in facilities design must be considered for proper driving:

- Walls must be closed in races and pens to avoid animals (mainly cattle) from seeing people, trucks, or human activities; if the animals are not distracted, they will easily move.
- Drainage must be outside races; if the animals must cross a sewage grating, they can refuse to continue.
- Dark areas: animals will refuse to move into building as they are darker than exteriors.
- Unloading ramps must have closed walls. In the upper part, ramps must be at least 2 m flat so that the animals have enough space to start walking when leaving the truck. Ramp slope must be not more than 20°. In case of a moving ramp, the maximum slope must be 25° (Grandin 1989, 1993).

15.4 Handling during Transport

It includes all activities from vehicle loading, transport to the slaughterhouse, and unloading (Becerril-Herrera and others 2007); in some cases, transport also includes animal stress recovery. Animal transport has implications on animal welfare. For this reason, transport from the commercial and scientific points of view is of prime importance (Earley and others 2006). In 2006, total losses for the pig industry in the United States, associated with animal transport, was $46 million. Losses are a multifactor problem including people, animals, facilities design, and environmental factors (Johnson and others 2010). Transport is stressful *per se*; animals are exposed to multiple factors affecting welfare (Mota-Rojas and others 2003). The European Union legislation establishes that animal handling and transport should be as less stressful as possible (European Economic Community 1986). Operations included during transport produce physical and social changes in the animal environment, together with deteriorative climatic changes in temperature, humidity, and air supply (López and Casp 2004; Madsen and Nielsen 2004; Mota-Rojas and others 2006; Terlouw and others 2008).

Stressors are classified into two types: physical and psychological (Warriss 1998). Physical stressors are injuries, extreme temperatures, vehicle vibration and acceleration, noise, confinement, and crowding; psychological stressors are unfamiliar off-odors, new environments, hunger, thirst, and fatigue. Both types of stressors have a deeper impact on young animals and alter the immune functions (Phillips and others 1989; Minton and Blecha 1990). In fact, diseases during transportation in young animals are frequent and have been related to stress and close association between animals (López and Casp 2004; Earley and others 2006). Although transport, in itself, causes stress, several variables also increase the degree of stress. Mazzone and others (2010) studied the effect of various load methods on welfare carcass and meat quality of hybrid commercial rabbits. They found that, after transport, most rabbits showed neutrofilia, lynfocitepenia, increase in aspartate aminotransferanse, alanine aminotransferase, creatin kinase activity, and twice the expected corticosterone concentration; the authors concluded that stress was affected by transportation, and not by the loading method.

15.4.1 Temperament

It affects temperature, as is shown in a study reported by Burdick and others (2010). The authors found that rectal temperature, and cortisol and epinephrine levels varied during transport in Braham bulls; at the beginning of the transport period, bulls with more temperament had higher temperature, cortisol, and epinephrine levels than bulls with less temperament. It was concluded that temperament is a stress reaction predictor.

Pigs' aggressiveness varies among individuals of the same population. D'Eath and others (2010) studied the aggressive behavior effect of pigs in mixed groups on stress and meat quality. Mixed groups of aggressive pigs showed more skin bruises and higher cortisol levels in plasma. Meat pH of aggressive pigs was higher after 24 h postmortem, loin CIE-Lab redness (a*) and yellowness (b*) were also higher.

15.4.2 Sex

Bass and others (2010) studied handling of steer and heifer diets, fed with Mg-supplemented diets. Each group showed different stress response, but none of them had Mg effect on physiological stress indicators. Heifers were easily excited, with short-term physiological stress response linked to acute suprarenal activation and producing a harder meat without increasing muscle pH. Steers showed more intense physical activity, associated with agonistic behavior; the carcass had low glycogen content and higher pH.

D'Eath and others (2010) studied the effect of pig plasma physiological variables level after transport to the abattoir; females had higher levels when slaughtered; a faster pH decrease postmortem and high lean meat content. The authors concluded that lactate and creatine kinase, and meat pH were affected by sex and temperament.

15.4.3 Seasonal Temperature

Miranda de la Lama and others (2010) studied the effect of preslaughter logistics chain, including transport, in sheep (Rasa Aragonesa) welfare and meat quality during summer and winter. The authors

concluded that during winter, cortisol and glucose levels were higher, while in summer, creatine kinase was higher; therefore, cold temperatures had a significant effect on bruising score. The results suggested cumulative effects of all factors associated to the logistics chain. Grandin (1994) also reported that the season of the year may produce high pale, soft, and exudative (PSE) meat incidence.

High temperatures during summer usually increase the animal response to a given stress, increasing concentrations of cortisol, adrenaline, noradenaline, and dopamine (Linares and others 2008). Dadgar and others (2010) reported the effect of temperature on poultry preslaughtering transport and meat quality. At temperatures <0°C, dark, firm, and dry DFD meat incidence increased in the breast muscle, and decreased PSE meat incidence.

15.4.4 Transport Duration

The European Union, in the regulation CE nº 1/2005, recommends transport duration as a function of animal type: suckling animals (9 h followed by 1 h for rest and watering); pigs (24 h, with permanent possibility for watering); horses (24 h with the possibility for watering every 8 h); cattle, sheep, goats (14 h, followed by 1 h rest and watering). The above-mentioned sequences can be repeated, provided the animals are unloaded, fed, given water, and rest in an authorized control station. Poultry are usually transported to the abattoir in craters or cages loaded onto a truck, and most are in adequate hygienic conditions (Berri and others 2005).

15.4.5 Load Density

This variable deeply affects animal welfare, since movement is severely restricted in high load densities (Gallo and others 2000). Overloading a truck results in evident physical stress (Grandin 1994; Tadich and others 2000). During transport, pigs must have enough space to stand in their natural position, as well as to lie down all at the same time (López and Casp 2004). With a load of 0.31 m^2/100 kg load densities, pigs do not have enough space to lie down (Warriss 1998); a suitable load density for pigs is not <0.42 m^2/animal (Fabregas and others 2001). High load densities cause problems such as fallen animals and high bruise incidence, in addition to stress (Mota-Rojas and others 2007). Pigs transported at high load densities (0.31–0.35 m^2/100 kg) showed high creatine phosphokinase levels, as compared to low load densities (0.41–0.50 m^2/100 kg) that show stress decrease (Warriss 1998; Warriss and others 1998).

De la Fuente and others (2010) studied the effect of low density (0.12, 0.2, and 0.25 m^2/animal) and transport time (30 min and 5 h) on physiological responses and meat quality of sheep. The authors found that load density did not significantly affect plasma physiological variables; however, some meat quality parameters significantly change. Meat from animals transported for 5 h had lower water-holding capacity and higher lipid oxidation than from animals subjected to a short transport period. Load density and transportation did not affect meat color and texture. Table 15.1 shows some recommended load densities (European Union 2009).

15.4.6 Disadvantages of Transporting Meat Animals

The main disadvantage in transporting meat animals for relatively long distances is weight loss (Lambooy and Engel 1991), which could reach 4.9% and 5.5% live weight for cattle and pigs, respectively, in a 500 km journey (Barranco 1988). These percentages increase even more with longer distances. Weight loss is mainly due to expelling liquids: transpiration, exhalation, feces, and urine (Mota-Rojas and others 2005c) and is usually replaced, once the animals reach the slaughterhouse facilities, in the holding pens when water is supplied (López and Casp 2004; Fergusson and others 2007). Physiological adaptation to stress promotes several responses, mainly an increase in catecolamines secretion inducing weight loss, increase in heart rate, oxygen consumption and body temperature, pH decrease, lactic acid accumulation, and glyconeogenesis increase leading to an increase in basal metabolism (Cárdenas 1987).

TABLE 15.1

Recommended Animal Load Density during Transport

Species		Floor Area/Animal (m^2)
Cattle	Adults	1–1.4
	Young animals	0.23
Pigs	Porker	0.3
	Baconer	0.4
	Adults	0.8
Sheep/goats		0.4
Poultry	1 day chicken	21–25 cm^2
	adults (1.6–5 kg)	100–105 cm^2
Ostrich		0.8

Source: Adapted from European Union. 2009. EU Council Directive 1009/2009/EC (2009). *Off J Eur Community*, L 303, 1–30; EU Council Directive 93/119/EC 1993. *Off J Eur Community* L 340:21–34.

Deaths occur with certain frequency during transport; it is an indicator of lack of animal welfare during antemortem handling. Mortality rate is affected by the interactions of the factors discussed before. Poorly designed and controlled transport can result in animal welfare and meat quality. Table 15.2 shows some of these effects of transport.

Several authors studied the method to prevent transport deleterious effects on meat quality. Onenc (2010) supplemented an electrolyte and glucose solution to young bulls, improving glycogen levels in the muscle, favoring final meat pH and quality (color and texture). Chen (2010) patented a solution made by magnesium aspartate, magnesium sulfate, calcium chloride, calcium propionate, organic selenium, vitamin E, glucose, sodium bicarbonate, and potassium bicarbonate for stress reduction in pigs, caused by transport.

15.5 Handling in the Abattoir

15.5.1 Preslaughtering Holding

Once the animals arrive at the abattoir, they should be left to rest in a holding pen since transport is a highly stressful experience, resulting in muscular congestion and glucose reserve depletion (Navarro

TABLE 15.2

Effects of Transport on Animals and Meat Quality

Effect	Comment
Stress	DFD and PSE meat
Bruises	It is the most important cause for production losses in the meat industry
Asphyxiation	Due to crowding in the trucks
Heart failure	In some pig breeds, and pigs not subjected to fasting
Heat stress	Due to high temperature and deficient ventilation
Stomach distention	Caused by tiding ruminant legs without turning the animal periodically
Dehydration	Due to log transport period, high temperature, and holding times without water supply
Fights	Mostly in cattle, when a pig transport stops nearby

Source: Adapted from European Union. 2009. EU Council Directive 1009/2009/EC (2009). *Off J Eur Community*, L 303, 1–30; EU Council Directive 93/119/EC 1993. *Off J Eur Community* L 340:21–34.

1994). In fact, holding is basic to produce high-quality meat, as glycogen is the substance mainly responsible for the conversion of muscle into meat, and the incidence of DFD and PSE meat. Mota-Rojas and others (2005b) reported that if animals are slaughtered immediately alter arrival at the abattoir, the meat does not reach the desired ultimate pH (5.5–5.8), resulting in a low-quality product. In addition, when holding periods are too short or are not allowed to the animals, vascular change does not return to its normal condition; therefore, muscle glycogen concentration cannot recover to a relaxed condition.

Several authors indicated the need for a resting period of 12–24 h after transport and before slaughtering, depending on the duration of traveling and transport conditions, so that glycogen can be completely restored (Dall and Barton 2001; Fergusson and others 2007). Conversely, Geverink and others (1996) suggested that, from the animal welfare point of view, it is better to stun and slaughter the animals as soon as they arrive at the abattoir. Rosmini (2006) reports that as the animals face an unfamiliar environment when arriving at the abattoir, fights to establish hierarchies are frequent, producing an increase in DFD. Mota-Rojas and others (2003) found that when no resting occurs, vascular changes do not return to normal conditions, and an increase in PSE can also occur (Rosenvold and Andersen 2003). Tadich and others (2000) studied 8 h resting periods during steers transport, resulting in a better recovery of the animals as a result of drinking and a positive effect on rumen filling on hunger sensation.

A brief holding period is also recommended for poultry once the animals arrive to the slaughtering plant (Fernández-López and others 2010). Different responses to transport stress have been found among poultry muscles; thighs are more sensitive than breast. If transport lasts <2 h, thigh pH decreases; significant changes were not observed in the breast (Berri and others 2005).

Injured animals deserve special attention; in these cases, they must be immediately stunned and slaughtered, or within 2 h maximum. They must also be unloaded first to separate pens, using rolling platforms to take them to the restraint device for stunning (López and Casp 2004).

15.5.2 Slaughtering

Slaughtering includes two operations: stunning or insensibilization, and bleeding. Both are important to obtain good-quality meat (Lawrie 1998). According to Rosmini (2006), stunning is the operation for eliminating any capacity for experience any perception through the senses by applying a mechanical, chemical, or electrical procedure to reach unconsciousness (Gregory 2005). In this way, the animal cannot recover before killing, and any suffering is prevented; later, death will be caused by bleeding (Grandin 1997). It causes unconsciousness condition when the animal is actually killed. Stunning before bleeding is aimed for reducing to a minimum, or eliminating, animal suffering during death (Linares and others 2008; European Union 2009). In the moment of stunning and killing, animals must be healthy and physiologically normal (European Union 2009). However, several disadvantages of this procedure has been described, such as broken bones, hemorrhages, muscle blood splash, safety risks for people, and so on (Cook and others 1992; Fernández-López and others 2010).

Barranco (1988) and Mota-Rojas and others (2005c) reported that deficient slaughtering methods, lack of sanitation, unsuitable facilities and animal rouge handling directly affect animal welfare, producing acute stress increasing the nervous system activity and release of catecholamines, noradrenaline, and adrenaline to the blood stream, increasing the heart rate, blood pressure, digestion arrest, and hepatic glucose transport to the blood. Once glycogen levels decrease, lactic acid production after death is decreased and meat pH has a higher pH (Ponce 2006; Rosmini 2006).

15.5.3 Restraint Devices

The animal must be immobilized for stunning; this ensures that the animal being in a stable position and the operation is carried out in a proper way. There are several types of restraint devices, according to the species. For cattle, sheep, goats, and pigs, stunning is carried out in boxes narrow enough to prevent animals to turn around; nonslip floors are also necessary (Grandin 1997, 2001). Poultry are held by the legs from shackles on a moving band, taking the birds to an electrically charged water bath (Mota-Rojas and others 2010). Animals should never be left alone in restraint devices or boxes (Grandin 2001).

15.5.4 Stunning Methods

There are three types of stunning methods, depending on the principle in terms of which they operate: by contusion, electrical stunning, and stunning with gases (Rosmini 2006). The main requirements a stunning device must fulfill are

- To ensure fast and prolonged unconsciousness, without causing pain
- Causing minimum physical damage
- No toxic residues left in the carcass
- Easy to apply
- Minimum economic cost

Contusion methods are usually applied to cattle and sheep, whereas electrical stunning is used in pigs, sheep, and poultry; gas stunning is applied to pigs and poultry (López and Casp 2004).

15.5.4.1 Contusion Methods

Stunning is caused by a rapid concussion, producing a trauma in the nervous system and inducing total or partial unconsciousness without affecting the brain structure. As a consequence, pressure waves of varying intensity and frequency diffuse through the brain tissue, depolarizing the cell membrane (Lambooy and others 1982). This method does not damage the brain tissue. After falling, the animal presents titanic spasms and rear limb paddling movements. In sheep unconsciousness occurs after 30–40 s. The precise position in the animal's head for contusion depends on the species; in sheep and some cattle breeds, such as Brahman, it is the tempoparietal joint (back of the skull); in most cattle breeds, it is in the forehead.

15.5.4.2 Captive Bolt Pistol

This method perforates the skull; bullets are replaced by a retractable puncher. Its action is a combination of functional disorder on the nervous system and a trauma due to severing a brain area. Gregory and others (2007) studied the effect of captive bolt on the depth of brain concussion, based on the presence of nystagmus and the absence of tongue protrusion. The authors concluded that nystagmus was associated with other indicators of a shallow depth of concussion, whereas absence of tongue protrusion was not associated with the depth of concussion, but could serve as a useful indicator of jaw relaxation and insensibility following exsanguination.

15.5.4.3 Electrical Stunning

Electric flow passing through the brain is the main factor affecting immediate insensibilization; it is inverse to electric flow resistance and, at the same time, depends on the type of tissue located between the electrodes. This is a reversible stunning method if applied with two electrodes, and irreversible if applied with three electrodes (López and Casp 2004). It causes immediate insesibilization by epileptiform seizure, where three stages occur: tonic, clonic, and recovery (McKinstry and Anil 2004). The epileptiform activity is due to the high neurotransmitter release into the brain, generated by electricity (Cook and others 1992). Muscle severe contraction and limb stiffness are caused at the same time as respiration arrest. After 10 s nonstop application, muscles relax and the animals remain flaccid; 45–60 s later, limb paddling movements start and breathing restarts.

Unconsciousness duration is independent of the applied voltage, but increases if the electrode position is correct. In pigs, if the stunning and bleeding time is >15 s, the animal can regain consciousness (López and Casp 2004). In studying sheep, Cook and others (1995) concluded that 1.0 A current (50 Hz, 500 V) for 0.2 s produces unconsciousness and analgesia to subsequent slaughter procedures, although maximum welfare benefits are attainable at 2.0 A for 20 s. The authors found that a seizure-like state and the levels of release of glutamate, aspartate, and gamma amino-4-butyric acid increased with stun duration until 4 s, where a peak in these parameters was seen.

Electrical stunning is the most widely used method for sheep (Bórnez and others 2010). Several authors studied alternatives to reduce electronarcosis negative effects, such as cardiac dysfunction or arrest, hemorrhages, and safety risks for operators (Kirton and others 1981; Vergara and Gallego 2000; Vergara and others 2005).

Meat quality is also related to electrical stunning. Vergara and Gallego (2000) concluded that electrical stunned sheep carcasses had shorter shelf life when packed than sheep stunned by other methods. Conversely, Velarde and others (2003) found no significant differences in color (L,a,b), muscle pH, dripping losses, and carcass weight loss after 45 min and 24 h in electrical stunned sheep carcasses. However, blood loss with respect to carcass weight and a higher incidence of heart petechia occur in these carcasses.

15.5.4.4 Carbon Dioxide Stunning

During 1959, in Denmark, animals were driven into a CO_2-filled chamber of 70–90% concentration, and they were held in the chamber for 4560 s, although gas concentration and application time depend on species and the animal age (Bórnez and others 2010). This system causes anesthesia; it is mainly applied to pigs (Holst 2001; Nowak and others 2007) and poultry (Gregory 2005). CO_2 does not leave toxic residues in the tissues, or accumulate in the blood; it reaches the brain causing unconsciousness. In addition, this gas reduces hemorrhage incidence, is safe for the operators, and prevents the reduction in meat quality (Channon and others 2002). In addition, CO_2 stunning favors better bleeding (Madsen and Nielsen 2004). Pigs are driven into 2–5-animal cages and lowered to a well filled with the gas, where they are allowed to stay for a given time. Stunning occurs after a neuronal function depression, it has three stages: (1) analgesic, approximately 20 s, is the animal response to pain, the stress is gradually reduced, and breathing becomes deeper and faster; (2) exciting, approximately 7 s, occurs after becoming unconsciousness; in some animals vocalization and random movements occur; and (3) anesthetics, skeletal and respiratory muscles become relaxed (López and Casp 2004).

Vergara and others (2005) studied the effect of electrical and CO_2 stunning, and nonstunned animals, on suckling lambs; they found that at 24 h postmortem, the highest pH was obtained from nonstunned lambs, whereas CO_2-stunned animals had the lowest final meat pH and the most tender meat. Bórnez and others (2010) compared different CO_2 concentrations and exposure time on hormonal physiological and hematological responses, and biochemical blood parameters. The authors concur with Linares and others (2007) in considering that CO_2 is an alternative for sheep stunning, producing good-quality meat.

Several alternatives to the use of CO_2 have been suggested, such as 90% argon, or a mixture of 60% argon 30% CO_2, decreasing the excitement reactions in pigs (Raj and Gregory, 1995, 1996) although CO_2 is still considered to be the best option for gas stunning.

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16

Postmortem Handling

Rosy Cruz-Monterrosa and Isabel Guerrero-Legarreta

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16.1 Introduction

After eviscerating and cleaning the carcass, a number of steps are taken to transform the muscle into edible meat. Obtaining a top-quality food will depend on the way these steps are followed. Carcass inspection and refrigeration, biochemical and physical changes involved in sensory characteristics, and microbial considerations should be understood and applied. This chapter describes carcass handling from the abattoir to the chiller, and the basic biochemical and microbial factors involved.

16.2 Biochemical Changes

After animal exsanguination and general circulatory and cardiac arrest, energy metabolism changes from aerobic to anaerobic; the final products stop being CO_2 and H_2O, instead lactic acid is produced as the pH gets reduced from around 7.0–5.3 to 5.9. At the same time, glycogen and creatine phosphate are depleted and body temperature decreases from 39 to 36°C. Changes in these concentrations depend on the animal *in vivo* metabolism, as well as premortem handling, slaughtering methods, and postmortem conditioning.

Rigor mortis starts shortly after death, when muscles became permanently contracted due to the polymerization of actin and myosin into actomyosin. Rigor onset initiates when ATP starts declining; when ATP is depleted to ADP and IMP, no rephosphorylation is possible and relaxation cannot occur. At this point, meat tenderness is at a minimum; therefore its physicochemical characteristics (water-holding capacity, emulsifying capacity, cohesiveness, and so on) practically do not exist. From the sensory point of view, meat is totally unacceptable due to its toughness, bland flavor, and dark color (Mottram 1998; Young and others 2001; Pérez-Alvarez 2006).

Rigor resolution gradually occurs during postmortem conditioning; muscles partly recover their tenderness and elasticity due to enzymatic activity that depletes, in various degrees and with different specificities, myofibrillar proteins such as desmin, troponin T, viculin, titin, nebuline, actine, and myosin,

resulting in loss of tension and changes in myofibrils. The Z-line is weakened due to desmine fragmentation and Z-disks also degrade as a result of phospholipid liberation, induced by Ca^{2+} release from the sarcoplasmic reticulum. The enzymes responsible of postmortem changes involving tenderization are of two sources, endogenous and exogenous. Endogenous proteases weakening the myofibrillar structure belong to two enzymatic groups: cathepsins and calpains. Cathepsins or acid lysosomal enzymes act upon myosin and actin; 13 cathepsins have been reported, although only seven are identified in striated muscle: A, B, C, D, H, L, J. Calpains, on the other hand, are of two types: m-calpains need high calcium concentrations (300 μM) to activate and μ-calpains need lower concentrations (5 μM), and are represented by calpains I and II, respectively. Both calpains have peak activities between pH 6.6 and 6.8. Another calpain, P94-calpain, has also been identified.

Exogenous enzymes, on the other hand, originate from native microflora, colonizing the meat surface. They originate during the slaughtering and storage stages and are selected due to the meat microecology (i.e., intrinsic meat parameters) and extrinsic storage conditions such as temperature and humidity.

16.3 Carcass Refrigeration

Keeping the meat at low temperatures after carcass evisceration and cleaning is the most important requirement in order to achieve a desirable shelf life, as it controls the microbial population type and growth rate (European Union 2004). Rapid chilling, applied in many commercial operations in order to speed up meat cutting, distribution, and commercialization, is desirable to reduce sanitary risks associated with microbial growth. Carcasses are cooled down to 5–7°C, as measured in specific muscles such as the legs (Gill 1995). However, rapid chilling may lead to meat toughening by severe shortening of unrestrained muscles.

The above-described biochemical changes during rigor mortis, involving glycolysis and protein polymerization, are temperature-dependent. Greaser (2001) reported ATP decrease from approximately 6.0 to 0.7 μm/g in 4 h postmortem in animals slaughtered under commercial conditions; pH decreased from 6.8 to 5.8 in the same time span, and creatine phosphate from 6.3 to 0 μm/g; however, rigor has a considerably longer duration if the temperature is lowered.

Storage at extreme temperatures (i.e., below 5°C or above 30°C) causes muscle shortening. This phenomenon is at a minimum if the carcass is stored between 12°C and 20°C during resolution of rigor mortis; storage at >30°C produces a tougher meat, and if stored at <10°C, the obtained meat will be at its toughest condition (Young and others 2001). However, this postmortem metabolism rate dependence on temperature is not applied to every muscle, as muscles with oxidative metabolism can undergo slow shortening at <10°C (Greaser 2001). Although the mechanisms are different at high and low temperatures, in both cases, the result is a tough, inextensible meat. Cold shortening (at <10°C) occurs due to the liberation of calcium, triggering muscle contraction; beef and lamb carcasses are the main species affected by this condition. Conversely, shortening at high temperatures (>30°C) occurs when the muscle enters rigor when still warm (Young and others 2001).

16.4 Microbial Contamination and Spoilage

In addition to the previously mentioned considerations on meat toughness, it is very important to have in mind that microbial growth is encouraged at temperatures above 15°C. The most common cause of meat spoilage is the presence of microorganisms together with metabolite production, although enzymatic degradation also accounts for carcass shelf-life reduction. Due to the diversity of chemicals that compose the meat substrate, a wide variety of microorganisms can be present; type and amount of microbial populations are also determined by intrinsic and extrinsic parameters, such as temperature, humidity, and time (Dainty 1996).

Pre- and postslaughtering handling is the main factor that determines the type and extent of microbial contamination; clothing, floors, walls, and instruments are also sources of contamination. The carcass itself is also a source of contamination, microorganisms involved mainly originate in the rumen, intestines, skin, ganglia, and feathers, and sometime faces, contaminating the carcass surfaces. When

the inner parts of the carcass are not rapidly cooled down, growth of *Clostridium perfringens* and *Enterobacteria* spp. are likely to occur (Masana and Rodríguez 2006).

16.4.1 Microbial Growth

Bacteria relevant to meat, meat products, and other foods are divided into three groups according to the temperature range within which they can grow: mesophiles (10–45°C), psychrophiles (0–28°C), and psychrotrophs (10–45°C). Mesophiles do not grow below 10°C, but psychrotrophs, of which *Pseudomonas* is the most important in meat, can grow even at 0°C. The nearer to 0°C, the slower the growth rate and the longer the shelf life. Many mesophiles cause spoilage, but since meat is generally kept under refrigeration, most spoilage is due to psychrophiles (Guerrero-Legarreta 2009).

Microbial populations initially colonize muscle surfaces, where its growth is based on the utilization of low-molecular weight compounds as energy source, such as glucose, glucose-6-phosphate, ribose, glycerol, amino acids, and lactate. When glucose utilization is higher than its diffusion from the muscle internal layers, amino acid utilization starts (Zamudio 2006). Therefore, glucose concentration in muscle is a critical factor for microbial colonization and meat alteration (Nychas and others 1988).

However, depending on their ability to degrade the meat substrate are growth requirements of a given microbial population, which are also affected by extrinsic parameters, mainly temperature and humidity (Masana and Rodríguez 2006). If stored under refrigeration, mesophile growth is inhibited in pork and beef, whereas psychrotroph growth and metabolism become the main cause for carcass deterioration (Guerrero-Legarreta 2009), mainly *B. thermosphacta*, *Carnobacterium* spp., *Lactobacillus* spp., *Leuconostoc* spp., *Weissella* spp., *Enterobacteria*, *Pseudomonas* spp., *Shewanella putrefaciens*, *Acinetobacter*, and *Psychrobacter inmobilis* (Borch and others 1996). At 0°C and high humidity conditions, storage promotes slime and odor development after 10 days, but can occur at only 3 days if stored at 5°C, due to the growth of aerobic psychrotroph Gram-negative bacilli, mainly *Pseudomonas* spp., *Acinetobacter*, and *Psychrobacter inmobilis* (Jay and others 2003), pseudomonads being the most abundant, such as *Pseudomonas fragi*, *P. lundensis*, and *P. fluorescens* (Zamudio 2006). At –1.5°C several lactic acid bacteria can grow in beef, such as *Carnobacterium*, *Lactobacillus*, *Leuconostoc*, and *Pediococcus*, among others (Jones 2004).

Most countries have already eradicated or controlled zoonosis; however, the highest risks are still associated with *Escherichia coli* O157:H7 in beef, *Salmonella* spp. in beef and pork, and *Yersinia* spp. in pork (FAO/WHO 2004). The most important microorganisms present in raw meat causing infections or intoxications are *Salmonella* spp., *Escherichia coli* O157:H7, several *Yersinia enterocolítica* serovars, *Campylobacter jejuni*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Clostridium perfringes*, *C. botulinum*, *Bacillus cereus*, and *Staphylococcus aureus. Salmonella* and *L. monocytogenes* are present in soil, forage, and feces, and find a niche in the animal's skin from where they contaminate the carcass surface; *Staphylococcus xylosus* predominates in the cattle skin, *S. xylosus* and *Staphylococcus lentus* in sheep and goat, and *Staphylococcus aureus* occurs in the udders and milk, especially in mastitis-infected animals. Salmonella and other feces-originated microorganisms, such as *L. monocytogenes*, *Y. enterocolitica*, and *E. coli* O157:H7, contaminate the skin during transportation; whereas the main source of *E. coli* (including *E. coli* O157:H7), salmonella, *Clostridium jejuni*, *C. perfringens*, and other clostridia are rumen and intestine-borne (Zamudio 2006). However, in chilled carcasses, meat pathogen proliferation such as *L. monocytogenes*, *Y. enterocolitica*, and *Aeromonas* is lower than spoilage microorganisms.

16.4.2 Viscera Storage

Viscera and blood share the characteristics of rapid spoilage due to their enzymatic content, pH close to neutrality, and the presence of easily metabolized compounds by microflora or that which can undergo chemical deterioration. Some tissues spoil more rapidly than others; for instance, adrenal glands, digestive system epithelium, pancreas, liver, kidneys, and central nervous system deteriorate first (Randall and others 2002). Several organs have high polyunsaturated fatty acid, cholesterol, and phospholipid levels that become easily oxidized (Hill and others 2004). With the exception of the nonruminant stomach, the smooth muscle pH is close to neutrality, allowing a rapid colonization of a wide number of pathogens and spoilage microorganisms. As mentioned before, animal intestines are highly contaminated

with microorganisms; they must be removed from the carcass, or if utilized, must be immediately cleaned. The fat is separated from the intestines, and the feces stripped out; the inner mucosa membrane is also separated from the casing. Blood is usually recovered from pigs, cattle, and lambs, and separated into plasma and red cells. In general, it is utilized as a raw material for a wide variety of food products (Guerrero-Legarreta in press).

16.5 Postmortem Inspection

Owing to the risk of zoonosis by meat consumption, postmortem inspection is carefully controlled by most legislation around the world. Food-transmitted diseases can be grouped into three categories: (1) animal infections feasible to be transmitted to man, such as helminthic zoonosis acquired through intestinal excretions and occupational excretions acquired due to meat handling in the abattoir; (2) infections and intoxications due to microbial contamination; and (3) chemical and toxicological contaminations.

The Codex Alimentarius code of practice for meat hygiene is the main document compiling international regulations encompassing sanitation throughout the meat production chain. It defines postmortem inspection as an essential component in meat control carried out after slaughtering; its main objective is to identify and confiscate all irregular material, as well as to detect possible contamination. In this way, suitability of meat for human consumption is ensured (Gracey 1989).

Time span between slaughtering and postmortem inspection must be as short as possible; carcasses should be cooled down to avoid microbial proliferation. Postmortem alteration occurs in a relatively short time depending on a number of factors, such as general condition of the animal and adipose panicles, presence of hair or wool, body temperature of the dead animal, room temperature, and so on.

Meat inspection legislation in most countries point out that the carcass parts (head, feet, viscera) must be identified separately, as, if any lesion or defect is detected, all parts are confiscated (Gracey 1989). Visual examination must be carried out under natural or artificial illumination allowing detailed identification; macroscopic characteristics are also complemented with more detailed examination. Inspection methodology must include

- Individual examination of each slaughtered animal and its organs.
- Examination of organs susceptible to defects or lesions.
- Incisions in certain organs and ganglia, if necessary.
- Search for anomalies in consistency, color, and odor.
- If any abnormality is suspected, chemical and histological analysis must be carried out, particularly to detect substances or hormones not allowed or limited by current legislations.

Table 16.1 shows the main laboratory tests necessary during postmortem inspection in the abattoir.

Inspection starts with a visual surface examination before carcass splitting or sectioning into primal cuts, with minimum handling. Visual inspection is particularly focused on any anatomical regions that may present signs of zoonosis or pathologies. Any cross-contamination to carcasses from the skin, hair, or viscera due to faulty techniques must be avoided. Carcasses must be separated from each other, avoiding any contact with walls, instruments, or other carcasses; or in general, any action that may contaminate the meat due to handling such as cutting, incision, or palpation.

In countries or regions where certain diseases such as tuberculosis, erysipelas, or parasitic infections are prevalent, general postmortem inspection should specifically look for these pathologies. However, depending on the animal species, postmortem inspection may vary, as described below. A complete inspection must be carried out in beef carcasses of 6 week animals or older, although younger animals are seldom slaughtered (Rhoades and others 2008).

16.5.1 Carcass Inspection

The initial carcass inspection is visual; it is focused on general carcass quality, injuries, edema, bleeding efficiency, bone swelling or deformation, muscle and joints, abnormal sensory characteristics (color,

TABLE 16.1

Most Frequently Applied Laboratory Tests Postmortem in Abattoirs

1. General Tests
Muscle pH
Specific and unspecific microbiology
Residual antibiotics, hormones, and drugs
Odor and flavor anomalies
2. Specific Tests in Species Feasible to Present Trichinosis
3. Specific Tests
Water-holding capacity
Bleeding efficiency
Yellowness due to pathologies (i.e., ictericia) or to feeds
Pig head and neck lymph ganglia staining

Source: Adapted from Moreno GB. 2006. *Higiene e Inspección de Carnes*. Madrid, Spain: Editorial Díaz de Santos. pp. 343–345.

odor, and texture), age, sex, and general animal hygiene. Other characteristics such as color, appearance, or general conditions of aponeurosis and cavity membranes (pleura and peritoneal) are also considered (García and others 2008).

The inner and outer carcass sides are inspected; lymphatic ganglia are inspected by palpation. Care must be taken not to harm the carcass in such a way as reducing its commercial value. In animals suspected of having any generalized pathology, the carcass is confiscated (Rhoades and others 2008).

Beef carcasses are vertically split along the spine with a saw and suspended by the hind legs; lamb carcasses are not split but are also suspended by hind legs exposing the abdominal and thoracic cavities, skinless, without the knuckles; the head is not separated. Inspection of sheep and goat carcasses is similar to beef, although the entire carcass is suspended by the hind legs, exposing the abdominal-thoracic cavity. Complete pork carcasses are also suspended from the hind legs, examination is similar to beef carcasses. Special attention must be taken to look for signs of tuberculosis, mainly in the submaxillary ganglia and cysticercosis. It is highly advisable to sample pork carcasses for possible triquinosis (FAO/WHO 2004).

One of the main problems in carcass inspection is detecting cysticercoids, even more difficult in beef than in pork carcasses since in beef, cysts are few and smaller and are located in the intramuscular connective tissue, where fat deposits mask their presence. Spanish regulations establish that external masseter muscles and the tongue must be inspected by making incisions and palpation (Real Decreto 1996). When triquinosis is suspected, samples must be taken and studied by microscopy techniques (Moreno 2006).

If a carcass is confiscated due to abnormalities because the abnormalities, lesions, or contamination were previously detected *in vivo*, the carcass can have the following destinations (Gracey 1989):

1. Totally confiscated. If on arrival to the slaughterhouse, the animal presented any acute infectious disease; subnormal temperature (hypothermia); comatose or dying state; chronic condition such as anemia, or degenerated organs; acute infection due to hemoparasites; septicemia or toxemia; abnormal color and odor associated with chronic diseases, feeds, or medicines; generalized edema; asphyxiated or death for any cause; diseases or pathologies in specific anatomical regions; pathologies due to microorganism or parasites, such as actinobacilli, actinomycosis, anthrax, *Cysticercus* spp., botulism, brucellosis, tuberculosis, rabies, *Salmonella* spp., tetanus, etc.
2. If the carcass is aimed for human consumption, the carcass can be
   - Inspected and authorized
   - Inspected and rejected
   - Inspected and authorized for meat processing (restricted use)
   - Inspected and authorized for frozen products (restricted use)

3. Totally rejected for human consumption
4. Partially authorized for consumption

16.5.2 Viscera Inspection

These are the main steps necessary to follow when inspecting viscera:

1. Head: Visual inspection, palpation, and sectioning of lymphatic nodules specially retropharyngeal, submaxillary and parathyroid to detect tuberculoses lesions, abscesses, and bacillosis (FAO/WHO 2004). The masseter muscle, tongue, lips, and gums are inspected inside and outside making incisions to verify foot and mouth lesions and necrotic stomatitis, as well as certain parasites, such as *Cysticercus bovis* (Gracey 1989).
2. Red viscera: Evisceration must be carried out in <30 min from the animal's death; if longer time is required, viscera must be subjected to bacteriological examination. Lymphatic ganglia are inspected only for tuberculosis; lungs are visually inspected as well as by palpation to detect lesions such as pleurisy, pneumonia, tuberculosis, and parasites such as fasciolasis and cysts. Heart is inspected for consistency, as softness is associated to septicemia; pericardium is inspected for pericarditis lesions, the heart is sectioned to exposed ventricles and to detect any clotting caused by cardiac insufficiency, or hidatidosis caused by poor sanitation. Liver is inspected by the same technique, focusing on color, firmness, and elasticity, must be negative for abscesses, fat degradation, parasites, adherence, calcification, congestion of bile conducts, necrotic infraction (blush-green, rancid odor), and telangiectasia; lymphatic nodules are also examined. Finally, kidneys are inspected; fat and capsule should be easily removed, otherwise it indicates the presence of a pathology. Petechiae in the renal cortex and necrosis must be absent (López y Casp 2004).
3. Green viscera: The esophagus is tided to avoid the carcass being contaminated by its content; the rumen and small and large intestines are also tided. For examination, these viscera are expanded; foreign material, local or general peritonitis, and lymphatic nodules are first inspected. Lesions related to tuberculosis or septicemia must be absent (Martínez 2010). Spleen parenchyma is specially inspected for septicemia (due to leucosis, tuberculosis, or anthrax) showed by changes in volume and consistency. In females, the uterus and mammary gland are also inspected by incisions in the supra mammary ganglia, and to verify absence of mastitis. The uterus is checked for pregnancy or recent parturition. In males, testes are also inspected (Gracey 1989).

Once a carcass is authorized, it can be transferred to storage and further commercialization.

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17

Electrical Stimulation in Meat Processing

Daniel Mota-Rojas, Patricia Roldán-Santiago, and Isabel Guerrero-Legarreta

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17.1 Introduction

During postmortem storage, the muscle undergoes a series of biochemical, histological, and physical events, all together called rigor mortis. If one or several of these events are modified, product acceptability may be altered (Stiffler and others 1982). Tenderness, in addition to color and flavor, is considered the most important characteristic of meat (Savell and others 1977; McKeith and others 1979; Elgasim and others 1981; Soria and Corva 2004; Castañeda and others 2005). Looking for an efficient postmortem tenderizing method is a result of consumers' demand for good tasting and uniform quality meat (Stiffler and others 1982). Several researchers developed methods to improve meat tenderness; muscle contractile proteins, connective tissue, or both are usually affected by these processes.

The factors determining the quality of raw meat, as well as meat subjected to refrigeration, freezing, or any other treatment involve its nutritive value, sanitation, sensory characteristics, and its possible use as raw material for further processing. These factors can be grouped under those originated before birth or genetic; occurring during the animal's growth or environmental; and those resulting from meat handling and processing, including meat ripening, storage, and specific operations for meat transformation into fabricated products. All of these factors determine meat preservation and quality (Stiffler and others 1982).

Electric stimulation (ES) is one step in meat transformation; it enhances sensory characteristics (color, odor and flavor, and tenderness). Early studies in ES were carried out during the 1940s in the United States, although without considerable success. Sams (1999) pointed out that ES meat was industrially produced in the 1950s, aimed for meat processing. During the 1970s, this method was used to avoid cold shortening in lamb carcasses. In 1975, some prototype stimulators were incorporated in American abattoirs; these prototypes were considerably improved during the following decades and at present some ES equipments handle up to 300 animals per hour (Guerrero and others 2004).

ES involves the application of an electric current to the carcass of recently slaughtered and eviscerated animals. By supplying an electric current, the muscle fibers extensively contract and suddenly undergo extension preventing further contraction or rigor mortis and, as a consequence, further muscle shortening. This effect is also known as physical disruption of the myofibrillar matrix or proteolysis acceleration; it increases the glycolysis rate and causes an immediate pH drop (Elgasim and others 1981; Byrne and others 2000; Hwang and others 2003).

The effect of ES on meat quality is discussed in this chapter, reported results are discussed, and biochemical and sensory characteristics of stimulated and nonstimulated carcass meat are compared.

17.2 Effect of ES on Meat Sensory Characteristics

Sensory characteristics are possibly the main factor determining meat quality. Failure in fulfilling characteristics expected by the consumer will result in economic losses for the producer; they depend on numerous factors including handling before and after slaughtering. Therefore, ES application also results in improving the sensory characteristics.

Radish and others (1983) reported that in a conventional slaughtering process, considerable time is consumed between slaughtering and getting the right carcass quality to be ready for marketing as meat. However, the time elapsed can be sensibly reduced by applying ES. On the other hand, Channon and others (2003a) comment that ES benefits largely depend on the stunning method as well as the voltage applied when electric stunning is used; time elapsed after killing also influences the optimal conditions for ES.

Studies reported by Elgasim and others (1981) on 459–510 kg American beef stimulated at 600 V, 7 A, 7 Hz for 1 min showed that muscle pH and temperature after rigor mortis affects meat tenderness as well as other sensory properties. Tenderness was analyzed using a Warner–Bratzler shearing device and by sensory evaluation methods. The panelists detected significant differences in the odor, flavor, appearance, and juiciness of the stimulated meat (Pospiech and others 2003).

Similar results were obtained in studies carried out by Savell and others (1978) on five conventionally slaughtered steer carcasses. After 1 h postmortem carcass, right halves were stimulated at 50 pulses for 0.5–1.0 s, 100 V, 5 A, 50–60 cycles/s; the left sides were taken as control. Both sides were then refrigerated, *Longissimus* muscle samples 20–24 h postmortem from both sides were analyzed for sensory characteristics and sarcomere length. The results indicated that samples taken from the stimulated half were less juicy, more tender, and with better flavor, having less detectable connective tissue; hardness and cooking loss were also reduced.

Davel and others (2003) studied sensory characteristics of meat obtained from stimulated carcasses of 22 castrated Dorper sheep (40–50 kg). Stimulation was at 20 V, 45 Hz for 45 s, the carcasses were then refrigerated for 24 h at 2°C, *Longissimus thoracis* and *lumborum* muscles were excised and cut into two, vacuum packaged and frozen at –30°C before the sensory evaluation. The right *Longissimus lumborum* was grilled at 160°C, 73°C internal temperature. Results showed higher acceptability of stimulated as compared to nonstimulated samples. ES did not affect cooking losses, associated with weight at sacrifice and carcass fat.

Studies carried out with 12 female alpacas, applying various stimulation voltages (500 and 600 V for 30 and 60 s) after 1–24 h postmortem, reported a significant effect on *Longissimus dorsi* pH between treated and nontreated meat. The difference was mainly due to time and applied voltage (500 V/30 s) causing a rapid decrease in pH_{24} (5.27), as compared to the control group (pH_{24} 6.31). In general stimulated meat has pH < 6.0 for the first 24 h poststimulation; conversely, nonstimulated meat has pH = 6.0. This phenomenon, according to the authors, could be due to the fact that ES increases the rate in muscle contraction cycles, accelerating exhaustion of glucose reserves and increasing lactic acid production (Guerrero and others 2004).

Similar results were obtained by Byrne and others (2000) in carcasses obtained from 47 calves; pH decreased during postmortem storage as a result of lactic acid accumulation due to glycolysis, that is, pH 6.56 after 2 h and pH 5.48 after 24 h postmortem. No DFD carcass meat was observed.

Young and others (1999) studied color changes in 96 chicken carcasses; half of the animals were electrically stimulated during stunning. The authors concluded that ES produced significantly brighter meat but less intense redness in the *Pectoralis major* muscle. pH in ES meat rapidly decreased, whereas pH decreased in control samples (non-ES) to the same levels in amount in 2 h. The bright color of the stimulated carcasses was probably due to rapid muscle acidification caused by protein denaturation which, in turn, caused higher light reflectance on the meat surface (Warriss 2000).

Voltage intensity and duration also affect the meat color. One hundred ES bovine carcasses were studied by Roeber and others (2000); the positive electrode was placed in the *Latissimus dorsi* muscle

and the negative in the *Biceps femoris* muscle. Average 100 V voltage was compared against high (300 V) voltage and 11 cps and 16 cps duration at 60 Hz. Color of stimulated *Longissimus* muscles was brighter, redder, and less blue as compared to nonstimulated muscles. Conversely, no differences were found with respect to water loss during cooking; the authors explained this observation to the fact that the total storage time in this study was 3 days, considerably less than in other studies that report an increase in water loss after 7 days total storage time. The authors also concluded that ES promotes a reduction in carcass-ripening time, in turn producing leaner and more desirable colored meat.

Studies carried out by Channon and others (2003b) concluded that with the application of 50–200 mA for 30 s constant current to previously bled swine carcasses, the time to reach final pH values was 40 min, as compared to 8 h in non-ES carcasses. However, the authors also reported that ES increased exudation.

It has been demonstrated that ES increases beef palatability (Stiffler and others 1982), improves color, and reduces ripening time; it also reduces the presence of dark-cutting beef (McKeith and others 1981). Warriss (2000) reports that ES affects meat acceptance due to flavor changes. Nonetheless, studies carried out by Guerrero and others (2004), showed flavor improvement in 600 V/30 s stimulated carcasses. Possibly, at this voltage stimulation free amino acids react with other chemicals, producing flavor-related compounds, such as those resulting from Maillard reactions (Gianelli and others 2003).

The effect of ES and evisceration on flavor was studied in 390 bovines (young bullocks and calves) by McKeith and others (1981). The carcasses were eviscerated and stimulated 5 min after bleeding, and 20 and 30 min after evisceration, and compared to a control (nonstimulated carcasses). ES was applied at 150 V or 550 V for 1 min (16 pulses) or 2 min (32 pulses); the impulses were for 1.8 s with 1.8 s intervals between them. The authors found that there was no flavor improvement when ES for 1 or 2 min was applied; however, when the carcasses were stimulated with 550 V, leaner, redder, and more tender with better flavor meat was obtained, as compared to meat from carcasses stimulated with 150 V. It was concluded that short time (1 min) and high voltage (550 V) ES can be applied during different dressing in order to increase meat palatability.

ES also affects meat odor. Data obtained by Guerrero and others (2004) indicated that on applying 500 V/60 s, the meat odor is significantly reduced. Conversely, studies by Owens and Sams (1997) in 36 electrically stimulated female turkeys (average weight 7 kg, 10 pulses in the neck region at 570 V, 45 mA, 2 s and 1 s) showed that muscular metabolism is accelerated after 2 h postmortem, decreasing pH and preventing excessive sarcomere shortening. However, ES had no effect on the cutting, water loss during cooking, fragmentation index, or color values, suggesting that postmortem ES provides benefits that justify applying this method in turkey processing.

Hertog-Meischke and others (1997) studied ES to 60 bull carcasses (2 years old, 368–410 kg carcass weight); eight carcasses were electrically stimulated (85 V, 14 Hz, 15 s immediately after bleeding). The results showed that ES caused high drip loss, possibly as a result of myosin denaturation caused by pH and temperature postmortem reduction.

Therefore, as shown by the results obtained from various authors, several sensory characteristics such as odor, color and flavor, juiciness and, mainly, tenderness are improved by the application of ES. However, other factors must be considered in these results such as animal species, genetics, handling, and animal age and breed.

17.3 Physiology and Biochemistry of ES Meat

ES accelerates two of the leading postmortem processes: pH decrease to values lower than 6.4 and rigor mortis onset by accelerating the glycolysis rate. During ES application, the rate of both processes is considerably increased but decreases when electricity application ceases.

ES also prevents thaw rigor in hot carcass, when meat is frozen before rigor mortis resolution. In some cases, ES is applied to excised muscles, although it should be carried out within 30 min after sacrifice, when muscles are still attached to the skeleton (Prändl and others 1994).

ES allows carcass or primal cuts to be refrigerated or frozen just at the end of the slaughter line, without previously allowing onset and resolution of rigor mortis. In this way, the advantage of using ES is mainly in avoiding carcass shorting during postmortem refrigerated shortening due to rapid temperature decrease.

From the physiological point of view, the mechanism responsible for ES meat tenderization is by Ca^{2+} ion-release from the sarcoplasmatic reticulum; myosin-ATPase is then activated promoting muscle contraction. Calcium liberated during muscular contraction stimulates calpains, specific sarcoplasmic proteases disrupting the Z-line. When this event occurs, muscle temperature is still high (around 30°C) and pH is above 6.5, and calpains are highly active. Lysosomes are also probably disrupted releasing cathepsins, other endogenous proteases, which also promote muscle proteolysis (Warriss 2000).

Conversely, during cold shortening, when hot carcasses are refrigerated (at approximately 10–15°C) in prerigor conditions, before dissipation of body heat, calcium ions are massively liberated from the sarcoplasm by disruption of the calcium pump, while myosin-ATPase is still active; at the same time, muscle ATP concentration is still high providing enough energy for contraction. As a result, the muscle severely contracts producing very tough meat, which is even more evident when cooked.

Toughening is even more marked when thaw rigor takes place. This occurs when the prerigor meat is frozen (at less than –10°C) (Lawrie 1991). In this situation, ATP has not depleted yet; calcium remains in the sarcoplasmic reticulum, even though the calcium pump is disrupted due to freezing. When thawed, massive calcium release occurs, while ATP is still at high concentrations, and the result is an extremely severe shortening, even more than in cold shortening. It has been reported that an excised muscle undergoing thaw rigor shortens up to 30% of its original length (Lawrie 1991). However, muscle length in thaw rigor meat as well as in cold shortened meat is prevented up to a certain point due to muscle attachment to the skeleton.

In addition, thaw rigor can be prevented by several methods, such as by allowing onset and resolution of rigor mortis at 14°C to 16°C, by suspending the carcass in altered positions such as pelvic suspension, or by ES (Prändl and others 1994).

ATP's, being the fuel for muscle contraction, presence allows contractile proteins to polymerize from actin + myosin to actomyosin. Protein polymerization means sarcomere reduction and, consequently, muscle toughening. During onset of rigor mortis, ATP depletes to ADP and finally IMP (inosinmonophosphate); this chemical species is not able to supply energy for polymerization; therefore, rigor mortis occurs when ATP is fully converted into IMP (Prändl and others 1994). ES fully depletes ATP to IMP, thus no energy is supplied for protein polymerization, hence for muscle contraction. In this situation, freezing does not affect meat tenderness as no further polymerization can take place (Savell and others 2005).

Studies reported by Kang and others (1983) in rabbit muscle subjected to ES using 50 mA showed changes in myofibrillar proteins. Once actin and myosin polymerize to actomyosin during contraction, this complex is dragged toward the meromyosin fraction of the main myosin helix; however, meromyosin also posses ATPase activity; when the actomyosin complex is at the meromyosin ATPase region, actomyosin splits into actin and myosin, and relaxation takes place. These authors reported a decrease in ATPase activity of ES-treated muscles. However, activity slowly increased during the 7 day storage. Actomyosin dissociation of ES muscles was lower than *in vivo* muscles; the authors conclude that ES minimized actomyosin changes.

Protein molecular mass, as analyzed by SDS-PAGE, showed pH 6.37 for nonstimulated and pH 6.01 for stimulated myofibrils of *Bos indicus* crossbred cattle. It was also found that muscle appearance improved, although no considerable degradation of I-band proteins occurred (titin, nebulin, desmin, and troponin-T) (Ho and others 1997). These authors also mention that the increase in tenderness is not a result of protein structural proteolysis of the Brahman and Simmental bull's (B × S) muscle tested. The most noticeable fracture frequency in electrically stimulated muscle I-bands suggested that it could be a factor associated to tenderness.

17.4 Meat Tenderness

Tenderness, together with color and juiciness, is one of the most important meat sensory characteristics. ES has been successfully used to improve tenderness and overall quality of meat, accelerating the tenderizing process during storage (Pospiech and others 2003; Acevedo 2004; Soria and Corva 2004).

Myofibrillar proteins and connective tissues constitute the structure of the meat. Connective tissue protein content varies between 2% and 6% in relation to total proteins. This difference is mainly caused by muscle type, species, and age of the animal, as well as the specific activity of a given muscle, although muscle metabolism depends on the animal genotype (Pospiech and others 2003). Solomon and others (1986) studied the effect of ES on 78 pure bred young bovine carcasses (10–18 month Angus and Brahaman, ES for 2 s at 550 V, 1.5 A, 20 pulses); loins were sensory evaluated for tenderness. The panel found a significant increase in tenderness and decrease in toughness due to connective tissue.

Meat reaches pH values around 6.0 at 10–12 h postmortem; but this value is reached at 1–2 h postmortem in stimulated meat. ES meat shows intense muscular contractions, resulting in fast rate glycolysis and pH decrease. However, this abrupt decrease also influences the development of early rigor mortis, followed by rapid resolution that promotes a fast relaxation rate. At the same time, intracellular calcium is rapidly liberated; as a consequence, calpain activity and proteolysis rate are increased, improving the meat tenderness (Elgasim and others 1981; Warriss 2000; Soria and Corva 2004).

According to Pospiech and others (2003), protein breakdown during ripening results in increasing the meat tenderness. This is due to three factors (Hedrick and others 1994) related to muscle fiber behavior. First, due to cold shortening prevention by reducing rigor mortis slow phase, but accelerating further contraction. Second, due to calcium liberation by ES, myofibrillar proteolysis occurs as a result of calpain activation. Finally, myofibril fracture occurs due to extreme contractions caused by the electric current.

Improved tenderness can be obtained by a variety of methods including the use of enzymes, mechanical methods, and ES, with early onset of rigor mortis, decreasing the ripening time and producing brighter and more tender meat that is more acceptable in restaurants, hotels, and other hospitality businesses (Yanar and Yetim 2003; Soria and Corva 2004).

Ripening is usually carried out to improve meat sensory characteristics such as tenderness, flavor, and odor; ES application reduces the ripening time enhancing quality. Cross (1979; cited by Sams 1999) discussed ES tenderizing effect as a possible result of several facts. First, ATP depletion is accelerated, resulting in cold shortening prevention; it could be also due to a fast postmortem pH decrease while muscle temperature is still high (30–32°C), to increased endogenous proteolytic enzyme activity, or to the physical disruption on muscular fibers. Ripening-stimulated carcasses yield more tender meat than nonstimulated ripened carcasses. Yanar and Yetim (2003) studied 14 (3–5 years) mixed breed Western male ovine (black, white faced) by applying ES at 350 V for 45 s, 15 pulses at 1.5 s intervals on *Longissimus dorsi* and S*emimembranous* muscles. Tenderness was analyzed by a trained panel, finding that the more tender samples were obtained from ES meat.

After 7 days ripening, stimulated meat obtained from 96 goats were more tender than nonstimulated meat (McKeith and others 1979). Studies by Zocchi and Sams (1999) on 7 week chicken ES carcasses (5 pulses, 450 V, 450 mA), stunned by head immersion in 1% NaCl and deboned after slaughtering, indicated that meat tenderness was equivalent to that in meat obtained 4 h after slaughtering, that is 50% time reduction to achieve the same tenderness. Similar results were reported by Kang and others (1991) on 14–16 week white Japanese female rabbits, stimulated immediately after slaughtering at low voltage (35–45 V, 50 mA max., 3 ms, pulses every 8 ms for 15 min) and at high voltage (200 V, 80–1000 mA max.). These authors suggested that penetration force increased with rigor mortis onset in ES muscles, this did not occur in muscles attached to bones.

Studies carried out by Castañeda and others (2005) on 54 female broilers, 1.5–1.6 kg, electrically stimulated at 450 mA, 450 V, 2 × 2 s, 7 pulses/s, showed that ES postmortem increases meat tenderness due to accelerated ATP loss, pH decrease, and muscular fiber physical disruption. The authors also reported onset of rigor mortis at 30–32°C; slow cooling may also cause pale color and water-holding capacity reduction. Normal final pH (5.9) meat, but showing PSE (pale–soft–exudative) condition was observed in meat stimulated after 3 h postmortem and subjected to slow cooling for 12 h (Elgasim and others 1981).

Zocchi and Sams (1999) and Castañeda and others (2005) pointed out that high voltage (450 mA, 450 V, 2 s × 2 s with 7 pulses) ES followed by rapid cooling (4°C) did not alter protein functionality or produce PSE meat. However, there is evidence that slow cooling after ES could negatively affect water-holding properties of the meat.

Soria and Corva (2004) and Savell and others (1977) studied the effect of ES on beef, lamb, and chevron. The two groups agreed that ES promotes postmortem glycolysis acceleration, causing the onset of rigor mortis in muscle fibers before cold shortening takes place, and significantly increasing sarcomere length. These observations were also reported by McKeith and others (1979) in chevron meat, showing 20% increase in *Longissimus dorsi* muscle tenderness, whereas nonconsistent results with respect to tenderness increase were observed in *Biceps femoral* and *Semimembransus* muscles. ES and slowly cooled (2°C for 24 h) Suffolk carcasses showed fast pH decrease in *Longissimus dorsi* muscle, as well as reduced cold shortening in the hemiaponeurotic muscle and increased tenderness in both muscles (Radish and others 1983). However, Elgasim and others (1981) reported no significant difference regarding sarcomere length between stimulated and nonstimulated beef carcasses, cooled at two different temperatures (2°C and l6°C).

Kerth and others (1999) reported the results obtained from 12 (90–120 days) lamb ES carcasses (Hampshire × Rambouillet, 59 kg). One side of each carcass was randomly assigned to an ES treatment of 550 V and 60 Hz of electricity for 2 s on and 2 s off 15 times. The authors concluded that stimulated meat showed a bright red color, fast pH decrease, no effect on myofibril fragmentation index, and sarcomere length was not affected in general with the exception of sarcomere length reduction in *Triceps braquial* muscle. Similar observations on sarcomere length in ES muscles were reported by Savell and others (1978). In addition, studies by Bouton and others (1978) carried out with male bovine (2–4 years, 150–180 kg, subjected to ES at 110 V maximum voltage), and later stored for 22–24 h, showed that low voltages can prevent the negative effects associated with rapid cooling or hot deboning. Conversely, Davel and others (2003) reported that stimulated meat was considerably less tender than the nonstimulated samples, since the ES was carried out at low voltage (20 V, 45 Hz, for 45 s). The results show that ES had an effect in reducing tenderness suggesting that ES could be applied to meat obtained from old animals to reduce variation in tenderness due to age, breed, species, slaughtering method, and nutritional factors.

Results obtained by Craig and others (1999), on 72 electrically stunned broilers, and later stimulating the carcasses, showed that stunning and stimulation significantly affect blood loss, pH decrease, and sarcomere length increase; stimulation at 440 V accelerates the rigor mortis onset. The results reported by these authors agreed with previous reports of Savell and others (1977) regarding sarcomere length.

The effect of sex was studied by Zywica and Katarzyna (2003). These authors studied 18 month heifers and bulls. ES was carried out on the left carcass half at 330 V, 17 Hz, 0.9 pulses for 120 s. The right half was the control. Twenty-four hours after stunning, the hemiaponeurotic muscle was separated from the carcass and divided into 1 kg sections. Sensory and instrumental analysis showed that high-voltage ES effect on cooked meat depends on the sex of the animal. Better meat quality was obtained from ES heifer hemiaponeurotic muscle.

Marination is a process whereby meat is subjected to salts affecting the electronic environment of muscle fibers or activating specific enzymes, such as calpains, and modifying the meat texture. Young and others (2004) studied the effect of ES on 93 poultry breasts marinated with polyphosphates. ES was applied in pulsed current, 220 V alternating current for 90 s, 0.5 s on followed by 1 s off. pH rapidly decreased and sodium tripolyphosphate absorption was increased; water loss after cooking was not affected. The same group (Young and others 2005) analyzed the force required to cut the *Pectoralis major* muscle of 40 (52–56 days) broilers electrically stimulating the carcass for 90 s with 220 V, 132–140 mA per carcass; after evisceration the carcass meat was cooled for 3 h on ice. Less force was required in stimulated meat; higher yields were obtained after cooking.

17.5 Effect of ES on Bacterial Growth

A major worldwide concern is meat sanitation when a specific process is applied. Several studies have been carried out regarding microflora present in the ES meat. Kotula and Emswiler-Rose (1981) studied the relationship between cut grades, hot or cold deboning, ES, and bacterial incidence in 10 American beef cuts. The authors reported that there was no effect of hot or cold deboning or ES in the growth of aerobic bacteria.

Butler and others (1981) analyzed microbial populations and pH in ground meat from *Biceps femoral* and *Infraespinatus* muscles of 279–378 kg bovines. The meat was previously inoculated with *Lactobacillus* spp., *Pseudomonas* spp., *Acinetobacter* sp., *Brochothrix thermosphacta, Erwinia herbicola* and *Moraxella* sp., and stimulated (550 V, 16 pulses at 1.8 s intervals). Inoculated and noninoculated samples with and without ES were analyzed for the same bacterial populations. Although pH was significantly reduced by ES, no significant differences in bacterial populations were observed between ES and nonstimulated meat. However, Guerrero and others (2004) point out that there is lower bacterial growth in ES meat, due to the fact that lactic acid content, which results in a rapid pH decrease, creates an unfavorable environment for the development of microorganisms. It can be concluded that ES has no influence on bacterial proliferation in meat; pH decrease is due to ES as discussed before and not due to the presence of lactic acid.

17.6 Implications

ES has been reported as an efficient technique to improve meat quality, especially tenderness in various animal species. However, it involves several complex technological factors to be controlled for being successful. This technique is mainly attributed to glycolysis acceleration, preventing excessive muscular fiber shortening. As onset and resolution of rigor mortis is accelerated in ES carcasses, it allows refrigeration, or even freezing processes, to be applied just after slaughtering and cleaning, and the processing time is considerably reduced. On the other hand, as the technique reduces the processing time and labor, it also represents employment loss when applied in developing countries, where more employment is necessary.

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18

Carcass Evaluation

Roger W. Purchas

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18.1 Introduction

This chapter starts by providing a definition of "carcass" so that characteristics that can play a role in determining the value per unit weight of that carcass for meat production can be considered. With this framework in place, some of the main situations where the evaluation of a carcass is required are outlined, and then in the main part of the chapter, methods available to make such evaluations are described and discussed.

18.1.1 What Is Meant by the Term "Carcass"?

The dictionary definition of "carcass" (or the equally acceptable spelling "carcase") as it applies to animals will often mean the whole dead body. For meat animals, however, the carcass refers to that part remaining after various dressing procedures have taken place. These procedures commonly involve removal of most of the internal organs (gastrointestinal tract, liver, lungs, heart, etc.), the feet, the head, and the skin. The exact nature of the items removed, however, will vary between species of meat animal and often between countries for the same species. Thus, whereas beef or lamb carcasses will usually have the feet, head, and skin removed (e.g., EUR 2006), these parts are often included as part of the carcass for pigs. An example of a difference between countries is that between New Zealand, where the kidney and pelvic fat is not included with beef carcasses, and the United States where these items are included as an aid to assessing carcass fatness. In order to be commercially realistic, the definition of a carcass used here is *that part of the meat animal that remains after normal commercial dressing procedures*, even though inconsistencies inevitably result from the use of this definition.

18.1.2 What Determines the Value of a Carcass?

In order to evaluate a carcass fully, it is necessary to be able to evaluate all those characteristics that can affect its average value per unit weight. This will be a weighted average, weighted according to the relative value of different carcass parts, and, where possible, according to measures of meat quality. A list of such characteristics or categories of characteristics is given in Figure 18.1. The extent to which they are commonly evaluated, however, varies widely, partly because of variable contributions to overall value, and partly because of variation in the accuracy and ease with which they can be measured or predicted. In this chapter, the emphasis is on methods of evaluating characteristics associated with the composition of the carcass (including the dressing-out percentage), and the palatability and appearance of the meat.

It can be argued that dressing-out percentage should not be included as a characteristic that affects carcass value because two carcasses that are identical in all respects other than dressing-out percentage will have the same value. It is important indirectly, however, as the carcass with a higher dressing-out percentage will have come from an animal with a lighter live weight, which may be very important to the overall economic viability of a meat production system.

Lean-meat yield (LMY%) is equivalent to carcass muscle percent and is shown in Figure 18.1 as being determined totally by carcass fat percent and the ratio of muscle weight to bone weight (M:B). This is shown by the equation: $LMY\% = (100\text{-fat}\%) \times [(M:B)/((M:B) + 1)]$, which means that LMY% can be increased by either decreasing fat% or by increasing M:B, as illustrated by the outputs in Figure 18.2. LMY% could also be shown as a function of fat% and bone%, but, because fat% is usually the most variable carcass component, it is useful to express the amount of bone in terms of M:B which, unlike bone%, is independent of fat% (Berg and Butterfield 1976).

Carcass shape is most often described in terms of conformation, but this term fell into disrepute when it was shown that carcasses with "superior" subjective conformation were often characterized by being fatter rather than by having a better LMY%. This problem was largely overcome by the introduction of several terms defining carcass shape that help distinguish between superior shape due to thick layers of muscle versus thick layers of fat (De Boer and others 1974). These terms included: *conformation* as the thickness of muscle plus fat relative to a skeletal dimension; *muscularity* as the thickness of muscle only relative to a skeletal dimension; and *fleshiness* as the thickness of muscle plus intermuscular fat relative to a skeletal dimension. An objective measure of muscularity based on weights and lengths of dissected muscles and bones has been proposed (Purchas and others 1991). Carcass shape can affect the value of a carcass partly through its relationship with LMY% (muscularity and M:B are usually positively related). In addition, superior muscularity is positively related to dressing-out percent, and more compact carcasses with thicker muscles may enable more valuable retail meat cuts to be prepared.

Lean-meat quality is difficult to define because it has to be in terms of the perceptions of the final consumer, which can vary between individuals, between groups of people, and between countries (e.g., Realini and others 2009). Characteristics contributing to the perception of meat quality in Figure 18.1 are subdivided into intrinsic characteristics of the meat, and external or extrinsic characteristics. Intrinsic

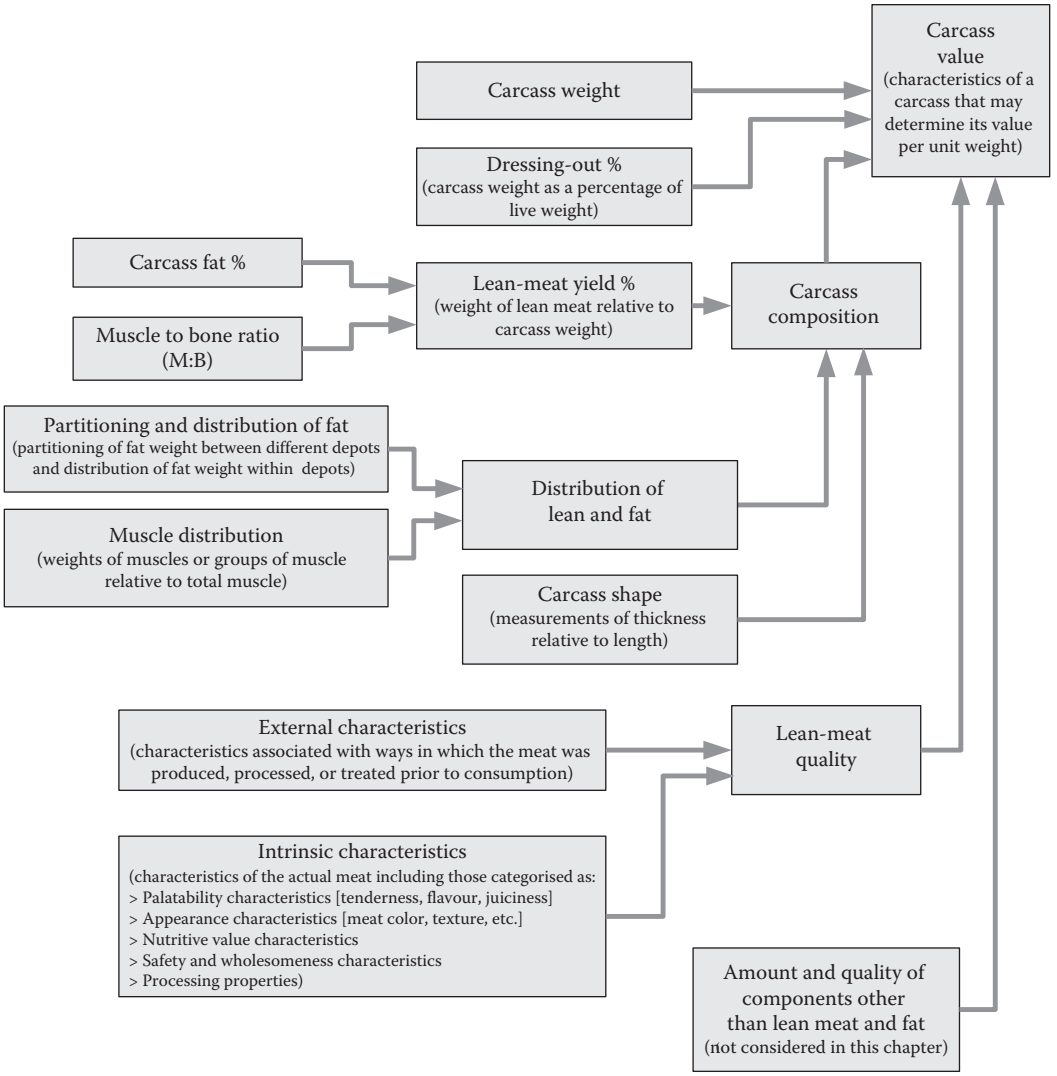

FIGURE 18.1 Characteristics and groups of characteristics contributing to the average value per unit weight of a carcass.

characteristics are those measurable at the time of consumption such as appearance, tenderness, flavor, and content of iron and other nutrients, while external characteristics relate to how the meat was processed and prepared and how the animals from which the meat was obtained were treated. An increasing number of consumers are becoming concerned about the external characteristics associated with meat (e.g., Bernués and others 2003). Branded meat products (e.g., organic products or breed-specific products) commonly have a number of extrinsic quality characteristics specified (Bredahl 2003). Methods of evaluating the nature or importance of extrinsic characteristics will not be considered further in this chapter.

Carcass value per unit weight will often increase with increasing carcass weight because the cost of processing a carcass is relatively unaffected by carcass weight and therefore these costs decrease per kilogram as carcass weight increases. This effect, however, may be offset if carcasses and cuts of a narrow weight range are favored by the retail sector so that prices per unit weight fall when carcass weights are above or below an optimum range.

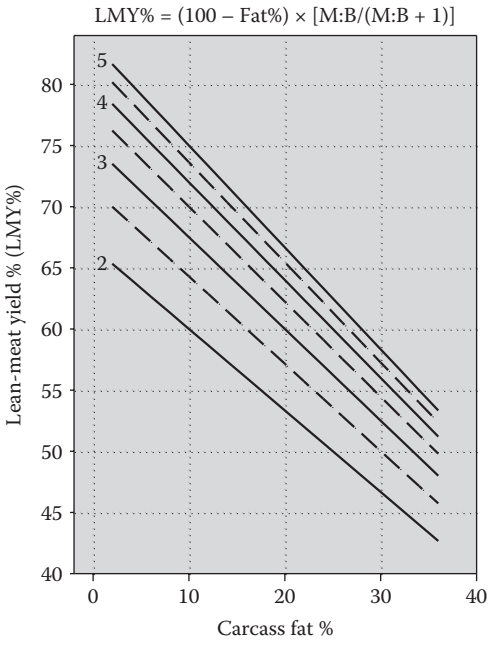

FIGURE 18.2 Patterns of decrease in LMY% with increasing carcass fat% at carcass M:B ratios of 2, 2.5, 3. 3.5, 4, 4.5, and 5 calculated using the equation shown above the figure. Increases in LMY% can be achieved in three ways: (1) By an increase in M:B (e.g., an increase from 2 to 4 at a fat% of 16 increases LMY% from 56 to 67.2); (2) by a decrease in fat% (e.g., a decrease from 30 to 10 at an M:B of 4 increases LMY% from 56 to 72); or (3) by a combination of increased M:B and decreased fat% (e.g., an increase of M:B from 2.5 to 5 and a decrease in fat% from 30 to 10 increases LMY% from 50 to 75).

18.2 Situations Requiring Carcass Evaluation

18.2.1 Carcass Evaluation with Live Animals

There are two main situations when an evaluation of the potential carcass characteristics of live animals is useful. The first is when meat animals are being selected for breeding purposes so that the direct evaluation of carcasses is not possible. This is most often of interest for male animals that are to be used as sires because they can potentially have a much greater influence on population characteristics than females. A limitation of assessing carcasses of breeding males is that gender has a significant effect on carcass characteristics, and for some species most of the animals used for meat production are castrate males or females. The second situation requiring carcass evaluation is as an aid in the selection of animals for slaughter in order to produce carcasses with the best possible returns per unit weight. Many more animals are involved than for breeding animals so that simpler and quicker methods of evaluation are generally required.

18.2.2 Carcass Evaluation for Carcass Grading or Classification

For carcass evaluation, the terms "grading" and "classification" are often used interchangeably, but sometimes a distinction is made. Thus, they both sort like-with-like so that items within a grade or class are more similar in certain respects than those in different grades or classes, but with grades there is an implication that one grade is better than another, while with classes they are just different (Kempster and others 1982). The constraints for the evaluation of an intact carcass, a carcass side, or a quartered carcass in the case of beef are much less than for live animals, but there are usually requirements that assessments be made quickly and in a nondestructive manner.

18.2.3 Carcass Evaluation for Research Purposes

Almost all the characteristics of interest (Figure 18.1) can be measured in some way, but in many cases the methods required are slow and/or destructive and/or expensive. Therefore, the appropriateness of making a particular measurement in a research project will often be determined by the questions being addressed and the research funds, expertise, and facilities available. However, opening up the possibilities to destructive methods that may not have time constraints means there are many more options as noted below. This is particularly the case for many meat-quality characteristics that are difficult to accurately measure or predict for meat in a live animal or on an intact carcass.

Some characteristics that need to be taken into account in choosing between different measurement methods are listed in Figure 18.3, along with an assessment of their importance in different measurement situations.

18.3 Methods Applicable for Live Animals

18.3.1 Subjective Assessments

Subjective assessments are sometimes referred to as "hand and eye" techniques and involve the assessment of characteristics without the use of any tools or instruments that would provide objective measurements. These are widely used in the assessment of carcass composition characteristics of live animals such as level of fatness, muscularity, and distribution of fat or muscle. Their accuracy and repeatability between assessors may be improved by the use of standard scoring systems (often termed condition scoring systems; e.g., Wagner and others (1988) for cattle; Shands and others (2009) for sheep) and with the help of standard photographs or drawings. The subjective assessment of carcass fatness of live animals is generally most effectively done at sites where there is mainly fat between the skin and the bone so that distinguishing between the contributions of the two soft tissues of fat and muscle is less important (e.g., over the brisket, over the ribs, in the flank region, and between the tail and pin bones). Conversely, muscularity is best assessed where there is little fat overlying the muscle (e.g., the shins and the lower round). Generally, subjective assessments of carcass composition characteristics are only reliable for detecting quite large differences although it is possible that some individual assessors may excel at detecting small differences. Conroy and others (2009) reported that subjective evaluations of muscular and skeletal scores of live cattle ($n = 336$ steers) were significantly related to subsequent carcass measurements of muscle percent and bone percent, while Fisher and others (1980–1981) reported that the average scores from several "expert" judges were better than any individual judge. Linear measurements of lengths or circumferences may be of some assistance, but the imperfect repeatability of such measures limits their value (Fisher 1975). The subjective detection of differences between very similar animals for dressing-out percent or meat-quality characteristics is generally not feasible.

18.3.2 From a Knowledge of Factors Affecting Relevant Characteristics

Considerable information is available on how factors such as genetics or gender affect the carcass and meat-quality characteristics summarized in Figure 18.1, so information on the nature and size of these effects can often be used to predict differences between groups of animals or individual animals that differ for these factors. A consideration of these factors is beyond the scope of this chapter, but it is noted that this information is of little help in detecting differences between individuals within homogeneous groups of animals.

18.3.3 From Information on an Animal's Genotype

Information on the genetic strengths or weaknesses of an animal for the characteristics of interest is of particular importance for those animals to be used for breeding purposes as that is the information that is passed on to the next generation. It will be of limited value for characteristics of low heritability,

Method characteristic	Situation where the measurement needs to be made				
	On live animals		For classification of carcasses		Research[a]
	Selection of breeding stock	Selecting animals for slaughter	Intact carcasses or sides	Quartered carcasses	
Cost of equipment (including depreciation)	***	****	***	***	**
Cost of training operators and/or maintenance staff	***	***	***	***	**
Cost of operating the equipment	***	***	****	****	***
Accuracy in terms of precision or repeatability	***	**	***	***	****
Accuracy in terms of closeness to the true value[b]	***	***	****	****	****
Accuracy in terms of absence of bias between groups[c]	***	***	****	****	****
Speed of measurement	**	***	****	****	*
Noninvasive (i.e., doesn't penetrate the carcass tissues)	na	na	**	**	*
Noncontact (doesn't come in contact with the carcass)	na	na	**	**	0
Applicability to live animals	*****	*****	0	0	0
Nondamaging or destructive	****	*****	****	****	*
Automatic operation without continuous human involvement	*	*	**	**	*

[a] When there are minimal constraints with respect to costs, time, and whether or not samples are destroyed.
[b] Measured in terms of parameters such as R^2 and/or RSD values relative to raw SD values.
[c] Assessed in terms of the size and statistical significance of differences in prediction relationships between groups (e.g., gender groups, or breed groups).

Key:		
	na	Not Applicable
	0	Of no importance
	*	Of minimal importance
	**	May sometimes be important
	***	Usually important
	****	Usually very important
	*****	Absolute requirement

FIGURE 18.3 An assessment of the importance of relevant characteristics of any measuring method for different situations where a measurement might be required.

however, because of significant environmental effects. The direct measurement of relevant characteristics on the progeny or other relatives of an animal that can be slaughtered provides some genetic information, but, during recent years, it has also become possible to directly determine which genes and alleles an animal possesses by DNA analysis. The number of genes or groups of genes that have been shown to be of importance to carcass value through composition or meat-quality characteristics is likely to increase over time. Examples relating to beef quality were described in a review by Hocquette and others (2007). The existence of genetic differences in the form of single-nucleotide polymorphisms (SNPs) can be related to animal characteristics even when the effects of single SNPs are unknown (Rothschild and Plastow 2008). This is possible by evaluating the relationships between thousands of known SNPs and animal characteristics using mathematical techniques. So-called "50k

snip-chips," for example, enable the simultaneous evaluation of 50,000 SNPs, but information on the characteristics of interest on a large number of animals is required in order to develop useful predictive relationships.

18.3.4 Ultrasonic Methods for Use on Live Animals

Ultrasonic pulse–echo methods are widely used with pigs, cattle, and sheep to obtain information on body composition of live animals. This is mainly in terms of depths of subcutaneous fat and cross-sectional areas of muscles, although intramuscular fat (marbling) levels can also be assessed. Fat depths are most often measured over the longissimus muscle in the region of the last rib, but fat depths at other sites, such as over the rump, have also been used. Ultrasonic waves, usually in the frequency region of 3.5 mHz, penetrate animal tissues and when they pass from one tissue to another with different acoustic properties, some of the sound waves are reflected and are detected by the same transducer that continues to emit the waves intermittently. The results are mainly in the form of fat depths if just a single transducer is used, or as a cross-sectional image of muscle, fat, and bone if a bank of transducers built into a head is used with what are termed real-time B-mode devices (Simm 1983). Ultrasonic measures of fat depth and eye-muscle area on live animals have been shown to be moderately good predictors of the matching characteristics on carcasses (although absolute values may differ slightly because of distortions resulting from the carcass being hung). The effectiveness of fat depths and eye-muscle area measurements as predictors of carcass LMY%, however, is not particularly good, as illustrated by the results from one study with cattle shown in Table 18.1. The results in this table are similar to many others with cattle, sheep, and pigs in showing that the percentage of the variation in LMY% (or other measure of carcass meat yield such as % retail yield in Table 18.1) accounted for by ultrasonic measurements is usually <70% (R^2% of 64% in Table 18.1), and that the variation (in terms of standard deviation (SD) values) remaining in LMY% after fitting the prediction equation is commonly still >2% points (e.g., the SD of % retail yield was reduced from 4.30 to a residual standard deviation (RSD) of 2.60 in Table 18.1).

In the study of Conroy and others (2009) referred above, the accuracy with which percent muscle and percent bone of the carcass of steers was predicted was significantly improved when ultrasonic measures of fat depth and muscle depth were added to the subjective muscular and skeletal scores. While less than

TABLE 18.1

An Example of a Study in Which the Value of Ultrasonic (US) Measurements on Live Cattle Plus Final Live Weight Were Assessed as Predictors of Percent Retail Yield from the Carcasses of 282 Steers

Characteristics of the 282 Steers (means ± SD)	
Final live weight (kg)	547 ± 63.7
Carcass weight (kg)	333.6 ± 40.4
Ultrasonic fat depth at ribs 12/13 (mm)	10.0 ± 3.5
Ultrasonic "eye"-muscle[a] area (cm^2)	77.0 ± 7.5
% Retail yield from the carcass (%)	65.2 ± 4.30
Characteristics of the Regression Equation for Predicting Retail Yield as a % of Carcass Weight	
Intercept	69.32
US-fat depth regression coefficient	–0.8502
US-eye-muscle-area regression coefficient	+0.135
Final live weight regression coefficient	–0.011
R^2 %	64%
RSD[b]	2.60

Source: Adapted from Greiner SP and others. 2003. *J Anim Sci* 81:466–73.

[a] The "eye" muscle is the longissimus muscle at ribs 12/13.

[b] RSD = residual standard deviation (given in the paper as RMSE).

perfect for accuracy, the widespread use of ultrasonic procedures to obtain information on the composition (particular fatness) of meat animals by well-trained and experienced operators is likely to continue because results are obtained quickly from portable equipment that is inexpensive compared with other scanning options. Some indication of muscularity is obtained from ultrasonic measures of eye-muscle shape, and indications of marbling levels may be indicative of certain meat-quality attributes. The velocity with which ultrasound travels though animal tissues, such as across a hind leg, also provides information on composition (Allen 1990, Fisher 1997), but this method has received little attention or use compared with the more convenient ultrasonic pulse–echo approach.

18.3.5 Computer-Assisted Tomographic Scan

Computer-assisted tomographic (CT) scans involve the use of x-rays to produce images of the whole cross section of the body that can be interpreted in terms of the fat, muscle, and bone present in the image (Allen 1990). Results from images taken at several sites along the body are usually combined in order to obtain an estimate of total body or carcass composition (e.g., Navajas and others 2007). This approach has been used mainly with sheep and pigs because they can be evaluated in medical CT scanners that have been designed for use with humans, but deer weighing up to 100 kg has also been evaluated using medical scanners, and a device has been reported for use with standing cattle (Nade and others 2005). CT scanning has the following disadvantages: (1) the equipment is much more expensive and less portable that ultrasound devices, (2) the animals have to be tranquilized, and (3) it does not produce an instant result. However, it has the major advantage of potentially being more accurate because complete cross sections of the body are obtained and many such cross sections can be analyzed. In the commercial sheep breeding context in New Zealand, farmers commonly make an initial selection of potential meat-breed sires using ultrasound results and visual assessments, and then a final selection among a much smaller group on the basis of CT scans. With regard to the characteristics listed in Figure 18.1, CT scans provide information on fatness, M:B, and muscularity (Navajas and others 2007), but not on dressing-out percent or meat quality (except to a limited extent indirectly through measures of intramuscular fat and fat cover). In an evaluation of the value of live-animal measurements as predictors of meat quality of lambs, Lambe and others (2009) reported only low-to-moderate correlations between meat-quality parameters and live-animal measures that included subjective scores, linear measurements, ultrasonic parameters, and CT scan data.

18.3.6 Magnetic Resonance Imaging

This method (previously referred to as nuclear magnetic resonance, NMR) is similar to CT scanning in that the tranquilized animal is passed through a cylinder (a large magnet in this case) and images of cross sections of the body are created, but it differs in that, rather than involving ionizing x-rays, the images are based on the differential response of tissues to a strong magnetic field. Results have shown that accurate measures of composition can be obtained in this way (Allen 1990), but because the equipment is significantly more expensive than CT scanners, it is not as widely used for carcass evaluation purposes.

18.3.7 Measures of Electrical Properties (Bioelectrical Impedance Analysis and Total-Body Electrical Conductivity)

Electrical properties of the animal body are influenced by its composition because adipose tissue is a poorer conductor of electricity than other tissues due to a lower content of water and ions. Bioelectrical impedance analysis (BIA) involves placing contacts on the animal and measuring the impedance and reactance of the current that flows between them. It is widely used as a simple and inexpensive means of assessing human body composition (Lee and Gallagher 2008). When tested under controlled conditions with lambs, Berg and others (1996) showed that measurements on live lambs predicted composition of carcasses with moderate accuracy, but its use with meat animals has been limited (e.g., with pigs; Mitchell and Scholz 2009).

Total-body electrical conductivity (TOBEC) of an animal is assessed by having an animal pass through a coil, which makes it more complex than the BIA equipment, and the results with live meat animals have not been particularly reliable (Allen 1990; Joyal and others 1987), because of inconsistent animal movement.

18.3.8 Other Methods for Use on Live Animals

A number of other methods have been used with meat animals in research studies or have been used successfully in human studies, but not with farm animals. These will not be discussed in any detail here, but many of them have been reviewed by Allen (1990) and by Lee and Gallagher (2008). They include

- The use of intrinsic markers, such as the natural radioactive isotope of potassium (^{40}K), that are at different concentrations in different tissues (^{40}K is at a higher concentration in muscle than other tissues), so if they can be measured they will provide an indication of the amount of that tissue present. Creatinine and 3-methyl histidine are other markers of muscle.
- The use of administered markers of body water (e.g., urea and tritiated water) that can be used to measure total-body water content by administering a known amount and measuring the degree to which it is diluted over a period of time.
- Measures of body density provide an assessment of body fatness because adipose tissue is less dense than other tissues. New methods of measuring body volume by air displacement plethysmography (Lee and Gallagher 2008) may make this approach more feasible for live animals than the older method for measuring the body volume by underwater weighing.
- Dual-energy x-ray absorptiometry (DEXA) is a scanning procedure used widely in body composition studies with humans, but one that would require farm animals to be tranquilized. This approach has been assessed mainly with pigs (Mitchell and Scholz 2009).
- Neutron activation analysis.
- Near-infrared reflectance (NIR) spectroscopy.

18.4 Methods Applicable to Intact or Quartered Carcasses

The main reason for measuring the characteristics at this stage is to allocate a carcass to a particular grade or class. The groups of characteristics that are assessed for this purpose are those relating to carcass composition and, to a lesser extent because of the measurement difficulties, meat quality (Lazzaroni and others 2007). Because the carcass is invariably weighed, a dressing-out percent can be calculated provided a live-weight just prior to slaughter is available. If this is not available, there is no satisfactory way of predicting or calculating dressing-out percent. The accuracy with which composition characteristics can be assessed will depend to some extent on whether the carcass has been quartered prior to classification and whether classification is done as the carcass or side leaves the slaughter floor or after it has been in the chiller for some time (usually overnight). The main composition characteristics assessed are carcass fat percent, M:B, and carcass shape. Limited assessments are also made of fat partitioning and distribution, and muscle distribution, but differences in muscle distribution are difficult to accurately detect because of limited variability in this characteristic (Berg and Butterfield 1976).

There has been a vast amount of research into methods that could be used to evaluate meat-quality characteristics of intact or quartered carcasses, with particular emphasis on measures of color and tenderness. Although this has been achieved to some extent as outlined below, it is likely that searches for improved methods will continue. There are some aspects of meat-quality characteristics that make their satisfactory measurement more difficult than most composition measures. These include the facts that

- Characteristics such as tenderness and flavor can change considerably after the time a carcass is graded, depending on storage conditions and other factors.

- Meat-quality characteristics differ considerably between muscles, and muscle-to-muscle correlations are often not close (e.g., Hildrum and others 2009).
- Measurements made on uncooked meat are often not a good indication of the same measurements following cooking. For example, the toughening of meat following cold-shortening is not apparent in raw meat (Purchas 1973).

Two approaches to the assessment of carcass characteristics that are equally applicable to intact carcasses as they are to live animals are from a knowledge of factors affecting the characteristic of interest, or from information about the genetic makeup of the animal that the carcass came from. The points made about these approaches for live animals in Sections 18.3.2 and 18.3.3 are also relevant for carcasses and will not be discussed further here.

18.4.1 Subjective Assessments

The disadvantages of subjective assessments of carcass composition characteristics of a hanging carcass or side are similar to those for the live animal, except that now the actual carcass can be observed and felt. As a result, the assessments are likely to be more accurate (Williams 1972). Well-trained classers using the European Union (EU) system of classification for beef, for example, can distinguish between at least 15 levels of fatness (5 levels, 1–5, with three subclasses within each) and carcass shape (conformation; six classes of S, E, U, R, O, and P, with three subclasses within each) with the aid of standard photographs or diagrams (EUR 2006, Hickey and others 2007). The accuracy with which carcass composition can be predicted in this way is less than the prediction from dissecting part of the carcass, as illustrated by the results of an Irish study with beef (Figure 18.4). Fat distribution within the subcutaneous depot can also be assessed subjectively.

Various sets of standard colors or photographs have been prepared as aids for subjectively assessing the color of meat for quartered carcasses, and yellowness of subcutaneous fat of intact carcasses (e.g., Aus Meat 2005). Palatability characteristics are more difficult to assess in a nondestructive way, but subjective assessments can be made of factors known to influence palatability such as measures of maturity (via ossification or dentition) and marbling in the USDA quality grading system for beef (AMSA 2001).

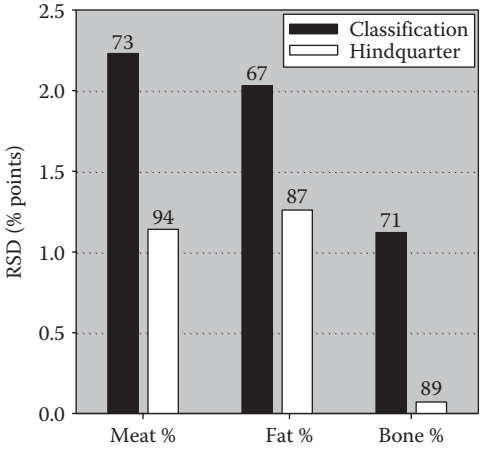

FIGURE 18.4 Results showing that prediction of carcass muscle, fat, and bone percent from 15-point classification scores for fatness and conformation of beef carcasses was less effective than prediction from hindquarter dissection results. Accuracy is expressed as both RSD values *(lower values are better)* as well as R^2 values (%) shown above each bar (*higher values are better*). Irish data from 662 animals. (Adapted from Conroy SB and others, 2010. *Animal* 4:234–41.)

18.4.2 Depths of Subcutaneous Fat

Depths of subcutaneous fat, measured either on the intact carcass after making a small cut or directly on a quartered carcass, have proved to be one of the simplest and most effective predictors of carcass fat% and LMY% for all the main mammalian meat-producing species. The relative accuracy of fat depths made at many anatomical sites have been shown to be comparable (reviewed by Kempster and others 1982, Fisher 1990), but those that are most widely used in various carcass grading systems around the world are taken over the longissimus muscle at the site of quartering. These may be defined as being a set distance from the midline (common for pork), at two-thirds or three-quarters of the distance from the medial edge of the muscle (common for beef), or at a site above the maximum depth of the muscle (common for lamb). Because this fat depth over the soft muscle may be difficult to measure accurately on lean carcasses that have not been chilled, an alternative measure that has proved to be similar with respect to prediction accuracy is the soft-tissue depth over the last or second-to-last rib at approximately half way around the carcass. This measurement is also predominantly of fat but may also include some muscle tissue. An example is the GR measurement over rib 12 at 110 mm off the midline that is used for lamb carcass classification in several countries (Kirton and Johnson 1979). An indication of fat distribution within the subcutaneous depot may be obtained by measuring the fat depths at several sites.

18.4.3 Color-Sensing Electronic Probes and Other Probes

A number of probes have been developed to facilitate the measurement of fat depths on intact carcasses. The Introscope, which was one of the earlier devices, is essentially a small periscope with a light source so that when it is inserted into the carcass the interface between fat and muscle can be seen by the operator. An automated version of this that avoids the need for an operator to view the interface is a device that measures the increase in conductivity as the probe passed from fat to muscle. Both of these, however, have been largely superseded by optical electronic probes with light-emitting and light-detecting diodes in the tip that produce a signal of the depth when the color changes from white fat to red meat. Several probes based on this principle have been produced and they are widely used for pig carcass classification (Fisher 1990). They have not proved as effective for beef or lamb carcasses, however, possibly because of the lower fat depths and the absence of the smooth, firm, and undamaged skin layer over the fat on pig carcasses. In addition to fat depths, it has been proposed that these probes have the potential to measure the muscle depths, intramuscular fat levels, and muscle color (e.g., Schinckel and others 2010, Hennessy Grading Systems, Auckland, New Zealand, http://www.hennessy-technology.com/).

18.4.4 Ultrasonic Methods for Use on Carcasses

In principle, ultrasound should work as well or better on a carcass as it does on a live animal, but in practice its application to carcass grading or classification has been limited because of the time it can take to get a good image and the expertise required to interpret these images. These limitations have been overcome to some extent with the development of devices giving quick results that are interpreted automatically. An advantage of ultrasonic devices over optical electronic probes, which may become more important in the future, is that they are nonintrusive, but they do need to have a close air-free contact with the carcass. Most of the research and development carried out in this area has been for the classification of pig carcasses. Results of a Canadian study that compared four options with pig carcasses are summarized in Table 18.2. In this case, the accuracies with which the four devices predicted saleable meat yield (SMY%) were similar, but it should be noted, that the accuracies of prediction were far from perfect with the RSD values still being 50% or more of the raw SD of SMY% in the calibration group. Thus, there is still scope for improvement in the accuracies with which the LMY% or SMY% of a carcass are predicted by ultrasonic methods for grading or classification purposes.

TABLE 18.2

Results of a Canadian Study with Pigs in which Four Devices for Predicting SMY% Were Compared

Device	Brief Description and Principle	Calibration (R^2%, RSD)[a]	Validation (R^2%)
Ultra-FOM 300	A hand-held device containing 64 ultrasonic transducers that detected average fat depth and longissimus muscle thickness at a site 70 mm off the midline in the region of the 3rd- and 4th-to-last ribs. Used on a hanging carcass. Nonintrusive.	71, 1.70	67
Auto-FOM	A horizontal trough that the carcasses passed through. The trough contained an array of 16 ultrasonic tranducers that took repeat measures of fat depth and muscle depth that were used to predict SMY%. Nonintrusive and automated.	75, 1.68	61
CVT-2	A hand-held device with a head containing many transducers that measured fat depth and muscle depth over a longitudinal span of 125 mm near the last rib and 50 mm off the midline. Used on a hanging carcass. Nonintrusive.	75, 1.57	79
HGP-2	The Hennessy optical–electronic grading probe, a hand-held device that was used for comparison with the newer ultrasonic devices. It measured the fat depth and muscle depth between the 3rd- and 4th-to-last ribs at a site 70 mm off the midline. Used on the hanging carcass. Intrusive, as the probe was pushed into the carcass.	74, 1.56	70

Source: Adapted from Fortin A, Tong AKW, Robertson WM. 2004. *Meat Sci* 68:537–49.

Note: Prediction equations for SMY% (mean ± SD of 59.8 ± 3.12) were developed from results with 194 pig carcasses (calibration), and the results validated with 72 pig carcasses.

[a] R^2% = the coefficient of determination, which provides an estimate of the percentage of variation accounted for, while RSD = residual standard deviation around the regression line with lower values indicating a greater accuracy.

18.4.5 Video Image Analysis

As the name implies, this technique involves the collection of video images of the carcass using a standard video camera with an appropriate charge-coupled device, converting this to a digital format, and then analyzing the resulting data in terms of linear measurements, areas, volumes, and colors usually with the aid of a computer (e.g., Oliver and others 2010). Information on volumes can also be obtained by measuring the curvature of a band of light that is projected at an angle onto the carcass from above the camera. Advantages of video image analysis (VIA) over other methods discussed above include the facts that it is not only nonintrusive, but also the measurements can be made without the carcass being touched. This means that it is a relatively simple matter to have the procedure fully automated. A shortcoming is that it does not "see" inside the carcass in the way that ultrasound or x-rays do. For carcass evaluation purposes, the images taken are most often the view from the side, back, or front of the hanging carcass as it leaves the slaughter floor, and/or an image of the exposed surface when the carcass or side is quartered (mainly for beef). The sort of information that can be extracted from these two types of images is summarized in Table 18.3.

A number of VIA systems for carcass evaluation are available commercially with the main application being with beef or lamb carcasses. With respect to accuracy, it is difficult to generalize, but when predicting carcass LMY% or SMY%, RSD values of less than two percentage points have seldom been achieved when representative groups of cattle or lambs with raw SD values of from 3 to 4 have been assessed. Examples include Allen and Finnerty (2001) and Cannell and others (2002) for beef, and Hopkins and others (2004) and Rius-Vilarrasa and others (2009) for lambs. This means that the residual SD remains at more than half the initial SD. It should be noted, however, that VIA is a fast procedure once the carcass has been orientated correctly, and while it has been seen mainly as a method to predict LMY%, it also has the potential to give information on other composition and meat-quality characteristics of importance (Tables 18.3 and 18.1). Jackman and others (2009, 2010), for example, used magnified images to characterize textural characteristics of the cut surface of beef.

TABLE 18.3

Examples of Information That Can Be Obtained from VIA Images of the Intact Hanging Carcass or of the Cut Surface That Is Exposed when a Carcass Is Quartered

Image of a Hanging Carcass or Side	Image of the Surface Exposed on Quartering
• Linear measures of length and width • Area and volume measurements that enable estimates of carcass shape • Fat depths (from the color of the underlying muscle) • Fat color (particularly yellowness) • Fat distribution over the carcass • Carcass defects or damage	• Muscle cross-sectional areas (especially that of the longissimus muscle) • Eye-muscle width and depth • Fat depths • Fat color • Meat color • Intramuscular fat (marbling) levels • Fat partitioning between the main three carcass depots

18.4.6 Eye-Muscle Area and Other Measurements on the Quartered Carcass

Measurements of value in carcass evaluation that can be made on the exposed surface when a carcass or side is quartered include some related to carcass composition as well as others related to meat quality, including those listed in the right-hand column of Table 18.3. Eye-muscle area and dimensions may also be measured by tracing the muscle and measuring the area using a planimeter or an image analysis system, but this is usually too slow for classification purposes. A result is obtained more quickly by measuring dimensions directly and the area by counting squares or dots on a transparent grid, but this is still much slower than by VIA.

Some indication of meat quality through subjective assessments of marbling, meat color, and fat color can be facilitated by the use of standard sets of photographs or colors (e.g., AMSA 2001, AUS-MEAT 2005).

18.4.7 Prediction Equations

Prediction equations usually take the form of multiple regression equations that have been developed from measurements made on a group of animals that are representative of the target population they are designed to be used with. Prediction equations have most often been developed as a means of estimating some measure of LMY% of a carcass. For example, yield grades for beef carcasses in the United States are estimated using an equation that includes four predictors weighted in such a way that they gave the best estimate of the percentage yield of closely trimmed (trimmed to a subcutaneous fat depth of half an inch) retail cuts from the round, loin, rib, and chuck (BIF 1990). The weighting factors are adjusted in order to use this equation to give yield grades. Actual weighting factors for this equation are given in Table 18.4 in both imperial and metric units, along with comparable results of a more recent study in which the accuracy of the same four predictors in predicting percent retail yield with fat trimmed to zero thickness for a group of 220 steers was evaluated (Apple and others 1991). These results indicate that the prediction accuracy was far from perfect with about half the variation unaccounted for and the RSD being more than half the raw SD (Table 18.4). Similar results were reported for a group of 87 steers by Farrow and others (2009) where the R^2% value was 57. Additional examples of prediction equations are given in BIF (2002). Even though prediction equations are effective in providing an optimum balance of weighting factors for a group of predictors, it is well recognized that those that have been developed to date provide only approximate estimates of SMY%, so that research in this area aimed at improving the accuracy is likely to continue.

18.4.8 Other Methods for Use on Carcasses

Other methods to be considered here can be divided into those that can be used on an intact hanging carcass and those that can be used when the cut cross section of a reasonably large muscle is exposed as

TABLE 18.4

Weighting Factors for the Prediction Equation Used to Calculate the Percent Yield of Closely Trimmed Retail Cuts from the Round, Loin, Rib, and Chuck of Beef Carcasses in the United States

Predictor	BIF (1990) (Imperial Units)	BIF (1990) (Metric Units)	Apple and others (1991)
Constant	51.34	51.34	54.11
Fat depth (at ribs 12/13)	–5.784 in.	–2.277 cm	–3.14 cm
% kidney, pelvic, and heart fat	–0.462%	–0.462%	–0.95%
Longissimus muscle area (at ribs 12/13)	+0.740 $in.^2$	+0.1147 cm^2	+0.12 cm^2
Hot carcass weight	–0.0093 pounds	0.0205 kg	–0.02 kg

Note: The weighting factors are changed when predicting yield grades (BIF 1990). The final column shows weighting factors for the same four predictors as calculated for a group of 220 steers to give prediction accuracy Parameters of 51% for R^2%, and 2.41 for RSD. (The raw SD was 3.44 percentage points for % retail yield.)

occurs when a carcass or side is quartered. Methods applicable to an intact carcass or side that have not been outlined above include

- *Measures of carcass density.* As with live animals a lower density of one carcass relative to another usually indicates that it contains more fat because fat tissue is less dense than the fat-free carcass (about 0.91 vs. 1.2 $g \cdot cm^3$, respectively), but a lower density may also be due to a higher M:B ratio as bone is more dense than muscle. Therefore, this approach to measuring fatness differences is based on the assumption that there is little variation in M:B (Pearson and others 1968, Garrett and Hinman 1969). Although this has been shown to be reasonably accurate approach, it has not been adopted commercially because of the difficulty in measuring carcass volume.
- *DEXA*: Dual energy x-ray absorptiometry has been shown to be an effective means of measuring the composition of live animals (including humans), so the limited evidence showing that it is also effective with carcasses (e.g., Clarke and others (1999) for lamb carcasses, and Mitchell and others (2003) for pork carcasses) was not unexpected. This technology is not widely used to classify meat carcasses, but it has proved to be an accurate method for online monitoring of fat content of boneless meat in cartons (e.g., Purchas and others 2007).
- *CT scans or magnetic resonance imaging scans*: As with live animals, both of these instruments are capable of producing multiple cross-sectional images of carcasses within which the muscle, fat, and bone can be distinguished, but generally the available instruments have been too slow and too expensive to be used for carcass classification purposes. CT scans have, however, been shown to be a relatively quick alternative to manual dissection for obtaining detailed composition information in an accurate and nondestructive manner for carcasses of beef (e.g., Navajas and others 2010a, although in this study the evaluation was made on wholesale cuts because whole carcasses or sides were too large for the equipment), lamb (e.g., Kongsro and others 2008), and pork (e.g., Vester-Christensen and others 2009). It is possible that CT equipment suitable for carcass classification purposes will be developed, in light of its potential accuracy for predicting, not only the proportions of muscle, fat, and bone, but also measures of carcass shape such as muscularity, of intramuscular fat levels (Karamichou and others 2006), and of ways muscle and other tissues are distributed between more and less valuable regions of the carcass. CT scans of individual cuts of beef have also been shown to provide useful information on overall carcass composition by Navajas and others (2010b).
- *Electrical properties*: As was the case with live animals, the main two methods used for the evaluation of intact carcasses are TOBEC and BIA. The measurements are simpler than with live animals because of the absence of movement and the fact that many potentially confounding structures are removed during dressing. Despite reasonably promising results in terms of

accuracy (e.g., BIA with lamb carcasses (Altmann and others 2004), and TOBEC with pig carcasses (Berg and others 1994)), these methods are not used widely commercially.

- *Intrinsic markers*: The use of intrinsic markers, such as ^{40}K as a marker for muscle, has not found wide application even though they can be quantified in a nondestructive and noninvasive manner. The likely reasons probably include the cost of the equipment and the fact that better options are available.

Methods applicable when a cross section of muscle is exposed, as is the case at the time of quartering for beef, are described and discussed in Table 18.5. It should be noted, however, that none of these

TABLE 18.5

Examples of Measurements That Can Be made on the Surface of the Cut Longissimus ("Eye") Muscle to Provide an Indication of Characteristics Affecting the Value of a Carcass, Including Composition Characteristics as Well as Meat Quality Characteristics

Measurement	Value Characteristic(s)
Meat pH	As noted by Monin (1998), pH is the characteristic most commonly measured in fresh meat because of the importance of the rate of pH fall to meat color and water-holding capacity, and the importance of ultimate pH to keeping quality as well as to palatability and appearance characteristics (Lawrie 1991). Methods for the prediction of ultimate pH from measurements soon after slaughter have been developed (Young and others 2004).
NIR (near-infrared reflectance)	The output from NIR reflectance has been shown in several studies to be closely related to the intramuscular fat content of meat (reviewed by Prieto and others 2009). Results from some studies have suggested that it may also be a useful predictor of differences in meat tenderness (Prieto and others 2009, Rosenvold and others 2009). An associated procedure involves the combination of NIR and reflectance in the visible spectra in hyperspectral imaging that may be carried out separately on each pixel assessed by the instrument (e.g., Konda Naganathan and others 2008).
Reflectance spectroscopy in the visible wavelength range	For meat, the most widely used method for expressing color differences is in terms of the CIE L*, a*, and b* color space, with these parameters being measured using a reflectance spectrophotometer (Hunt and others 1991). Color measured in this way will also reflect differences in ultimate pH and indirectly other quality characteristics such as tenderness (e.g., Vote and others 2003).
Physical resistance	Various mechanical devices have been developed to test the resistance of muscle tissue to actions such as penetrating or biting forces in the hope that these would be indicative of cooked meat tenderness, but none have proved very satisfactory, probably because the relationship between raw and cooked meat tenderness is affected by a number of factors (e.g., Parrish and others 1973, Purchas 1973, Stephens and others 2004).
Fluorescence	Measures of fluorescence characteristics within meat by means of a probe have been shown to reflect certain collagen properties, and it is known that the amount and nature of collagen is one determinant of meat tenderness (e.g., Egelandsdal and others 2002, Swatland 2005). It has not proved to be a useful predictor of cooked-meat tenderness, possibly because only one of several determinants of tenderness is being assessed.
Raman spectroscopy	Raman spectroscopy is based on the way in which monochromatic light from a laser is scattered. This relatively new approach has been shown to detect changes that occur as meat ages using a hand-held instrument (Schmidt and others 2009).
Conductivity	The conductivity of muscle depends on its water content and on the distribution of that water between and within muscle fibers, so that it is indicative of water-holding capacity and to some extent pH and color (e.g., Warriss and others 1991). It has been seen as a possible alternative to the measurement of pH, but is not widely used commercially.
NMR (nuclear magnetic resonance)	Portable one-sided NMR sensors have been developed that are capable of measuring some distance into meat (Manz and others 2006), but the extent to which the information obtained is indicative of useful meat characteristics has yet to be determined.
VIA (video image analysis)	Explained in the second column of Table 18.3.

measurements are possible when carcasses or sides are not quartered, as is usually the case for carcasses from species other than cattle and with hot-boning beef plants or beef plants that practice side boning rather than quarter boning.

18.5 Methods that Involve Destruction of All or Part of the Carcass

18.5.1 Physical Dissection into Muscle Fat and Bone

The physical dissection of a carcass using a scalpel or boning knife into muscle, fat, bone, and "other" is generally considered the "gold standard" with regard to carcass composition characteristics (except dressing-out percentage). Ideally, this is the method that any new indirect method should be tested against for accuracy. Unfortunately, however, this is a very time-consuming and expensive approach, and has not always been used in assessing new approaches. Furthermore, the detailed procedures followed during dissection are not always consistent across laboratories, particularly in the case of what is included in the "other" component. In a survey of European laboratories, Williams (1976) found considerable variation in dissection procedures with, for example, nervous tissue and blood vessels being included as "other" in some meat laboratories but as part of lean (muscle) or fat in others. Examples of published descriptions of dissection procedures include those for sheep by Fourie (1962), for cattle by Butterfield and May (1966), and for pigs by Jones and Burson (2000), and Nissen and others (2006).

In addition to providing the relative weights of muscle, fat, and bone, with a little extra recording, the dissection procedure can also give information on

1. The relative weights of subcutaneous and intermuscular fat depots (an important fat partitioning parameter).
2. The relative weights of all individual muscles or of a selection of the major muscles (a measure of muscle distribution).
3. The relative weights of some or all individual bones (of limited commercial interest).
4. The weights of muscle, fat, and bone in different anatomical parts of the carcass or within all or some commercial cuts (measures of muscle distribution, fat partitioning, and fat distribution within depots).
5. Estimates of measures of carcass shape by measuring the lengths of selected muscles and bones as well as their weights (Purchas and others 1991).

A widely used simplification of physically dissecting a whole carcass or side has been to dissect a sample cut and develop equations to predict carcass composition from the composition of a cut (Williams 1976, Kempster and others 1982). An example of the effectiveness of this approach is provided by the results in Figure 18.5 from a study where an assessment was made of the relative effectiveness with which the muscle percentage in 11 cuts predicted the percentage of muscle (LMY%) in a whole side of beef. A perfect sample cut would have an RSD of zero. None of the cuts evaluated came close to that, and the best three had RSD values between 30% and 40% of the raw SD (Figure 18.5). These three cuts (top piece, pony, and coast) were also the three heaviest cuts in this study, which illustrates the general tendency for the best sample cuts to be those that make up the largest proportion of the whole. The results in Figure 18.5 came from a study involving over 700 steers in 17 groups. When differences between groups in the slopes and intercepts of the relationships were taken into account, and side weight was included in the prediction equations, the pooled within-group RSD values were reduced by an average of 15%, which indicated that, for this group of cattle, using overall prediction equations would have produced biased results for some of the breed groups.

Another modification of the basic detailed dissection method is where the main measurement is of SMY% rather than LMY%. The main advantages of this approach are that it is usually faster because it can often be carried out in commercial boning/cutting rooms, and the resulting cuts can pass into normal commercial channels. These advantages need to be set against the disadvantages of a lower

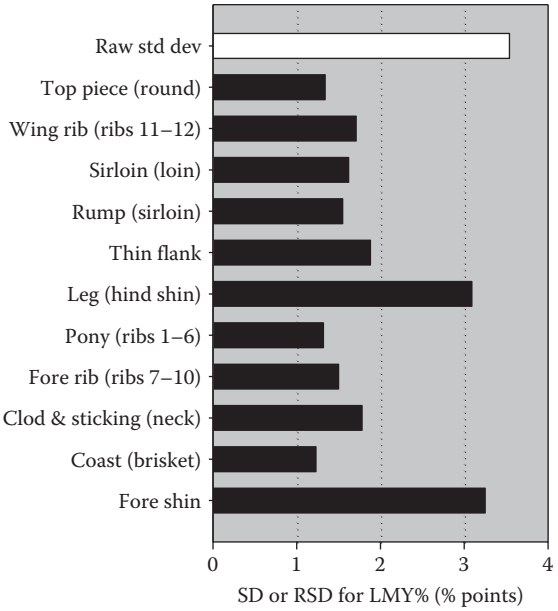

FIGURE 18.5 An example of results from a study in which the accuracy of predicting the LMY% in a side of beef from the lean% in each of 11 cuts was assessed in terms of RSDs. (Based on the data from Kempster AJ, Jones DW. 1977. *J Agric Sci, Camb* 88:193–201.) The open bar shows the raw standard deviation of LMY% for this population of 753 steers made up of 17 breed groups. Within the brackets are alternative cut names or an indication of the position of a cut.

accuracy because of the difficulty of accurately trimming cuts consistently to a specified thickness of subcutaneous fat, and the fact that some trimmed cuts may include bone. The protocol followed in terms of commercial cuts considered and the level of fat trim will vary between countries and over time, so that a further disadvantage is that SMY% values for the same species from different studies will often not be strictly comparable. There are many examples of the sorts of specifications set out in the estimation of SMY% values (e.g., Perry and others (2001) for beef, and Marcoux and others (2007) for pork). Values for SMY% will usually be higher than LMY% because of the fat included in the former, but this will depend on the trimming specifications. For example, in the study of Perry and others (1993) with 156 prime cattle, the SMY% and LMY% values were 66.9% and 57.0%, respectively.

18.5.2 Chemical Analysis of a Representative Sample

In order to obtain a representative sample of a carcass for chemical analysis, the carcass (or one side of a carcass as bilateral symmetry can usually be safely assumed) must be homogenized in some way. This commonly involves slicing a frozen carcass or side into small pieces with a bandsaw, and then passing these pieces through a mincer/grinder several times with decreasing hole sizes in the plates used. Mixing the mince between runs either mechanically or by hand is very important in order to get a representative sample. At this stage, a subsample is taken and homogenized further in a laboratory homogenizer so that quite small samples of a few grams are representative of the whole. A relatively simple analysis of this sample is by proximate analysis to give the content of water, lipid, protein, and ash. A description of the methods involved is beyond the scope of this chapter (for examples of methods see AOAC 2000). As an example, in the study of Field and others (1985), the proximate analysis of carcass sides from 33 prime lambs gave the following results (average ± standard deviation; coefficient of variation (%)):

1. Water: 52.7 ± 5.8; 11.0%
2. Lipid: 25.2 ± 7.7; 30.6%

3. Protein: 17.4 ± 1.7; 9.8%
4. Ash: 4.7 ± 0.5; 10.6%

These results illustrate, first, that the main component of carcasses is usually water and, second, that the most variable component is usually lipid (as shown by the largest CV%).

The procedure outlined above for a whole carcass or side is equally applicable to a part of a carcass (e.g., a sample cut) or to one component of the carcass that has been separated from the rest by physical dissection, such as the lean meat or the adipose tissue (fat). The component of most interest in this respect is the lean meat or muscle. Lean meat with all visible fat except intramuscular fat removed by dissection consists mainly of muscle fibers (usually more than 90%), but will also contain some intramuscular fat as adipocytes (fat cells) outside the muscle fibers and as lipid within the fibers, and some connective tissue that provides a framework for the muscle fibers and fat cells to fit within. The connective tissue, which is primarily made up of collagen and elastin fibers, also serves to transfer the forces generated within the muscle fibers via the tendons to the skeleton. An indication of the chemical composition of lean meat is given in Figure 18.6 (based on the data from Lawrie 1991). The two items in Figure 18.6, which vary most between muscles within an animal, or between animals, are the lipid content, which is usually <5%, but may be more than 30% (see, e.g., the levels of more than 33% for the longissimus muscle of Japanese Black heifers in the study of Okumura and others (2008)), and connective tissue. For example, Dransfield (1977) reported that the concentration of collagen (the main fibrous component of muscle connective tissue) for 18 beef muscles varied more than twofold from 0.5% to 1.3%. More detailed analyses of certain subitems within the categories listed in Figure 18.6 have also shown considerable variation between muscles, often as a reflection of the balance of the different muscle fiber types present. For example, in the study of Purchas and Busboom (2005) with bovine muscle, the concentration of the amino acid taurine (mg.100 g^{-1}), which tends to be higher in red-type muscle fibers, varied from 48 in the longissimus muscle, to 108 in the triceps brachii, and to 611 in cheek muscle.

The chemical composition of lean meat is relevant with respect to the meat-quality aspects of carcass evaluation because the chemical characteristics and related metabolic machinery of meat have an important effect, not only on the nutritive value of the meat, but also the palatability and appearance characteristics. In addition there is increasing evidence that meat contains bioactive compounds that are potentially beneficial to the health and wellbeing of consumers in addition to any contribution they make as nutrients (e.g., Purchas and others 2004).

Another way in which different muscles can be characterized, in addition to their anatomical location (by physical dissection) or chemical features (by chemical analysis), is by histological analysis of the proportions of different muscle fiber types. Such proportions, which vary widely between different muscles within an animal as well between animals of different species, have been shown to be related to meat composition and quality characteristics (Lefaucheur 2010), and therefore will sometimes be a relevant measurement to make in a carcass evaluation project.

18.5.3 Measurement of Meat Quality Characteristics

For the purpose of this chapter, meat quality is defined as: "The combination of all those intrinsic and external characteristics of meat that affect its level of acceptability or excellence for a consumer." A brief indication of specific characteristics involved was provided in Figure 18.1 and the accompanying text, but with respect to carcass evaluation "meat quality" will encompass all those intrinsic characteristics of the edible part of the carcass that can influence the value of that carcass per unit weight. External meat-quality characteristics (Figure 18.1) are excluded here because the measurement of those is usually straightforward. The list of intrinsic characteristics of meat in Figure 18.1 is expanded in Table 18.6. Methods of measuring these characteristics include a range of approaches that can be categorized as

- Sensory methods, particularly for palatability and appearance characteristics.
- Instrumental methods, particularly for characteristics such as tenderness, color, juiciness, and, to lesser extent, flavor (Chikuni and others 2010).
- Chemical methods for most nutrients, bioactive compounds, and some wholesomeness and safety characteristics.
- Biological methods, particularly for aspects of microbial safety, and some nutrients by bioassays.

Details of measurement methods for individual quality-related characteristics are covered in other chapters in this book.

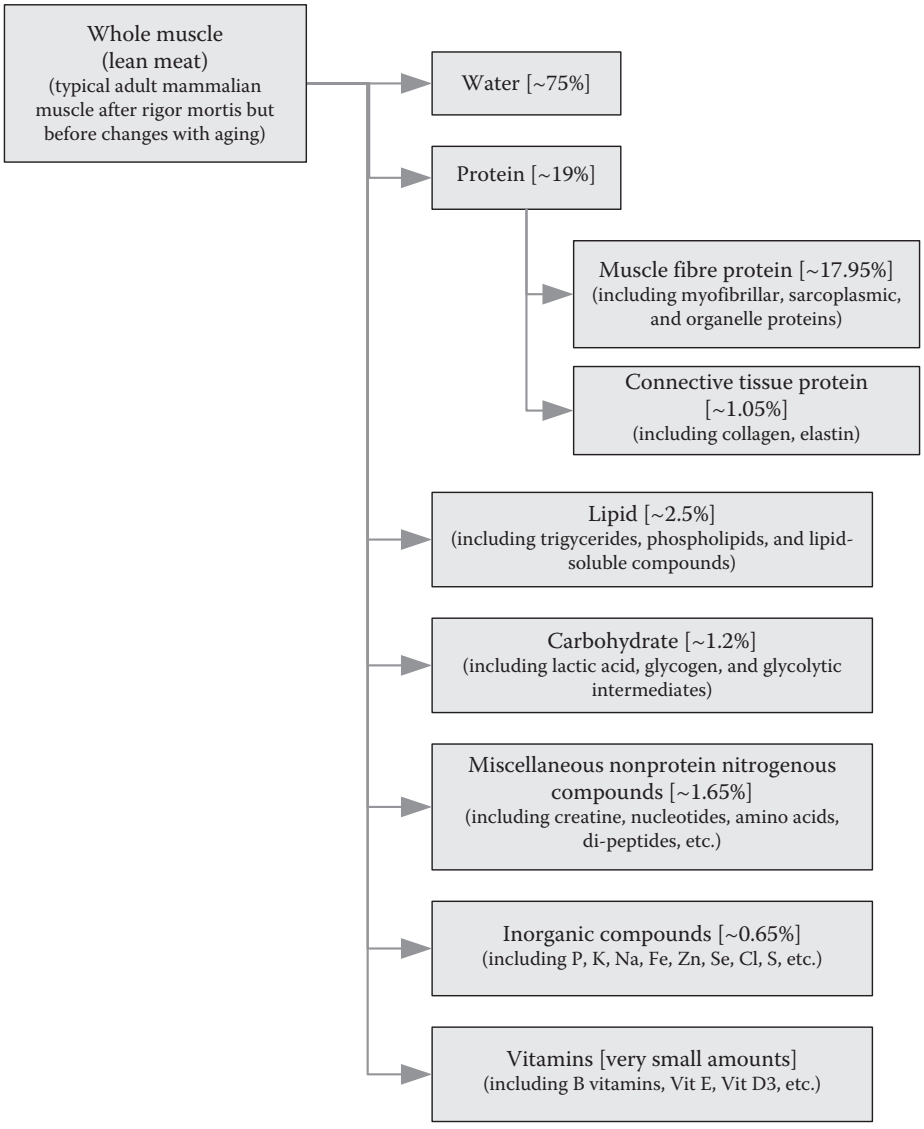

FIGURE 18.6 The chemical composition of a typical mammalian skeletal muscle in broad terms (Based on Lawrie RA. 1991. *Meat Science*. 5th ed. Oxford: Pergamon Press. 293pp, Table 4.1.) The symbol "~" means "approximately."

TABLE 18.6

Examples of Intrinsic Characteristics of Meat That Contribute to Its Quality when Meat Quality Is Defined as "The Combination of All Those Intrinsic and External Characteristics of Meat That Affect Its Acceptability or Level of Excellence for a Consumer"

Category of Meat Quality Characteristics
Palatability characteristics:
• Tenderness (sometimes treated as a component of meat texture)
• Flavor (incorporating the two senses of taste and olfaction or aroma)
• Juiciness (a function of both water content as well as the extent of salivation)
Appearance characteristics:
• Lean meat color
• Fat color
• Meat texture in terms of fineness/coarseness of a cut surface
• Firmness (ability to retain shape on display)
• Composition in terms of proportions of visible muscle, fat, and bone
Nutritive value characteristics:
• Energy (kJ/100 g), particularly relative to other nutrients (nutrient density)
• Protein content
• Protein quality in terms of amino acid balance and digestibility
• Mineral content (e.g., Fe, Zn, Se, etc.)
• Mineral bioavailability (particularly for iron where meat excels)
• The fatty-acid composition of intramuscular lipid
• Vitamins (particularly the B group plus vitamins A, D3, and E)
• Bioactive compounds (those that enhance health and/or wellbeing)
Safety and wholesomeness characteristics:
• Presence of pathogenic microorganisms
• Presence of spoilage microorganisms
• Presence of dangerous components (may be intrinsic to the meat or extrinsic)
• Presence of unsightly items
Processing properties:
• Water-holding capacity
• Binding capacity (between pieces of meat)
• Emulsifying capacity

18.6 Conclusions

Carcass evaluation, which is taken here to mean the estimation of the average value per unit weight of a carcass from a meat animal, involves assessing many characteristics associated with the composition and quality of all or part of that carcass (Figure 18.1). Having accurate information on all these characteristics would facilitate the assessment of live animals, intact carcasses, and individual meat products at the retail level. Although many satisfactory methods have been developed to obtain this information, as outlined in this chapter, there is still considerable scope for developing improved methods, particularly with respect to their accuracy, ease of use, speed of operation, cost, and nondestructiveness.

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19

Chilling and Freezing Meat

Mike F. North and Simon J. Lovatt

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19.1 Introduction

The meat industry uses refrigeration for food preservation. In many countries, animals slaughtered for meat were, and in some cases still are, immediately distributed, sold, and consumed. Preservation was therefore unnecessary. As producers began to produce surplus meat, however, preservation methods were required so that excess product could be held and used later, or in a distant location.

Chilling meat preserves it for a time without changing its form or state. Freezing is a logical progression from chilling that gives much longer preservation times, though it involves making some (largely reversible) changes to the meat.

The main factors that affect the storage life of meat are microbial growth and deleterious chemical reactions. Microbial growth can make the meat less pleasant to eat (spoilage) and it can make the consumer ill if the numbers of pathogenic microorganisms are too high or enough toxins are produced. Even if microbial growth is limited, meat may become unacceptable due to chemical reactions that cause flavor and textural changes, or due to color deterioration.

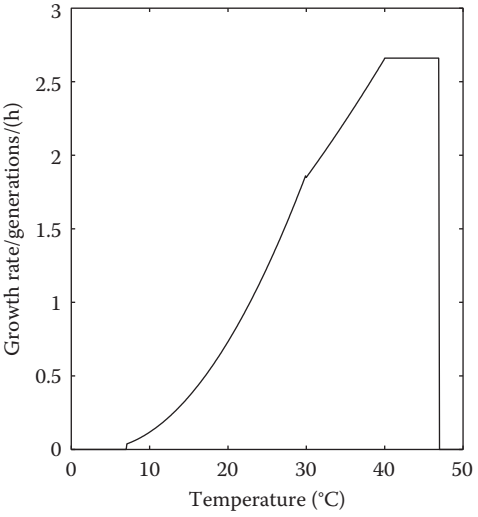

FIGURE 19.1 Growth rate of *E. coli* versus temperature, plotted from the model described by Gill and others (1991).

Bacteria and moulds, like all living things, have a range of temperature in which they prefer to grow. As the temperature moves away from their preferred level, their growth rate slows. Once they get too far away from that level, growth stops completely and the bacteria may die.

Figure 19.1 shows how the growth rate of a commonly studied bacterium varies with temperature. By cooling the meat from the initial body temperature of the animal (about 38°C) to 10°C, the growth rate drops by about 95%. Below 7°C, Figure 19.1 suggests that this particular bacterium (*Escherichia coli*) does not show sustained growth. The growth rate at a given temperature varies between different microorganisms, but the principle remains the same. Cooling meat is a very good way to slow or even stop microbial growth and thereby reduce the rate of microbial spoilage or the growth of pathogens.

Chemical reaction rates are also affected significantly by temperature. Figure 19.2 shows how the rate of a typical chemical reaction varies with temperature. Unlike the microbial growth rate, this reaction

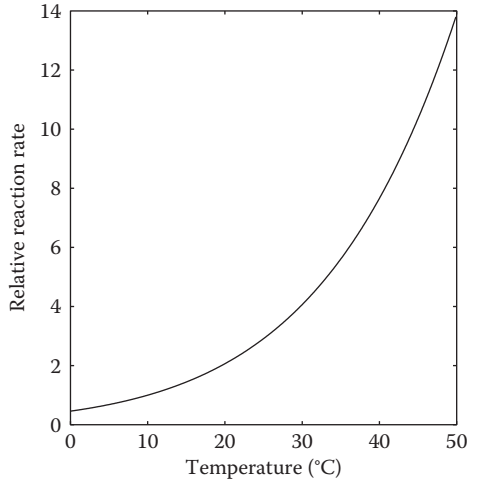

FIGURE 19.2 How the rate of a typical chemical reaction varies with temperature, assuming an Arrhenius relationship between reaction rate and temperature and an activation energy of 50 kJ/mol.

will still take place at low temperatures, albeit very slowly. Thus, even meat cooled to a very low temperature will slowly deteriorate, thereby setting a finite shelf life regardless of other factors.

19.2 Mechanisms of Heat Transfer

Refrigeration depends on removing heat from meat and then maintaining it at a lower temperature than its surroundings. Heat is a form of energy, measured in units of Joules (J). There are three fundamental mechanisms through which heat is transferred: conduction, convection, and radiation. All of the possible methods for cooling are based on one or more of these mechanisms.

Conduction is the transfer of heat through physical contact, either within a body or between two bodies, without the particles of those bodies moving significantly. Heat flows by conduction from a body that has a higher temperature to a body that has a lower temperature.

Convection transfers heat when higher temperature portions of a liquid or gas move and mix within lower temperature portions.

Radiation transfers heat from a body at a higher temperature to one at a lower temperature by electromagnetic waves passing through space. For radiation to occur the bodies need only be in line of sight of each other. Heat transfer by radiation is generally, only important when one or more of the bodies is much warmer than room temperature. In the chillers and freezers of a meat processing plant, heat transfer by radiation therefore usually has only a very small influence on the rate of cooling.

19.3 Surface Heat Transfer

Meat product cooling is most commonly achieved by placing the meat into a cold liquid or gas. When the cold fluid comes into contact with the warm meat, heat is transferred from the meat to the fluid by conduction. This cools the surface of the meat while warming part of the fluid. The warm portion of the fluid will then move away from the surface, either naturally or by an external means such as a fan, and be replaced by more cold fluid. The rate of heat flow to the cold fluid will decrease over time as the meat surface gradually cools. Cooling will then continue as long as the temperature of the meat surface exceeds that of the fluid or until the meat is removed from the cold fluid.

There are two ways by which the fluid can move away from the meat surface. Natural convection (sometimes called "free convection") occurs naturally during the cooling process. When the fluid at the meat surface is heated, it becomes less dense than the surrounding fluid. The warm fluid therefore rises due to its buoyancy and the surrounding cold fluid sweeps in from the sides to replace it. Forced convection occurs when the fluid is moved by an external force, for example, if a fan blows air or a pump pushes liquid across a surface. Forced convection increases the rate of heat removal because the fan or pump moves the fluid at a higher velocity than is achieved by natural convection alone.

The rate of heat removal in either forced or natural convection depends on "Newton's Law of Cooling," discovered by Sir Isaac Newton in 1701. This is described as

$$Q = hA(T_{\text{surface}} - T_{\text{fluid}}) \tag{19.1}$$

where

Q is the rate of heat removed from the surface (W)
h is the surface heat transfer coefficient (W/m^{2}K)
A is the area over which heat is being transferred (m^2)
T_{surface} is the temperature of the surface (°C)
T_{fluid} is the temperature of the fluid (°C).

"Newton's Law of Cooling" demonstrates mathematically which variables affect the rate of heat removal from the product surface (Q). The temperature difference between the surface and the fluid ($T_{\text{surface}} - T_{\text{fluid}}$)

TABLE 19.1

Approximate Surface Heat Transfer Coefficients in Various Convective Situations

Mechanism	Surface Heat Transfer Coefficient h(W/m^{2}K)
Forced convection, water	250–15,000
Forced convection, air	25–500
Natural convection, air	5–50

Source: Adapted from Welty JR. 1978. *Engineering Heat Transfer* (SI version). Singapore: Wiley.

affects the rate of heat removal and, as the fluid and surface approach the same temperature, the rate of heat removal approaches zero. The rate of heat removal also relies on the area (A) over which the heat transfer is occurring, so exposing a larger meat surface area to the cold fluid will increase the rate of heat removal. The surface heat transfer coefficient (h) also has an impact—its value depends on the characteristics of the fluid and its velocity. A higher surface heat transfer coefficient is typically found in convective heat transfer to liquids when compared with gases and it increases with fluid velocity. The heat transfer coefficient also depends on the level of turbulence in the fluid.

Table 19.1 shows some typical ranges for h in a variety of situations. For situations that arise in meat processing plants, the heat transfer coefficients are commonly near the bottom end of the ranges shown in Table 19.1. Methods for estimating h for common product cooling cases are discussed further in the section on "Predicting Chilling Times."

19.4 Internal Heat Transfer

When the surface of a meat product is cooled, a temperature difference arises between the surface and internal parts of the product. This temperature difference drives internal heat transfer by conduction, where the warmer parts of the product transfer heat to the cooler surface parts. For a slab-shaped piece of meat cooled from the top surface only, the rate of internal heat flow (Q) from the bottom to the top is given by the equation:

$$Q = \frac{kA}{x}(T_{\text{top}} - T_{\text{bottom}}) \tag{19.2}$$

where

k is the thermal conductivity of the material (W/m K)
A is the area over which heat is being conducted (m^2)
x is the thickness of the slab (m)
T_{top} is the temperature of the top slab surface (°C)
T_{bottom} is the temperature of the bottom slab surface (°C).

Equation 19.2 is strictly correct only when the heat flow is steady, which is not the case when meat is cooled because the temperatures within the meat are constantly changing. Despite this, Equation 19.2 indicates the factors that are important to internal heat transfer. It shows that a greater rate of cooling will occur when the slab thickness is small, the slab surface area is large and the difference between the top and bottom surface temperatures is large. The thermal conductivity of a material specifies how easily heat can be transferred through the material by conduction. Highly conductive materials such as metals will easily transfer heat from one side of a slab to the other. In contrast, a material with a low thermal conductivity will make heat flow difficult.

19.5 Surface versus Internal Heat Transfer

In practice, meat cooling typically involves a combination of conduction and convection, where internal conduction carries the product's heat content to the surface, and then convection (natural or forced) carries that heat away from the surface. This means that the relationship between overall cooling rate and the parameters discussed earlier is not completely intuitive. For example, if a high rate of surface heat transfer already exists, then increasing the heat transfer coefficient will increase the rate of heat removal from the surface, but it may not significantly increase the total rate of heat removal from the product because that rate will be limited by the rate of internal conduction. On the other hand, if there is a high rate of internal heat conduction through a body, reducing the body's thickness may not significantly increase the heat flow rate because the total rate of heat removal will be limited by the convection at the surface.

The way in which the external resistance to heat transfer (due to convection) is balanced against the internal resistance (due to conduction) is indicated by the Biot number (Cleland 1990). The Biot number, abbreviated *Bi*, is the ratio of the conductive (internal) resistance (X/k) to heat transfer to the convective (external) resistance ($1/h$), as defined by

$$Bi = \frac{hX}{k} \tag{19.3}$$

where

X is the distance from the center of the meat product to the surface (m).

The Bi can range from 0 to infinity. If Bi is low (much less than 1.0), the external resistance controls the process. In this case, changes to h will have a large effect on the cooling time and changes to X will have little effect. If Bi is high (much more than 1.0), then the internal resistance controls the process. In this case, changes to h will have very little effect on the cooling time, but changes to X will have a large effect.

19.6 The Chilling Process

Chilling is the process of cooling meat while the meat remains above its freezing temperature. The temperature of the cooling medium (air or water, for instance) does not matter as long as it is colder than the meat but, as soon as the meat starts to freeze, the process is no longer considered to be chilling.

A well-designed and controlled chilling process is very important to producing meat of satisfactory quality. An appropriate chilling regime is critical to producing tender meat, and it is just as critical to producing meat with low levels of bacterial growth, and therefore a long shelf life (Savell and others 2005).

Therefore, the following sections on chilling will focus on (1) the impact of chilling rate on meat quality, (2) how to select and predict the chilling rate of meat products, and (3) how to predict the heat that must be removed from the product as it chills (the "heat load"), which will help determine the required capacity of the refrigeration system.

19.6.1 Impact of Chilling Rate on Meat Quality

After an animal is slaughtered, blood circulation stops and muscles lose their oxygen supply. Without oxygen, the muscle turns to anaerobic glycolysis to generate energy. Anaerobic glycolysis produces energy to contract the muscle and it also produces lactic acid, which causes the muscle pH to fall. As glycogen supplies are depleted, the energy for muscle contraction is lost and the actin and myosin filaments lock together in a permanent contraction called *rigor mortis*. An animal with low glycogen stores at slaughter, which may be caused by stress or lack of feed/water, will consequently result in meat with an elevated pH that is often dark, firm, dry, may have problems aging, and may spoil faster than normal pH meat (see chapters on muscle biology, meat composition, and postmortem chemistry and biochemistry).

When meat is chilled so that its temperature falls below about 10°C while its pH is still above 6.2, the muscle will contract (Honikel and others 1983). This is known as cold shortening, and the meat becomes tough in approximate proportion to the amount of shortening that takes place. If the muscle pH is less than about 6.0, it can be cooled fairly rapidly without shortening, although if it is then frozen it may shorten when thawed. For tenderness, chilling must be slow enough to prevent "cold shortening" (see Chapters 4 and 8–10).

It is essential to subsequently chill the meat in a way that ensures that there is enough time for aging to take place before the meat is frozen or consumed. Aging of meat is a tenderization process caused by the activity of enzymes that are already present in the muscle when the animal is killed. These enzymes act on the muscle and break down some of the proteins within the meat. Electrical stimulation does not accelerate aging as such but, because stimulated meat goes into rigor more quickly, it starts to age sooner and it does so while the meat is still relatively warm, which therefore accelerates aging.

To minimize bacterial growth, meat should be chilled as quickly as tenderness considerations allow. Bacterial growth depends on the meat surface temperature, but it also depends on the water activity at the surface of the meat. Fortunately, the surface temperature typically drops quickly during chilling. Air chilling can also dry the surface of the meat, thereby reducing the water activity, and bacteria do not grow as well in low water activity environments. These factors combine to keep bacterial growth to an acceptable level in a well-designed and operated process.

More detail on meat quality issues is provided in the chapters on quality assessment, texture and juiciness, and sensory evaluation. Although meat quality may not always be mentioned throughout this chapter, it should always be borne in mind when discussing chilling or freezing of meat.

19.6.2 Thermal Properties

The amount of heat that has to be removed from a meat product during chilling and the rate at which that heat can be removed depend on the thermal properties of the meat. The important thermal properties are the thermal conductivity (measured in W/m K), the heat content or enthalpy (J/kg), and the density (kg/m^3) of the meat. Above its freezing temperature, the enthalpy of a meat product increases at a rate, that is, almost constant with temperature, called the heat capacity (J/kg K).

Values of thermal properties for various meat products may be found in Miles and others (1983), Pham and Willix (1989), Pham and others (1994), Willix and others (1998), Amos and others (2008), and *ASHRAE Handbooks* (e.g., ASHRAE 2010), or may be estimated from its composition using the following equations:

$$\rho_l = \frac{1}{W/\rho_{lW} + S/\rho_S + F/\rho_F} \tag{19.4}$$

$$k_l = \rho_l \left(\frac{k_{lW}}{\rho_{lW}} W + \frac{k_S}{\rho_S} S + \frac{k_F}{\rho_F} F \right) \tag{19.5}$$

$$C_l = C_{lW} W + C_S S + C_F F \tag{19.6}$$

where:

ρ_l is the unfrozen meat density (kg/m^3); $\rho_{lW} \approx 1000$, $\rho_S \approx 1300$, and $\rho_F \approx 850$ kg/m^3.
k_l is the unfrozen thermal conductivity (W/m K); $k_{lW} \approx 0.58$, $k_S \approx 0.25$, and $k_F \approx 0.18$ W/m K.
C_l is the unfrozen heat capacity (J/kg K); $C_{lW} \approx 4180$, $C_S \approx 1400$, and $C_F \approx 1900$ J/kg K.

W, F, S are the mass fractions of water, fat, and other solids respectively (i.e., for a typical piece of lean beef, $W \approx 0.70$, $F \approx 0.10$, $S \approx 0.20$) and the subscripts indicate the thermal properties of each component.

It should be noted that, while Equations 19.4 and 19.6 provide reliable estimates of the density and heat capacity of meat, the "parallel" model shown in Equation 19.5 includes only some of the factors that are important to estimate thermal conductivity. Carson (2006) provided a comprehensive review of methods

to predict the thermal conductivity of food products that included more sophisticated methods than the parallel model.

19.6.3 Selecting a Chilling Rate

Three process factors can be changed in order to change the chilling rate: (1) object size or shape, (2) external heat transfer resistance, and (3) temperature-driving force.

Changing the object size or shape changes the length of the internal pathway along which the heat must be conducted. The pathway may be shortened by cutting the object into smaller pieces. This can greatly reduce the chilling time for a meat product, but it has several disadvantages. Reducing the sizes of the meat pieces will increase the surface area for a given volume, resulting in much greater evaporation rates and greatly increased weight loss, even given the shorter time spent in the chiller. A greater surface area also exposes more meat to oxygen in the air, which will increase the rate at which "oxidative rancidity" develops, and therefore increase "warmed-over" flavor. Repacking densities after chilling will also be lower than for the original undivided meat. Finally, it becomes more difficult to identify the original form of the meat as the size of the meat pieces is reduced.

Increasing the object size will increase the chilling time. This can occur when many smaller products are packaged inside one larger package. Chilling meat in very large blocks (e.g., several hundred millimeters thick) inevitably means that the center temperature will remain high for many hours, with a consequent high growth of microorganisms in that part of the package. The tenderness of meat in the center of the package will also vary considerably from the tenderness at the surface, particularly if a high rate of heat transfer is used at the surface in an attempt to reduce the overall chilling time. Even in more moderate package sizes, for example a 25 kg carton that is 160 mm thick, the variation between the center and the surface can be considerable (see Figure 19.3).

The amount of external resistance to heat transfer in meat cooling can be changed by changing the heat transfer medium (e.g., using forced convection instead of natural convection, or water instead of air), or by changing the packaging. One heat transfer medium not yet discussed is plate cooling (which is typically used for freezing), where two opposite surfaces of the package are placed in direct contact with refrigerated metal plates. In this case, the external resistance will be negligible, except for any packaging that may surround the product.

The final parameter that affects the cooling rate is the temperature-driving force, but there is a limit to how cold the cooling medium can be during chilling. In principle, to avoid all risk of freezing, the cooling medium should not be any colder than the freezing temperature of the meat. In practice, however, if the cooling medium is no more than a few degrees below the freezing temperature, there will be very little risk of freezing the surface.

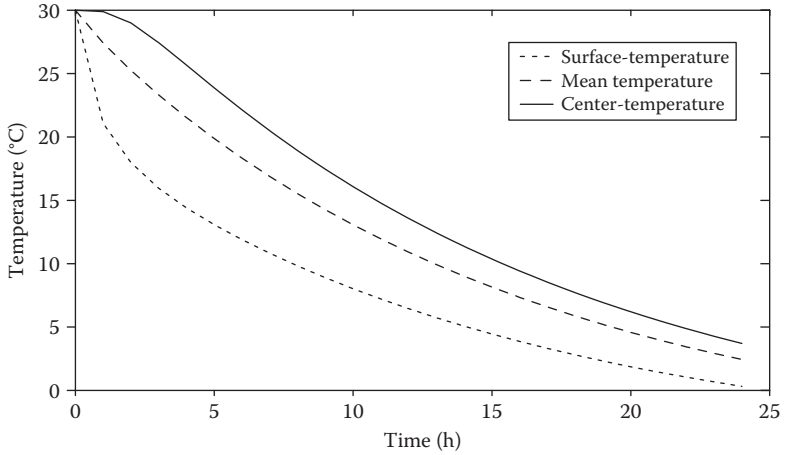

FIGURE 19.3 Temperature profiles at different positions within a 160 mm thick carton of meat.

19.6.4 Predicting Chilling Times

To design a chilling process, it is important to be able to calculate the length of time that the process will take and the heat loads that will have to be removed.

The surface heat transfer coefficients (h, in W/m^{2}K) for meat chilling or freezing in air due to forced convection can be estimated using the following equations, derived from equations (5-197) and (5-193) of Welty (1978) by assuming an average air temperature of about 7°C:

$$h = 6.1\, L^{-0.2}\, v^{0.8} \text{ for flat surfaces of length } L \text{ meters} \tag{19.7}$$

$$h = 6.7\, D^{-0.4}\, v^{0.6} \text{ for spheres with diameter } D \text{ meters} \tag{19.8}$$

where

v is the air velocity (m/s)

Equation 19.8 is an adequate approximation for heat transfer near the deep butt or deep leg points of beef sides, and pork or lamb carcasses. Equation 19.7 should be used for cartons of meat products.

These equations are valid down to the air velocities where natural convection starts to have a significant influence on the heat transfer coefficient. In general, they should be used in air velocities of more than 0.4 m/s. For air velocities below 0.4 m/s, it is generally safe to assume that the heat transfer coefficient at the surface of a meat product due to natural convection is ~5–10 W/m^{2}K for much of the chilling process. Equations for estimating heat transfer coefficients of natural convection in either air or water can be found in heat transfer textbooks, such as those by Incropera and others (2011) or Kreith and others (2010).

The chilling process reduces the temperature of a product from some initial temperature (T_i) toward the ambient temperature of the surrounding cooling medium (T_a). The best way of expressing how far through the chilling process the product has gone is to state what fraction of that temperature difference the meat still has to pass through. This is called the fraction unaccomplished temperature change, and it has the symbol *Y*. *Y* can be calculated for any point in a meat product but the average temperature or the thermal center temperature are usually of most interest. The thermal center is the slowest-cooling point in the object. This is often the same as the point in the object that is furthest from the nearest surface, though that may not be the case if the rate of heat transfer varies around the surface of the object. The fraction unaccomplished temperature change at the thermal center, Y_c, is therefore:

$$Y_c = \frac{T_c - T_a}{T_i - T_a} \tag{19.9}$$

The average fractional unaccomplished change for the object is calculated in the same way, but T_c is replaced with T_{av}:

$$Y_{av} = \frac{T_{av} - T_a}{T_i - T_a} \tag{19.10}$$

Alternatively, if the value of Y_c or Y_{av} is known, we can rearrange Equations 19.9 and 19.10 to find the center or average temperature:

$$T_c = (T_i - T_a)\, Y_c + T_a \tag{19.11}$$

$$T_{av} = (T_i - T_a)\, Y_{av} + T_a \tag{19.12}$$

As discussed above, chilling requires a temperature-driving force, which is the difference in temperature between the surface of the object and the surrounding medium. As the difference in temperature

between the object and the surrounding medium becomes smaller, the rate at which the temperature falls will become slower. As a result, if a piece of meat initially at 35°C is chilled in cold air at a temperature of 0°C, the meat will never quite reach 0°C.

Although the meat will never reach the ambient temperature, we can calculate how long it would take for the meat to get part-way there. The time required for Y_c to change from 1.0 to 0.5 is the time required for the thermal center of the object to go half way from its initial temperature to the ambient temperature. This is known as the half-cooling time and is denoted $t_{1/2}$, which should not be confused with half of the total cooling time. The total cooling time depends upon the final temperature that is selected (i.e., some temperature above T_a) as the point when the meat is to be taken out of the chiller. The half-cooling time, on the other hand, depends only on the physical conditions of the meat in the chiller.

For a given set of conditions, it is possible to calculate the half-cooling time in several different ways but we will use the method of Cleland and Earle (1982). For a simple shape such as a slab or a sphere, the calculation is straightforward. We calculate the *Bi* for the situation and use Figure 19.4 to find the Fourier Number at the half-cooling time ($Fo_{1/2}$) for that value of *Bi*. We can then use Equation 19.13 to calculate the half-cooling time:

$$t_{1/2} = \frac{Fo_{1/2}\,\rho_l\,c_l\,X^2}{k_l} \tag{19.13}$$

where

X is the depth of the thermal center, in meters.

Given the half-cooling time, we can calculate the time required to cool to any temperature.

19.6.5 Cooling to an Average Temperature

For an average temperature, T_{av}, to which we want to cool, we can use Equation 19.10 to calculate the fractional unaccomplished temperature change, Y_{av}. From Y_{av}, we can find the number of half-lives, N, required to cool to that Y_{av} value using Figure 19.5. Finally, we can find the total time to cool to the specified average temperature using

$$t = N t_{1/2} \tag{19.14}$$

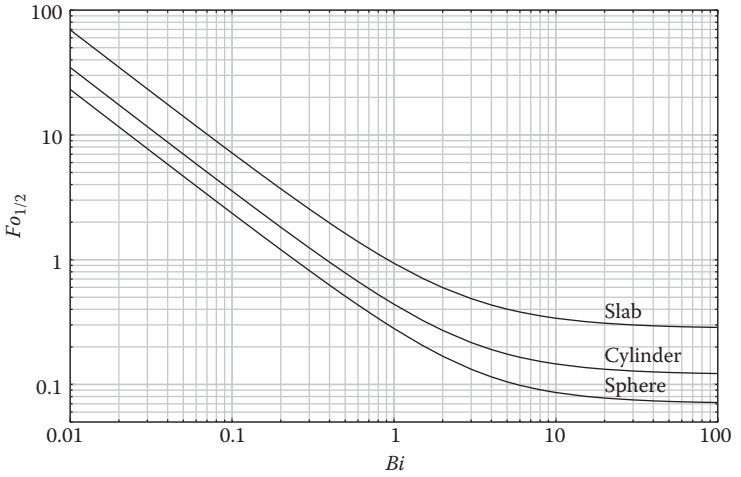

FIGURE 19.4 Half-cooling Fourier numbers for slabs, cylinders, and spheres with different *Bi* values.

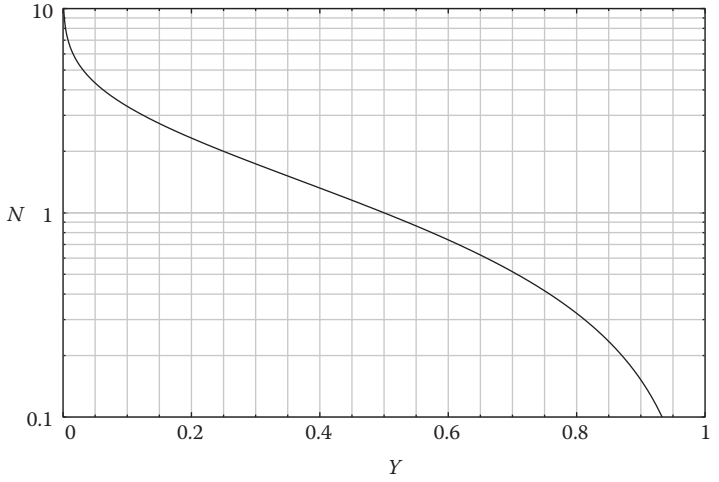

FIGURE 19.5 Number of half-lives, *N*, required to cool to a given fractional unaccomplished temperature change, *Y*.

For shapes other than a slab or a sphere, we cannot just read the Fourier number from Figure 19.4. Instead, for a flat-sided shape such as a carton, we can find the $Fo_{1/2}$ for a slab, and for an oval shape such as a carcass or side, we can find the $Fo_{1/2}$ for a sphere from Figure 19.4. Once we have the $Fo_{1/2}$ for a slab or sphere, we can calculate the $Fo_{1/2}$ for the shape using either equations

$$Fo_{1/2,\text{carton}} = \frac{Fo_{1/2,\text{slab}}}{E} \tag{19.15}$$

$$Fo_{1/2,\text{carcass}} = \frac{3Fo_{1/2,\text{sphere}}}{E} \tag{19.16}$$

The factor *E* is called the equivalent heat transfer dimensionality. This factor relates the cooling time for a complicated shape to the cooling time for a simple shape such as a slab or a sphere. It is possible to calculate *E* for a complicated shape (Hossain and others 1992a–c) but some values for relevant shapes are shown in Table 19.2 (Cleland and Earle 1982). Where a range is shown in Table 19.2, the exact value depends on the precise shape. In general, a larger *E* value applies for an object that is thicker relative to its width and length. This means that a deep carton will have an *E* value of 1.5 while a shallow carton will have an *E* value of 1.3.

For shapes other than a slab, cylinder, or sphere, the depth of the thermal center may have to be measured or estimated. For a box of meat that is (for instance) 160 mm thick, $X = 0.08$ m (i.e., half the thickness). For an irregular shape such as a carcass or beef side *X* should be measured directly. If this is not

TABLE 19.2

E Values for Products of Interest to the Meat Industry

Product	***E***
Lamb (shoulder)	1.4
Lamb (deep leg)	2.2
Ewe (deep leg)	2.0
Beef carton (plate freezer)	1.0
Beef carton (air blast freezer)	1.3–1.5
Beef side or quarter (deep leg)	1.3

possible, Equation 19.17 gives an approximate correlation between beef side weight and thermal center depth. For beef quarters, double the weight of the quarter to get the weight of the equivalent side.

$$X = 0.026\,M^{1/3} \tag{19.17}$$

where

M is the mass of the mass or weight of the beef side (kg).

19.6.6 Finding the Center Temperature

To calculate the chilling time for the thermal center of an object, the difference between the thermal center temperature profile and the other temperature profiles in the object must be taken into account. The thermal center is the slowest cooling part of the object, so it lags behind the other temperature profiles.

For the thermal center, rather than using Figure 19.5 to find the number of half-lives, N, we should use Figure 19.6 instead, which takes this lag into account. We can find the value of E at the bottom left, and move straight up the graph until we reach the value of Bi for this situation. Then we can move straight across to the right until we are above the value of Y that we need. This will give us the number of half-lives, N, to use in the remainder of the calculation.

19.6.7 Effect of Packaging on Heat Transfer Coefficient

Up to now, the chilling time estimation methods that we have discussed have dealt with unpackaged objects where the heat transfer coefficient was estimated from Equations 19.7 and 19.8. However, when a meat product is packaged, the effective heat transfer coefficient is reduced by the thermal resistance of the packaging and by any trapped air layer between the package and the product. The thermal conductivities of some example packaging materials are shown in Table 19.3.

Although the impact of the packaging material on heat transfer can be very important, often the most important factor is any layer of still air that is trapped in the packaging. Table 19.3 shows that still air has a lower thermal conductivity than any of the packaging materials. In many packages, there is an air layer of 0.5–2 mm thickness around most of the product in the package, with a larger air gap on top.

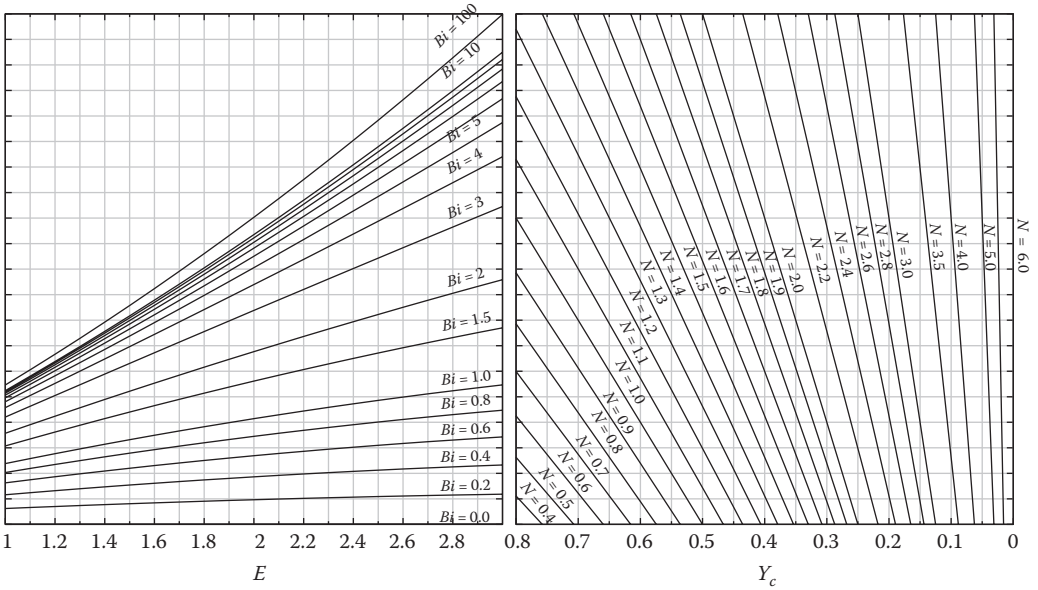

FIGURE 19.6 Alignment chart to calculate N, the number of half-lives for the center temperature from E, Y_c, and Bi.

TABLE 19.3

Typical Thermal Conductivities (W/m K) of Packaging Materials and Still Air

Solid cardboard	0.06–0.10
Plastic film	0.08–0.15
Corrugated cardboard	0.04–0.06
Still air	0.03

The overall effect of the packaging on the heat transfer coefficient can be calculated from

$$\frac{1}{h} = \frac{1}{h_{\text{surface}}} + \frac{x_{\text{package}}}{k_{\text{package}}} + \frac{x_{\text{air}}}{k_{\text{air}}} \tag{19.18}$$

where

h is the heat transfer coefficient to use in your calculations
h_{surface} is the surface heat transfer coefficient
k_{package} is the thermal conductivity of the package from Table 19.3
x_{package} is the packaging material thickness (m)
k_{air} is the thermal conductivity of still air from Table 19.3
x_{air} is the thickness of the still air layer (m)

In practice, the surface heat transfer coefficient varies significantly between different surfaces of the product, and even between different parts of the same surface. The most important common example is in a carton with an air gap under the top surface. The heat transfer coefficient under that surface can be much lower than it is under surfaces with little or no air gap. The simple methods for estimating cooling times only use one heat transfer coefficient, however, so they do not account for this sort of variation. One solution that is often satisfactory is to use a weighted average heat transfer coefficient rather than any single value. A rough guideline is to estimate the upper and lower surface heat transfer coefficients and use the average of the two, ignoring the coefficients of the carton sides and ends.

19.6.8 Worked Examples of Chilling Time Prediction

Example 1

Calculate the time required to chill a carton containing a 160 mm thick layer of lean beef, initially at 36°C, to a center temperature of 2°C in a forced convection air chiller set to a temperature of −5°C and air velocity of 2 m/s. The carton is constructed of a single layer of 3 mm thick corrugated cardboard and has a 6 mm air gap at the top.

Therefore, $X = 160$ mm^2 = 0.08 m; $T_i = 36$°C; $T_c = 2$°C; $T_a = -5$°C; $v = 2$ m/s; $x_{\text{package}} = 0.003$ m; $x_{\text{air}} = 0.006$ m.

Step 1. Find thermal properties in the literature or estimate using Equations 19.4 through 19.6: Assuming for lean beef that $W = 0.70$, $S = 0.20$, $F = 0.10$, then

Using Equation 19.4

$$\rho_f = \frac{1}{0.70/1000 + 0.20/1300 + 0.10/850} = 1029.3 \text{ kg/m}^3$$

Using Equation 19.5

$$k_f = 1029.3\left(\frac{0.58}{1000}0.70 + \frac{0.25}{1300}0.20 + \frac{0.18}{850}0.10\right) = 0.479 \text{ W/mK}$$

Using Equation 19.6

$$C_l = 4180 \times 0.70 + 1400 \times 0.20 + 1900 \times 0.10 = 3396 \text{ J/kg K}$$

Step 2. Calculate the heat transfer coefficient (h).
Using Equation 19.7, for a flat surface with assumed carton length of 0.55 m

$$h_{\text{surface}} = 6.1 \times 0.55^{-0.2} \times 2^{0.8} = 12.0 \text{ W/m}^2\text{K}$$

Using Table 19.3, $k_{\text{package}} = 0.05$ W/m K; $k_{\text{air}} = 0.03$ W/m K.
Using Equation 19.18 to account for the packaging and air gap at the top of the carton:

Top surface, h_{top}

$$\frac{1}{h} = \frac{1}{12} + \frac{0.003}{0.05} + \frac{0.006}{0.03} = 0.343; \quad \text{so } h_{\text{top}} = 2.91 \text{ W/m}^2\text{K}$$

Bottom surface, h_{bottom}

$$\frac{1}{h} = \frac{1}{12} + \frac{0.003}{0.05} + \frac{0}{0.03} = 0.143; \quad \text{so } h_{\text{bottom}} = 6.98 \text{ W/m}^2\text{K}$$

Average of h_{top} and h_{bottom}

$$h = \frac{2.91 + 6.98}{2} = 4.94 \text{ W/m}^2\text{K}$$

Step 3. Calculate *Bi*.
Using Equation 19.3

$$Bi = \frac{4.94 \times 0.08}{0.479} = 0.825$$

Step 4. Calculate the $Fo_{1/2,\text{ carton}}$.
Using Figure 19.4, for slab with $Bi = 0.825$

$$Fo_{1/2,\text{slab}} = 1.08$$

Using Table 19.2, for beef side in air blast

$$E = 1.4$$

Using Equation 19.15

$$Fo_{1/2,\text{carton}} = \frac{1.08}{1.4} = 0.771$$

Step 5. Calculate the half cooling time.
Using Equation 19.13

$$t_{1/2} = \frac{0.771 \times 1029.3 \times 3396 \times 0.08^2}{0.479} = 36{,}009 \text{ s}$$

Step 6. Calculate the fraction unaccomplished temperature change (Y_c) when the center reaches 2°C.
Using Equation 19.9

$$Y_c = \frac{2 - (-5)}{36 - (-5)} = 0.1707$$

Step 7. Estimate the number of half-lives (N).

Using Figure 19.6 because center temperature is required, for $E = 1.4$, $Bi = 0.825$, and $Y_c = 0.1707$

$$N \approx 2.7$$

Step 8. Calculate the total chilling time.

Using Equation 19.14

$$t = 2.7 \times 36{,}009 = 97{,}224 \text{ s} = 27.0 \text{ h}$$

Example 2

Calculate the time required to chill a carton of lean beef to an average temperature of 7°C under the same conditions as in Example 1.

Steps 1 through 5. Same as for Example 1.

Step 6. Calculate the fraction unaccomplished temperature change (Y_{av}) when the average temperature reaches 7°C.

Using Equation 19.10

$$Y_{av} = \frac{7 - (-5)}{36 - (-5)} = 0.2927$$

Step 7. Estimate the number of half-lives (N).

Using Figure 19.5 because average temperature is required, for $Y_c = 0.2927$

$$N \approx 1.8$$

Step 8. Calculate the total chilling time.

Using Equation 19.14

$$t = 1.8 \times 36{,}009 = 64{,}816 \text{ s} = 18.0 \text{ h}$$

19.6.9 Chilled Product Heat Loads

The product chilling heat load is the total amount of heat that must be removed from the product spread over the length of the operation. In practice, however, because the temperature difference between the product and the cooling medium is high at the start of chilling and low at the end, the heat load will also be greater at the start than at the end. There are methods that can be used to take all of this into account and to calculate a smooth heat load profile, but the simplest technique for hand calculation is to use the average-temperature cooling times as shown in the previous section.

As an example, to chill a beef side with a heat capacity of about 4000 J/kg K in air at 0°C from an average temperature of 40°C to 5°C, the total amount of heat to be removed would be $(T_1–T_2) \times C_1$, that is, (40–5°C) × 4000 J/kg K = 140,000 J/kg. Assuming this heat is to be removed over a period of about 24 h (86,400 s), the average heat load would be 140,000 J/kg/86,400 s = 1.62 W/kg.

If this 24 h process took three half lives ($N = 3$), it would mean that the average temperature would drop half-way from 40°C to 0°C (i.e., to 20°C) after 8 h; it then dropped three-quarters of the way to 0°C (i.e., to 10°C) after 16 h; and then dropped seven-eighths of the way to 0°C (i.e., to 5°C) after 24 h. So, the amount of heat removed during the first 8 h would be (40–20°C) × 4000 J/kg K = 80,000 J/kg. This was a period of 28,800 s, so the average heat load during the first 8 h would be 80,000 J/kg/28,800 s = 2.78 W/kg.

During the second 8 h, the temperature would drop from 20°C to 10°C, so the heat removed during this time would be (20–10°C) × 4000 J/kg K = 40,000 J/kg. The average heat load during this period would be 40,000 J/kg/28,800 s = 1.39 W/kg. During the last 8 h, the temperature would drop from 10°C to 5°C, so the heat removed during this time would be (10–5°C) × 4000 J/kg K = 20,000 J/kg. The average heat load during this period would be 20,000 J/kg/28,800 s = 0.69 W/kg.

To check that this calculation is correct, we can add up the amounts of heat removed in each third of the process: 80,000 W/kg + 40,000 J/kg + 20,000 J/kg = 140,000 W/kg. This is the same as the total amount of heat removed for the whole process, so the calculation was correct, assuming that the parameters were correct.

Although these results were given in terms of W/kg, they can be calculated for the whole chiller by multiplying by the number of sides in the chiller and by the weight per side.

In the example above, the heat load in the chiller was calculated at three points in time (after 1, 2, and 3 half-lives) because this happened to be convenient. The average heat load can, however, be calculated over any period during a chilling process as long as we know the average temperature at the beginning and the end of that period. To estimate the average temperature part way through a half-life, we can use the following equation to find Y_{av}:

$$Y_{av} = 0.5^N \tag{19.19}$$

To calculate the instantaneous heat load at the start of the cooling process, we would need to know the product surface area in addition to the other factors that have been discussed above, and we can then calculate the initial heat load from:

$$Q = h A_{\text{surface}} (T_{\text{surface}} - T_a) \tag{19.20}$$

where

Q is the initial heat load (W)
A_{surface} is the meat surface area (m^2)
T_{surface} is the surface temperature of the meat (°C)
T_a is the ambient temperature (°C)

So for the example above, if we assume an air velocity of 1 m/s and a thickness through the thermal center of 0.21 m (and hence a heat transfer coefficient of 12.5 W/m^2K using Equation 19.8), a surface area of 2 m^2/side and 200 sides in the chiller, then the initial heat load Q = 12.5 $W/m^2K \times 200$ sides $\times$ 2 m^2/side $\times$ (40–0°C) = 200,000 W, or 200 kW for this chiller.

In addition, to the product load in a chiller, there will also be loads due to other factors. These include

- Heat infiltration through the walls, floor, and ceiling
- Warm air entering through open or unsealed doors
- Heat load due to fans, lights, and machinery (e.g., forklifts)
- Heat loads due to people in the chiller
- Heat loads due to the structures in the chiller

The total heat load to be removed from a chiller is the sum of the product heat load (which varies with time as the chilling process progresses) and these additional loads, most of which do not vary over time. Cleland (1990) describes in detail how these other heat loads can be calculated. It is important to minimize these additional heat loads to ensure that the refrigeration system has as much capacity as possible to handle the product heat load and thereby achieve the desired result.

19.7 The Freezing Process

Freezing is the process of removing heat so that the water in meat is converted into ice. To make this change from liquid water to ice, a large amount of heat must be removed in addition to the amount that is required to change the temperature. This extra amount is known as the *latent heat of freezing.* When a pure substance freezes, this latent heat is removed without any change in temperature. Meat is not a pure substance, however, so the latent heat is removed over a temperature range.

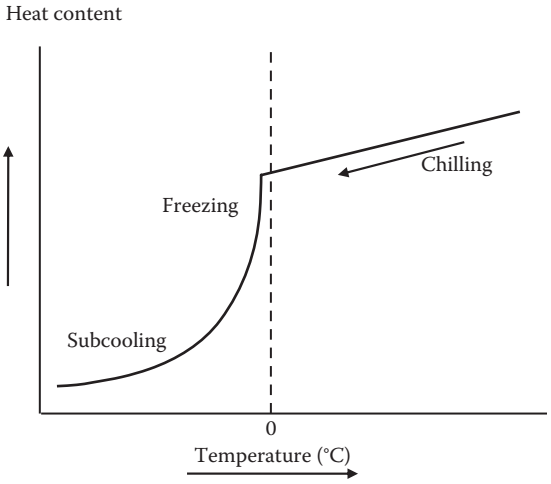

FIGURE 19.7 The variation in heat content (enthalpy) of meat with temperature.

Figure 19.7 shows that the amount of heat removed during freezing is quite a lot more than the amount removed during chilling to effect a given temperature change. This means that the freezing part of the process will take longer than the chilling part if the same temperature difference and heat transfer coefficient are used.

Eventually, freezing can be regarded as finished and the meat moves into the final stage of the process, known as subcooling. At this point, most of latent heat will have been removed.

19.7.1 The Freezing Front

Meat does not start to freeze until its surface temperature drops below the initial freezing temperature of the meat. At that time, the piece of meat will start to freeze from the outside toward the inside. The surface layer freezes first and then the frozen layer will become thicker over time. The inner boundary of the frozen layer is known as the freezing front. This freezing front moves gradually inwards toward the center of the meat, with frozen meat on the outside and unfrozen meat on the inside, as shown in Figure 19.8.

Once the freezing front has started moving, the temperature of the unfrozen region will usually have dropped to the freezing temperature of the meat. This means that the temperature at the thermal center of the meat remains constant for most of the freezing process, at the freezing temperature of the meat, a little below 0°C. One consequence of this behavior is that it is difficult to calculate the extent to which a piece of meat is frozen by measuring its thermal center temperature unless the meat is almost completely frozen and the thermal center temperature has already started to drop below the freezing temperature.

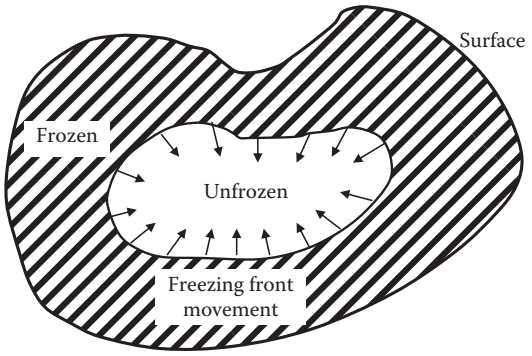

FIGURE 19.8 The freezing front.

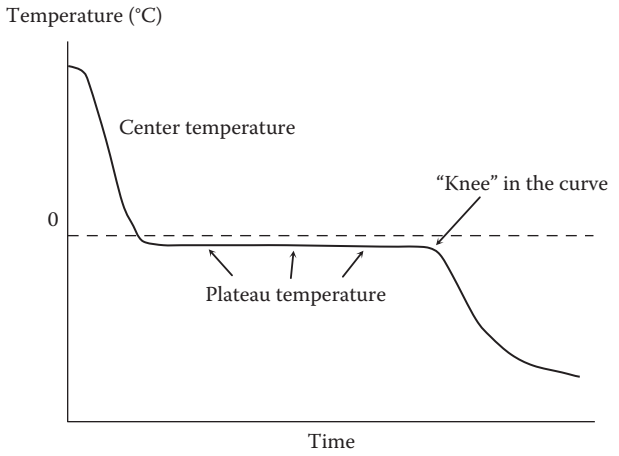

FIGURE 19.9 Center temperature during freezing.

If we measure the temperature of a point that is not actually at the thermal center, the plateau temperature that we measure will equal the freezing temperature of the meat early in the freezing process. However, the temperature at a noncentral point will start to drop below the freezing temperature before the center temperature begins to drop. In addition, a noncentral point will drop in temperature more slowly than the center point because it is not just releasing its own heat content outward into the rest of the meat (and hence into the surroundings), but it has other heat passing through it from deeper in the meat. As a consequence, the "knee" in the temperature plot is sharper as the measurement position is moved closer to the center of the meat. Figure 19.9 shows a typical center temperature–time profile during meat freezing, indicating how the freezing time is well-defined if the temperature is measured at this position. The bottom part of the tail on Figure 19.9 indicates where the meat temperature is becoming close to the air temperature and so the rate of cooling, and therefore the rate of temperature decrease, has slowed.

19.7.2 Quality Considerations

Under normal freezing conditions, the water content of meat forms discrete ice crystals around nucleation sites. The sizes of these crystals depend upon the rate of freezing. If the freezing is done quickly, then the ice crystals will stay small. If the freezing is done more slowly, the crystals grow (Reid 1983).

Ice crystals can grow within and between meat cells. The crystals have the potential to cause mechanical damage to cell membranes and the concentration of solutes into unfrozen portions of the meat during the freezing process can damage proteins, causing denaturation. These effects may lead to "drip loss" during thawing, which is unattractive to customers and causes the defrosted meat to lose weight (Sahagian and Goff 1996).

The process of freezing aged meat, if sufficiently rapid, generally does not in itself have any demonstrable effect on the cooked color, flavor, odor, or juiciness of that meat (Petrović and others 1993). Indeed, the process of thawing can result in a certain amount of tenderization (Lagerstedt and others 2008).

Most nutrients are retained during freezing and subsequent storage. Soluble proteins can be lost in the drip during thawing, but often the fluid loss is similar to the amount of fluid lost when fresh meat is cooked, anyway.

The fastest freezing rates are associated with the least damage because they result in small ice crystal sizes and they do not provide an opportunity for the chemicals dissolved in the moisture content of the meat to move from their original locations. On the other hand, a slow freezing rate does allow the solutes to move away from the growing ice crystals. This results in ice crystals that are composed of almost pure water rather than the solution of water and various chemicals that normally makes up the moisture in the meat.

Even if the meat is frozen quickly to start with, a long period of cold storage will allow the ice crystals to grow to a large size (Reid 1983). Ice crystal growth occurs more quickly at higher storage temperatures, and is further encouraged by temperature fluctuations.

19.7.3 Thermal Properties

As for chilling, it is important to know the thermal properties of any meat for which you want to calculate a freezing time or heat load. Thermal property data can be obtained from the same sources as for chilled meat data. Thermal properties can also be calculated in the same way as for chilled meat from W, S, and F (the fractional water, solids, and fat contents). However, there are more parameters to calculate in the case of freezing. The ice fraction (I) is important, as is the composition of the solids, given by X_a, X_p, and X_o (the fractional ash, protein, and "other" contents). Typically the solid content of unsalted meats is 90% protein (i.e., $X_p = 0.9\ S$) with the remainder made up of ash and "other" components.

The initial freezing temperature, T_f (°C) can be estimated from (Pham 1996):

$$T_f = -4.66\frac{X_o}{W} - 46.4\frac{X_a}{W} \tag{19.21}$$

Most unsalted meats have an initial freezing temperature of −0.6 to −1.2°C. The ice fraction when fully frozen, I, can be estimated from (Pham 1996):

$$\frac{W - I}{W} = 0.342(1 - W) - 4.51X_a + 0.167X_p \tag{19.22}$$

Ice is less dense than water, so the density of frozen meat, ρ_f (kg/m^3) is a little less than that of unfrozen meat:

$$\rho_f = \frac{1}{W - I/\rho_{lW} + I/\rho_{fW} + S/\rho_S + F/\rho_F} \tag{19.23}$$

where

$\rho_{fW} \approx 916$ kg/m^3

The thermal conductivity of frozen meat, k_f (W/m K) can be estimated from

$$k_f = \rho_f\left(\frac{k_{lW}}{\rho_{lW}}(W - I) + \frac{k_{fW}}{\rho_{fW}}I + \frac{k_S}{\rho_S}S + \frac{k_F}{\rho_F}F\right) \tag{19.24}$$

where

$k_{fW} \approx 2.25$ W/m K

The heat capacity of frozen meat, C_f (J/kg K) can be estimated from

$$C_f = C_{lW}\ (W - I) + C_{fW}\ I + C_s\ S + C_F\ F \tag{19.25}$$

where

$C_{fW} \approx 2027$ J/kg K

The latent heat of freezing of a meat product is released over a range of temperatures, rather than at a single temperature as is the case with a pure substance (such as water). The total amount of latent heat change due to freezing, L (J/kg), can be estimated by

$$L = 333{,}600\ I \tag{19.26}$$

Note that the ice fraction when the meat is fully frozen is not equal to the moisture content. Some of the water within the meat is bound so tightly to the proteins that it never freezes no matter how cold the meat gets. This is called the "bound water" content of the meat.

19.7.4 Predicting Freezing Times

Predicting the chilling time of meat is made quite difficult by the changing temperature profile within the product. However, during freezing, the temperature profile in the meat is much simpler. Inside the freezing front, the meat is unfrozen, but it can be assumed to be at its freezing temperature. Outside the freezing front, the meat is already frozen and its temperature is gradually dropping toward the temperature of the surroundings. Most of the heat to be removed is latent heat and this is released from a given part of the meat as the freezing front passes through that part.

The task of predicting the freezing time is therefore simplified to the task of predicting how long it will take for the freezing front to get from the surface of the meat to the center, and then adding factors to account for the initial chilling period and the final subcooling period. Both of these periods are typically short compared to the freezing period, as can be seen from Figure 19.9, so there is no need to predict their lengths very accurately as long as the length of the freezing period is well predicted.

Equation 19.27 is an adaptation of an equation developed by Pham (1986) and can be expected to predict the freezing time of meat to within ±15%.

$$t_f = \frac{1}{E}\left(\frac{\Delta H_1}{\Delta T_1} + \frac{\Delta H_2}{\Delta T_2}\right)\left(\frac{X}{h} + \frac{X^2}{2k_f}\right) \tag{19.27}$$

where

$$\Delta H_1 = \rho_l C_l (T_{in} - T_{fm}) \tag{19.28}$$

$$\Delta H_2 = \rho_l L + \rho_f C_f (T_{fm} - T_c) \tag{19.29}$$

$$\Delta T_1 = \frac{T_{in} + T_{fm}}{2} - T_a \tag{19.30}$$

$$\Delta T_2 = T_{fm} - T_a \tag{19.31}$$

$$T_{fm} = 1.8 + 0.263\,T_c + 0.105\,T_a \tag{19.32}$$

where

t_f is the freezing time (s)
E is the equivalent heat transfer dimensionality
X is the shortest distance from the thermal center to the surface (m)
h is the surface heat transfer coefficient (including packaging) (W/m^{2}K)
k_f is the frozen meat thermal conductivity (W/m K)
ΔH_1 is the heat released during chilling (J/m^3)
ΔH_2 is the heat released during freezing and subcooling (J/m^3)
ΔT_1 is the temperature-driving force during chilling (°C)
ΔT_2 is the temperature-driving force during freezing and subcooling (°C)
ρ_l, ρ_f are the densities of unfrozen and frozen meat (kg/m^3)
C_l, C_f are the heat capacities of unfrozen and frozen meat (J/kg K)
T_{in} is the initial temperature of the product (°C)

T_{fm} is the mean freezing temperature of the product (°C)
T_c is the final center temperature of the product (°C)
T_a is the temperature of the cooling medium (°C)
L is the latent heat of freezing the ice fraction (J/kg)

One problem that arises more frequently in freezing time prediction than in chilling time prediction is that of asymmetric heat transfer. Often, for instance, the heat transfer coefficient at the top of a meat carton is different from that at the bottom of the carton, due to the air gap that is intentionally left at the top to accommodate the meat as it freezes and expands.

Pham (1987) found a straightforward solution to this problem for slab-shaped food products. It applies quite well to products that are almost slab-shaped too, such as meat cartons in plate or air-blast freezers. When different h values apply at each side of a slab, the freezing fronts progress inwards at different rates. Instead of meeting in the geometric center of the meat product, the freezing fronts will meet at a point $X(1 - a)$ from the top and $X(1 + a)$ from the bottom. Now if

$$r = \frac{h_{\text{bottom}}}{h_{\text{top}}} \tag{19.33}$$

where

h_{bottom} and h_{top} are the heat transfer coefficients at the top and bottom of the carton, respectively.

. . . then the value of a is given by the following equation:

$$a = \frac{r - 1}{r + 1 + 2\,Bi_{\text{bottom}}} \tag{19.34}$$

where

Bi_{bottom} is the Biot number calculated with h_{bottom} and k_f.

Having calculated a, we can then calculate the freezing time for the product using Equation 19.27 but setting $h = h_{\text{bottom}}$ and $X = (1 + a)\,X_{\text{actual}}$, where X_{actual} is the depth of the thermal center for the actual product.

19.7.5 Worked Example of Freezing Time Prediction

Calculate the time required to freeze a carton containing a 160 mm thick layer of lean beef, initially at 4°C, to a center temperature of –12°C in an air blast freezer set to a temperature of –25°C and air velocity of 4 m/s. The carton is constructed of a single layer of 2 mm thick solid cardboard and has no air gap at the top.

Therefore, $X = 160$ mm$^2 = 0.08$ m; $T_i = 4$°C; $T_c = -12$°C; $T_a = -25$°C; $v = 4$ m/s; $x_{\text{package}} = 0.002$ m; $x_{\text{air}} = 0.006$ m.

Step 1. Find thermal properties in the literature or estimate using Equations 19.4 through 19.6 and 19.21 through 19.26:

Assuming for lean beef that $W = 0.70$, $S = 0.20$, $F = 0.10$, then the unfrozen thermal properties are the same as for Example 1 above ($\rho_l = 1029.3$ kg/m^3; $k_l = 0.479$ W/m K; $C_l = 3396$ J/kg K). We may also assume that the composition of the solids is ~90% protein, 5% ash, and 5% "other," giving $X_p = 0.18$, $X_o = 0.01$, and $X_a = 0.01$.

Using Equation 19.21

$$T_f = -4.66\,\frac{0.01}{0.70} - 46.4\,\frac{0.01}{0.70} = -0.73$$

Using Equation 19.22:

$$\frac{0.70 - I}{0.70} = 0.342(1 - 0.70) - 4.51 \times 0.01 + 0.167 \times 0.18 = 0.0876; \quad \text{so } I = 0.64$$

Using Equation 19.23

$$\rho_f = \frac{1}{(0.70 - 0.64)/1000 + 0.64/916 + 0.20/1300 + 0.10/850} = 970.7\,\text{kg/m}^3$$

Using Equation 19.24:

$$k_f = 970.7\left(\frac{0.58}{1000}(0.70 - 0.64) + \frac{2.25}{916}0.64 + \frac{0.25}{1300}0.20 + \frac{0.18}{850}0.10\right) = 1.62\ \text{W/mK}$$

Using Equation 19.25:

$$C_f = 4180 \times (0.70 - 0.64) + 2027 \times 0.64 + 1400 \times 0.20 + 1900 \times 0.10 = 2018\ \text{J/kgK}$$

Using Equation 19.26

$$L = 333{,}600 \times 0.64 = 213{,}504\ \text{J/k}$$

Step 2. Calculate the heat transfer coefficient (*h*).
Using Equation 19.7, for flat surface with assumed carton length of 0.55 m

$$h_{\text{surface}} = 6.1 \times 0.55^{-0.2} \times 4^{0.8} = 20.8\,\text{W/m}^2\text{K}$$

Using Table 19.3, $k_{\text{package}} = 0.08$ W/m K; $k_{\text{air}} = 0.03$ W/m K.
Using Equation 19.18 to account for the packaging and any air gap:

$$\frac{1}{h} = \frac{1}{20.8} + \frac{0.002}{0.08} + \frac{0}{0.03} = 0.073;\ \text{so } h = 13.7\,\text{W/m}^2\text{K}$$

Step 3. Calculate the mean freezing temperature of the product.
Using Equation 19.32

$$T_{fm} = 1.8 + 0.263 \times (-12) + 0.105 \times (-25) = -3.981$$

Step 4. Calculate the temperatur-driving force during freezing and subcooling.
Using Equation 19.31

$$\Delta T_2 = -3.981 - (-25) = 21.02°\text{C}$$

Step 5. Calculate the temperatur-driving force during chilling.
Using Equation 19.30

$$\Delta T_1 = \frac{4 + (-3.981)}{2} - (-25) = 25.01°\text{C}$$

Step 6. Calculate the heat released during freezing and subcooling.
Using Equation 19.29

$$\Delta H_2 = 1000 \times 213{,}504 + 970.7 \times 2018 \times \left(-3.981 - (-25)\right) = 2.547 \times 10^8\,\text{J/m}^3$$

Step 7. Calculate the heat released during chilling.
Using Equation 19.28

$$\Delta H_1 = 1029.3 \times 3396 \times \left(4 - (-3.981)\right) = 2.790 \times 10^7\ \text{J/m}^3$$

Step 8. Calculate the total freezing time.
Using Table 19.2, for beef side in air blast

$$E = 1.4$$

Using Equation 19.27

$$t_f = \frac{1}{1.4}\left(\frac{2.790 \times 10^7}{25.01} + \frac{2.547 \times 10^8}{21.02}\right)\left(\frac{0.08}{13.7} + \frac{0.08^2}{2 \times 1.62}\right) = 73{,}864\,\text{s} = 20.5\,\text{h}$$

19.7.6 Frozen Product Heat Loads

The total amount of heat released during freezing can be calculated from the following equation:

$$\Delta H = \frac{\Delta H_1}{\rho_l} + \frac{\Delta H_2}{\rho_f} \tag{19.35}$$

where:
ΔH_1 is the heat released during chilling (J/m^3) from Equation 19.28
ΔH_2 is the heat from freezing and subcooling (J/m^3) from Equation 19.29
ρ_l, ρ_f are the densities of unfrozen and frozen meat (kg/m^3)
ΔH is the heat released during the whole process (J/kg)

The average product heat load is therefore given by

$$\varphi_f = \frac{\Delta H}{t_f} \tag{19.36}$$

where
φ_f is the average product heat load per kg of meat in the freezer (W/kg).

The average total heat load can be calculated by multiplying φ_f by the total weight of meat in the freezer.

Although this gives the average product heat load during freezing, it does not show how the load varies with time during the process. While product freezing times are easier to calculate than product chilling times, unfortunately the reverse is true for product heat loads, at least for hand calculation. To estimate the freezing product heat load at any given time during the process, it is necessary to trace the position of the freezing front all of the way from the surface to whatever position it has reached by that time. This can be done in a computer program using one of several different techniques (e.g., Lovatt and others 1992), but it is laborious to do by hand, so will not be covered here.

Although it is difficult to calculate accurately, the product heat load during freezing follows a similar pattern to the heat load during chilling—it starts off very high, drops sharply, and then declines over the rest of the process. The main difference between the freezing and chilling heat load profiles is that, while the chilling heat load profile forms a smooth curve throughout the process, the freezing heat load profile can become almost flat over time while the freezing front is moving and then drops away once the freezing front has moved close to the center.

The sources of additional heat loads in freezers are the same as for chillers. However, because of the lower temperature inside a freezer, the heat load due to infiltration through the walls, floor, and ceiling is generally greater. Higher air velocities in air-blast freezers compared with chillers also result in the fan power being a more important heat load in freezers than in chillers. Heat loads from lights, machinery,

and people in a freezer are generally negligible in comparison to the large heat loads resulting from the product, heat infiltration, and fans.

19.8 Software to Estimate Cooling Times and Heat Loads

It is often more practical to use a software program to carry out the calculations outlined above for chilling and freezing rates and heat loads. Food Product Modeller™ and Refrigeration Loads Analyser™ (AgResearch Limited, New Zealand) are examples of two software packages that allow more complicated situations to be calculated and the temperature profiles and heat loads in all parts of the product to be displayed at any time during the cooling process.

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Irradiation of Meat

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20.1 Introduction

Foodborne diseases remain responsible for high levels of morbidity and mortality in the general populations but particularly for at-risk groups such as infants, young children, pregnant women, elderly, or immunocompromised peoples. The presence of microbial pathogens on human foods is a serious global problem. Even in highly industrialized and developed countries, pathogen-contaminated foods

and the resulting health and economic impacts are significant (Smith and Pillai 2004). According to the 2011 CDC estimates, 48 million people fall sick, 128,000 are hospitalized, and 3000 die from foodborne diseases in the United States (CDC 2010). Meanwhile, in the European Union, 190,566 confirmed human cases of campylobacteriosis, 131,468 cases of salmonellosis, and 3159 VTEC infections were reported in the year 2008 (EFSA 2010).

Meat is a rich nutrient matrix that provides an ideal environment for the growth and propagation of meat spoilage microorganisms and common foodborne pathogens (Aymerich and others 2008, Zhou and others 2010). Several massive recalls of ground beef as well as raw and other processed meat products, because of contamination with *Escherichia coli* O157:H7 or with *Listeria monocytogenes*, were reported in the United States. This resulted in losses of millions of dollars from destroyed products as well as from the recall itself, disposal of suspect products, and decreased consumer confidence (Molins 2001, USDA/FSIS 2010a,b). On the other hand, consumers demand high-quality, convenient, innovative, regular, and safe meat products with natural flavor and taste as well as an extended shelf life (Aymerich and others 2008).

Considering a series of outbreaks of pathogenic bacteria in meat, the expanded application of irradiation technology in meat and meat products becomes especially important to improve safety and public confidence (Lim and others 2008). The Center for Disease Control and Prevention estimated that irradiating half of all ground beef, poultry, pork, and processed meat would reduce food poisoning by 1 million cases and prevent 6000 serious illnesses and 350 deaths caused by the main microorganisms involved in foodborne infections, such as *E. coli* O157:H7, *Campylobacter*, *Salmonella*, *Listeria*, and *Toxoplasma*, only in the United States (Tauxe 2001). Irradiation is the most effective technology for inactivating pathogens in meat and meat-based products. In addition, irradiation can be performed at ambient or lower temperatures that guarantee better preservation of nutritive values and physicochemical properties of food products. A major advantage of irradiation is that the food can be processed after it has been sealed in its final packaging, thereby reducing or eliminating entirely the possibility of recontamination following treatment (Borsa 2006, O'Bryan and others 2008). There has been increasing recognition of the importance of irradiation in the food industry in both developed and developing countries. Food irradiation is approved for use in more than 55 countries worldwide for various applications and purposes in a wide variety of foodstuffs (IAEA 2009, Stefanova and others 2010).

20.2 Treatment of Food by Ionizing Radiation

Food irradiation is one of the few technologies that address both food quality and safety by virtue of its ability to control spoilage and foodborne pathogenic microorganisms. Foods are irradiated to provide the same benefits as they provide on being processed by heat, refrigeration, freezing, or treatment with chemicals, but irradiation has several advantages: (1) it does not significantly raise food temperature and the food does not "cook," (2) unlike chemical treatments, irradiation does not leave potentially harmful residues, and (3) it can be used to treat packaged foods, which will remain safe and protected from microbial contaminations after treatment (Blackburn 2011).

For commercial radiation processing of food products, three types of ionizing radiation are used. These are: radiation from high-energy γ-rays, x-rays, and accelerated electrons. Only three sources of ionizing radiation are authorized for food irradiation according to Codex General standard for Irradiated Food (Codex Alimentarius Commission 2003):

1. γ-Rays emitted by the radioactive elements Cobalt-60 (^{60}Co) or Cesium-137 (^{137}CS).
2. X-rays generated from machine sources operated at or below an energy level of 5 MeV.
3. Electrons generated from machine sources operated at or below an energy level of 10 MeV.

During the irradiation process, food is exposed to the ionizing energy source in such a way that a precise and specific dose is absorbed. The absorbed dose is the mean energy imparted by ionizing radiation to the matter in a volume element divided by the mass of the matter in that volume element. The dose of irradiation or the amount of absorbed radiation is measured in Gray (Gy) in the SI unit. 1 Gy is equal

to 1 joule (J) of absorbed energy per kilogram. 1 kilogray (kGy) equals 1000 Gy. Energies from the authorized radiation sources are too low to induce radioactivity in the irradiated food; thus, the irradiated food does not become radioactive.

20.3 Radiosensitivity of Microorganisms in Meat and Meat Products

The radiation resistance and sensitivity of the different species of microorganisms varied. The sensitivity of any microorganism to radiation is expressed in terms of D_{10}-value which is defined as the dose of radiation that kills 90% of microorganisms. The D_{10}-value provides a good indication of which microorganisms will remain following irradiation of a meat product. The D_{10}-values were determined by several authors for different microorganisms in different meat products at different conditions. Psychrotrophic bacteria were found to be extremely sensitive to irradiation. The D_{10}-value for *Pseudomonas fluorescens* was 0.12 kGy in low-fat ground beef (Maxcy and Tiwari 1973), while Lefebvre and others (1992) showed that *Pseudomonas* spp. (mainly responsible for spoilage) were radiosensitive in ground beef with a D_{10}-value of 0.035–0.153 kGy. Tarkowski and others (1984b) reported D_{10}-values of 0.15–0.40, and 0.16 kGy for strains of *Campylobacter jejuni* in ground beef. In another study, Clavero and others (1994) found that the D_{10}-values for *C. jejuni* were 0.24 ± 0.02 and $0.18 \pm$ kGy in raw low-fat ground beef (8.2–13% fat) at − 17 − 15°C or at 3–5°C, respectively. While in raw high-fat ground beef (26.8–27.1% fat), the D_{10}-values for the same organism were 0.21 ± 0.02 and 0.20 ± 0.02 kGy at −17 − 15°C or at 3–5°C, respectively.

Yersinia enterocolitica was highly susceptible to radiation injury. The D_{10}-values for this pathogen ranged between 0.10 and 0.21 kGy in ground beef at 3°C (Tarkowski and others 1984a), while its D_{10}-value was 0.25 kGy in a homogenate of 10% raw pork meat (Kamat and others 1997). Tarkowski and others (1984b) indicated that the D_{10}-value for *Salmonella typhimurium* in ground beef was 0.37 kGy. Clavero and others (1994) reported that the D_{10}-values for a mixture of *Salmonella* serovars averaged 0.64 kGy in ground beef irradiated at 2–5°C, whereas it ranged from 0.241 to 0.307 kGy for *E. coli* O157:H7. The authors indicated that *E. coli* O157:H7 may be more sensitive to irradiation than *Salmonella*. Elshenawy and others (1989) observed that the D_{10}-values for three isolates of *L. monocytogenes* ranged from 0.51 to 1.0 kGy in minced beef irradiated at 20–21°C. The D_{10}-value for a mixture of four pathogenic isolates of *L. monocytogenes* averaged 0.47 kGy in vacuum-packaged beef, pork, and lamb meats irradiated at 5°C as reported by Thayer and others (1995). In another study, Tarte and others (1996) observed that the D_{10}-values were 0.447, 0.424, 0.445, 0.372, and 0.638 kGy for *L. monocytogenes* NADC 2045 Scott A, *L. monocytogenes* NADC 2783, *L. monocytogenes* ATCC 15313, *L. ivanovi*, and *L. innocua* in ground pork, respectively. Grant and Patterson (1992) determined the D_{10}-value to be 0.93 kGy for *Staphylococcus aureus* NCTC 10655 in roast beef irradiated at a temperature of 0–3°C, while a study by Thayer and others (1995) showed that the D_{10}-values that required for inactivating *S. aureus* were 0.46 ± 0.02, 0.40 ± 0.03, and 0.43 ± 0.02 kGy, respectively, when inoculated onto sterile beef, lamb, and pork meats and irradiated at 5°C. Jo and others (2004) examined the D_{10}-value of *S. aureus*, *Bacillus cereus*, *S. Typhimurium*, and *E. coli* in raw marinated beef rib and found that they were 0.663 ± 0.01, 0.594 ± 0.05, 0.636 ± 0.02, and 0.538 ± 0.01 kGy, respectively. The D_{10}-value required for inactivating *B. cereus* vegetative cells in the components of a chilled ready meal containing roast beef was found to be 0.126–0.288 kGy (Grant and Patterson 1992). However, Thayer and Boyd (1994) observed that the D_{10}-value was found to be 2.78 kGy when they examined the radiation resistance of a mixture of the spores of six strains of *B. cereus* in ground beef, ground pork loin, and beef gravy. *Clostridium* spp. are very resistant to ionizing radiation. The radiation resistance of *Clostridium botulinum* spores, measured by D_{10}-value, varied between 3.36 and 2.24 kGy according to the meat substrate (Anellis and others 1965, 1967, 1969, 1972). Grecz and others (1971) reported that the D_{10}-value for *C. botulinum* E spores was found to be 3.45 – 3.60, 3.73–3.85, and 4.30–4.34 kGy at +25, 0, and −50°C in cooked beef, respectively. However, Barnhart (1977) found that the D_{10}-values for *Clostridium perfringens* cells and spores in ground beef were 0.3 and 2.5 kGy, respectively. The dose required for 12D inactivation of these bacteria were examined through several studies aimed at producing sterilized vacuum-packaged meat products for storage at room temperature. The process is applied to vacuum-packaged, enzyme-inactivated meat products, while they are deeply frozen.

20.4 Factors Influencing Radiosensitivity of Microorganisms in Meat and Meat Products

The sensitivity of microorganisms to ionizing radiation in meat products was found to be affected by many factors including the organism itself, components of meat and meat products, presence of oxygen (atmosphere in packages), temperature during irradiation, and dose rate.

20.4.1 The Organism

A broad spectrum of microorganisms exists with regard to radiation sensitivity. Radiation sensitivity of microorganisms differs with species and even with strain, although the range of resistance among strains of a single species is usually small enough to be ignored for potential purposes. Radiation resistance of microorganisms generally follows the sequence Gram-negative < Gram-positive ≈ molds < spores ≈ yeasts < viruses. The presence of large populations of microorganisms in the meat product being irradiated reduces the effectiveness of the applied irradiation dose. Thus, a number of microorganisms can affect their destruction by irradiation, and therefore, initial good microbiological quality for meat and meat products increases the effectiveness of their decontamination by irradiation. The physiological state of bacteria was found to have significant effect on its radiation resistance. The sensitivity of exponential-phase cells to irradiation is higher than that of lag-phase or stationary-phase cells.

Adaptation of bacteria to different stresses significantly affects their radiation resistance. It has been illustrated that *E. coli* has a capability to develop an increased acid resistance due to preincubation of cells at sublethal low pH. The increased acid resistance of *E. coli* O157:H7 results in cross-protection against ionizing radiation (Benjamin and Datta 1995, Buchanan and others 1999, Leyer and others 1995, Lin and others 1996). Therefore, growing this potential meatborne pathogen under conditions that induce a pH-dependent stationary phase would also result in higher radio-resistance (Buchanan and others 1999). In addition, Yuk and Marshall (2003) showed that preincubation of *E. coli* O157:H7 at sublethally high temperature of 45°C induced heat adaptation and consequent increased radiation resistance. Furthermore, preincubation of this organism under sublethally high ionic strength (salt content) in meat products induced cross-protection, leading to an increased radio-resistance to electron-beam irradiation (Black and Jaczynski 2007). A recent study by Levanduski and Jaczynski (2008) showed that *E. coli* O157:H7 has a capability to develop increased resistance to electron-beam irradiation if the products are repetitively subjected to e-beam processing at sublethal levels. These authors indicated that the D_{10}-values for this pathogen that inoculated in ground beef and repetitively subjected to e-beam resulted in a significant increase from 0.24 ± 0.03 to 0.63 ± 0.02 kGy. Following four cycles of e-beam processing, the microorganisms were able to resist doses of 3 kGy.

20.4.2 Meat Components

The composition of meat may affect the radiation resistance of microorganisms. Its high protein content may provide protection for microorganisms against the radiation destruction effect through the neutralization of free radicals. In addition to proteins, other meat constituents such as natural antioxidants are present, and all can compete for free radicals, produced by the radiolysis of water, leading to decreases in the radiation antimicrobial efficacy (Diehl 1995). On the other hand, irradiation of meat may produce free fatty acids, carbonyl compounds, hydrogen peroxide, and hydroperoxides from fats. The produced hydrogen peroxide and other oxygen products should increase the microbial destruction effect of irradiation in the presence of fat (Diehl 1995). However, studies have shown that fat levels in meat had no significant effect on the extent of microbial destruction by irradiation (Clavero and others 1994, Monk and others 1994, Thayer and others 1995). The ineffectiveness of fat levels to influence the radio-resistance of certain meatborne pathogens may be due to the other protective constituents such as proteins (Diehl 1995). A study by Sommers and Fan (2002) showed that dextrose at concentrations up to 8% had no effects on the radiation resistance of *L. monocytogenes* in beef bologna, although irradiation of dextrose may result in the production of peroxides (Kawakishi and others 1971) that can theoretically contribute to microbial inactivation during irradiation of meat products containing sweeteners.

20.4.3 Atmosphere in Packages

The atmosphere in package can affect microbial destruction by irradiation as well as postirradiation recovery in the irradiated meat product. The presence of oxygen increases the microbial destruction by irradiation. However, Hastings and others (1986) reported that various lactobacilli strains that inoculated into minced meat were more sensitive to irradiation when meat packaged under 100% CO_2 than packaging under vacuum, air, or nitrogen, and packaging in nitrogen seemed to increase the microbial radioresistance. The D_{10}-values were determined for two *Y. enterocolitica* isolates on pork packaged with air or under modified atmosphere (25% CO_2 and 75% N_2). Modified atmosphere significantly increased the radiation resistance of one of the isolates from 0.164 to 0.176 kGy (Grant and Patterson 1991a). Other studies, however, indicated that packaging atmosphere (under air versus vacuum) had no effects on the survival of *E. coli* O157:H7 during irradiation of meat and ground beef (Fu and others 1995b, Thayer and Boyd 1993) or showed little effect (López-González and others 1999). It has been suggested that more complete information is needed to optimize the use of vacuum packaging or modified atmosphere packaging combined with irradiation for insuring the microbial safety of fresh meats (Lee and others 1996).

20.4.4 Temperature during Irradiation

The temperature of meat during irradiation appeared to be an important factor influencing the extent of microbial destruction by irradiation. Irradiation of ground beef at temperatures of 25°C and –35°C showed an increased D_{10}-value for *Y. enterocolitica* from 0.195 to 0.388 kGy, respectively (El Zawahry and Rowley 1979). Another study by Sommers and others (2002) showed that the D_{10}-value for a mixture of four *Y. enterocolitica* strains inoculated into ground pork increased as product temperature decreased with values of 0.19, 0.19, 0.21, 0.40, 0.40, 0.38, and 0.55 kGy at irradiation temperatures of +5°C, –5°C, –10°C, –15°C, –20°C, and –70°C, respectively. Lag-phase cells of *C. jejuni* had D_{10}-values of 0.17, 0.16, and 0.32 kGy in ground beef irradiated at temperatures of 30 ± 10, 0–5, and -30 ± 10°C, respectively (Lambert and Maxcy 1984). Thayer and Boyd (1994) observed that vegetative cells of *B. cereus* showed a greater radiation resistance when irradiated in meats at a temperature of –20°C than irradiation at +20°C, whereas the effect of temperature on the radiation resistance of spores was small. Monk and others (1994) indicated that irradiation temperature did not influence the D_{10}-value of *L. monocytogenes* in frozen and refrigerated ground beef patties. In contrast, Thayer and Boyd (1995) observed a D_{10}-value of 0.45 kGy for *L. monocytogenes* in refrigerated ground beef (+5°C) versus a D_{10}-value of 1.21 kGy in frozen ground beef (–20°C) showing increased radiation resistance as the radiation temperature decreased. Several studies regarding radiation resistance of *E. coli* O157:H7 showed temperature as the main factor affecting the radio-resistance of this pathogen and survival was much higher at subfreezing temperatures (Clavero and others 1994, Fielding and others 1994, Fu and others 1995b, Thayer and Boyd 1993). The D_{10}-values of *E. coli* O157:H7 were found to be 0.28 and 0.44 kGy in beef irradiated at temperatures of +5 and –5°C, respectively (Thayer and Boyd 1993), 0.41 and 0.62 kGy in ground beef patties irradiated at temperatures of +5 and –15°C, respectively (López-González and others 1999), and 0.39, 0.98, and 1.11 kGy in ground beef irradiated at temperatures of +4, –20, and –76°C, respectively (Thayer and Boyd 2001). In their study, Thayer and Boyd (2001) added that the D_{10}-values of *S. aureus* also increased in the frozen ground beef being 0.51 ± 0.02 and 0.88 ± 0.05 kGy at 0°C and –20°C, respectively. The general observation was that microorganisms exhibit a greater radiation resistance on irradiation at subfreezing temperatures. Freezing reduces the water activity in meat due to converting the water into ice, which in turn, increases the microbial radiation resistance by drastically reducing the generation of free radicals from radiolysis of water (Diehl 1995). Furthermore, beyond the limited production of free radicals, frozen state of meat impedes the migration of free radicals to other parts of the frozen meat product (Taub and others 1979).

20.4.5 Dose Rate

The microbial destruction by ionizing radiation depends on the total absorbed dose. Large microbial populations need higher doses of radiation for their inactivation. Generally, microbial death from exposure

of populations to ionizing irradiation is logarithmic in nature. Thus, when suspension of microorganisms is exposed to a consistent flux of lethal-ionizing radiation, a constant fraction of the population will be killed at equal time intervals, regardless of the total number (ICMSF 1980). The dose rate may be a factor affecting the microbial sensitivity to irradiation; however, little is known about the effect of dose rate on microbial destruction in meat and meat products. In one study, a greater D_{10}-value for *E. coli* O157:H7 was observed in irradiated hamburger patties when the energy source was γ-rays than when the source was electron beams (which has a greater dose rate than γ-rays), suggesting that the effect of dose rate may be of importance (López-González and others 1999). In contrast, another study by Fu and others (1995b) showed that dose rate had no significant effect on the radio-resistance of *E. coli* O157H:7.

20.5 Current Regulations for Irradiation of Meat and Meat Products in the United States

For control of foodborne pathogens in, and extension of the shelf life of, refrigerated or frozen, uncooked meat products within the regulation of 9CFR301.2 (rr), meat by-products within the regulation of 9CFR301.2 (tt), or meat food products within the regulation of 9CFR301.2 (uu), with or without nonfluid seasoning, which are otherwise composed solely of intact or ground meat, meat by-products, or both meat and meat by-products. The dose should not exceed 4.5 kGy maximum for refrigerated products, and should not exceed 7.0 kGy maximum for frozen products according to USDA-FDA (2003).

20.6 Potential Application of Meat Irradiation

Meat and meat products may be irradiated for three objectives: (1) elimination of meatborne pathogens so that there is no potential health hazard (radicidation); (2) reduction of the number of spoilage microorganisms sufficiently to extend their shelf life (radurization); and (3) producing sterilized shelf-stable products, in which spoilage does not occur regardless of storage conditions (radappertization). Each of these objectives imposes a distinctly different radiation dose and processing requirement.

20.6.1 Destruction of Pathogens (Radicidation)

20.6.1.1 Bacteria

The application of irradiation treatment for inactivation of specific pathogens in meat and meat products has been extensively investigated. Lebepe and others (1990) stated that naturally occurring *L. monocytogenes* could survive and multiply in vacuum-packaged fresh pork loins irradiated at 3 kGy and stored at 2–4°C, while *Yersinia* spp. survived but did not multiply in the same pork loins. Meanwhile, Fu and others (1995b) observed that irradiation at a dose of 2 kGy reduced the contents of *Y. enterocolitica* inoculated at 10^5 onto the surface of beef steak to undetectable levels. Irradiation dose of 1–3 kGy, however, was found to be more adequate for control of *E. coli* O157:H7 and other radiation-sensitive nonsporeforming pathogens on chilled meat cuts (Thayer and Boyd 1993). Kanatt and others (1997) showed that Enterobacteriaceae and faecal coliforms were not detected in buffalo and lamb meats irradiated at 2.5 kGy and stored at 0–3°C, while these bacteria were present and their counts increased during storage of the control samples. They added that *Staphylococcus* spp. had an initial log cfu/g of 4.45 and increased to 6.15 log cfu/g after 2 weeks of storage in the control nonirradiated meats. However, *Staphylococcus* spp. were not detected in the irradiated meat during storage up to 3 weeks, and during the fourth week colonies were recovered at levels of 2.9 log cfu/g but were not potentially pathogenic. Jo and others (2004) investigated the efficacy of irradiation treatment for inactivating four pathogens of public health significance (*S. aureus*, *B. cereus*, *S. typhimurium*, and *E. coli*) in samples of radiation-sterilized marinated beef rib that inoculated at 10^6 cfu/g with each of the studied pathogens. They observed that the studied inoculated pathogens were not detected during refrigerated storage of samples irradiated at 4 kGy. However, a dose of 5 kGy may not be enough to control the remaining viable cells

from multiplying in the raw marinated beef rib when the storage temperature is not maintained (20°C). Dempster and Lahola (1983) examined the effect of irradiation at doses of 0.5 and 1 kGy on *S. aureus*, *E. coli*, and *Streptococcus faecalis* when inoculated into ground beef at levels of 3.5 and 2.5 $\log_{10}$ cells/g. They found that irradiation at 1 kGy dose reduced the counts of *S. aureus* from $\log_{10}$ 3.5 cells/g to 1.5 cells/g, while the applied dose completely eliminated this pathogen when its initial inoculum was $\log_{10}$ 2.5 cells/g. However, the lower irradiation dose was ineffective against *S. aureus*. The 1 kGy dose also eliminated the inoculated *E. coli* at both levels. *S. faecalis*, however, was the most radio resistant for which 0.5 kGy dose had no effect, while the dose of 1 kGy reduced its counts by only a fraction of a $\log_{10}$ cycle. In another study by Dempster and others (1985), *Salmonella* and *Staphylococci* were eliminated by irradiation of beef burgers at doses of 1 and 1.5 kGy, whereas a low number of Enterobacteriaceae survived and remained viable in the irradiated samples, but they did not increase in number. Irradiation of ground beef at a dose of 0.75 kGy reduced an average inoculum of $10^{4.8}$ cfu/g to <10 cfu/g, whereas survivors were not detected in samples irradiated at a dose of 1.5 kGy even after temperature abused at 35°C for 24 h and no measurable verotoxin was found (Thayer and Boyd 1993). Clavero and others (1994) showed that γ-irradiation at 2.5 kGy dose sufficiently reduced the counts of *E. coli* O157:H7, *Salmonella*, and *C. jejuni* in ground beef patties by ~8, ~3, and ~10 log cycles, respectively. A study by Fu and others (1995b) indicated that irradiation at a dose of 2 kGy reduced the counts of *Y. enterocolitica* inoculated at 10^5 onto ground beef to an undetectable level. Furthermore, no viable counts were observed for aerobic bacteria, total coliforms or *E. coli* during storage of beef patties irradiated at doses of 5 and 10 kGy using electron beam (Wong and others 2005). Another study by Schilling and others (2009) showed that electron beam and x-ray irradiation dose of 2 kGy inactivated *E. coli* O157:H7 inoculated in frozen ground beef at 10^3 cfu/g to below the limit of detection. Tarkowski and others (1984a,b) found that irradiation of inoculated "filet Americain" (raw meat + mayonnaise) and raw beef at a dose of 1 kGy reduced the number of *Salmonella* by 1.6–2.7 $\log_{10}$ cycles and 1.3–1.8 $\log_{10}$ cycles in filet Americain and raw beef, respectively, while reduced the count of *Y. enterocolitica* and *C. jejuni* by more than 4 $\log_{10}$ cycles. In the other study using raw beef for consumption as "filet Americain," these authors showed that one sample was positive for *Salmonella* after irradiation at a dose of 1 kGy, compared to 19% of the nonirradiated controls in addition to the absence of *Yersinia* and *Campylobacter* in all irradiated samples. Furthermore, the counts of Enterobacteriaceae were reduced in the irradiated beef samples by 4 $\log_{10}$ cycles. Dickson and Maxcy (1985) irradiated minced meat at doses up to 5 kGy for production of fermented sausages. The total aerobic bacterial counts reduced by $\log_{10}$ 2.2 in the irradiated commercial batters in addition to the reduction of coliforms and staphylococci populations to levels that permitted fermentation with the added lactic acid starter culture (*Pediococcus cerevisiae*) to be carried out more slowly and/or with a lower inoculum. In a study using roast beef ready meal, *S. aureus* was not detected in the roast beef, which had been inoculated with approximately 10^2 cfu/g of this pathogen and irradiated at a dose of 2 kGy, during storage for 7 days at 15°C. This dose also reduced the population of this pathogen by 3–4 log cycles and significantly delayed the detection of toxin under abuse conditions when greater inoculum was used. In addition, the applied 2 kGy dose delayed the toxin production by *B. cereus* which inoculated in the roast beef with approximately 10^2 cfu/g when temperature abused at 22°C (Grant and others 1993). Kamat and others (1997) discovered that *Y. enterocolitica* that had been inoculated in salami and cooked ham at 10^6 cfu/g could be eliminated by an irradiation dose of 3–4 kGy, whereas the pathogen was not eliminated from raw pork. The authors added that low inoculums (<10^3 cfu/g) could be effectively eliminated by a dose of 1 kGy even at –40°C. The effectiveness of low γ-irradiation doses in the destruction of *E. coli* O157:H7 and *L. monocytogenes* in raw beef sausages was investigated by Badr (2005). Irradiation at doses of 1 and 2 kGy significantly reduced the counts of these pathogens, while 3 kGy dose effectively eliminated these bacteria by more than 4 and 3 log units, respectively, and could keep their counts below the detection level during storage at 4 ± 1°C. In another study by Samelis and others (2005), irradiation at a dose of 2 kGy decreased the counts of *E. coli* O157:H7 in fermented sausage to 2 log cfu/g, while 4 kGy dose completely inhibited the organism. Yildirim and others (2005) observed that irradiation at a dose of 2 kGy was sufficient to inhibit the coliform bacteria and *E. coli* in raw meatballs, while 4 kGy dose was sufficient for elimination of *Staphylococcus* spp. They concluded that an irradiation dose of 7 kGy was needed for the safety of raw meatballs. The microbiological quality of irradiated meatballs was also

studied by Vural and others (2006). They found that the counts of coliform bacteria were reduced to undetectable levels after irradiation at 2 kGy dose, while 3 kGy reduced the counts of *S. aureus* and sulfite-reducing clostridia and improved the hygienic quality of samples. Gumus and others (2008) examined the effect of γ-irradiation on the inactivation of *E. coli* O157:H7, *S. Aureus*, and *S. typhimurium* that inoculated into Tekirdag meatballs and irradiated at the absorbed doses of 1, 2.2, 3.2, 4.5, and 5.2 kGy. The counts of *E. coli* O157:H7 in the 1 kGy irradiated meatballs was 4 log cfu/g lower than the count in control samples after 7 days of refrigerated storage. *S. aureus* counts were decreased to 4 log cfu/g after exposing to irradiation at 1 kGy dose. They also added that irradiation at a dose of 3.2 kGy reduced the counts of *E. coli* O157:H7 and *S. aureus* to undetectable levels, but was ineffective in elimination of *Salmonella*. However, none of the examined organisms was detected in samples exposed to 4.5 kGy dose.

20.6.1.2 Parasites

Foodborne parasitic diseases are also an important cause of illness and economic loss worldwide. The public health is imposed by parasitic diseases such as toxoplasmosis, trichinellosis, cysticercosis, and trematodosis are substantial even in developed countries (Murrell 1995, Roberts and others 1994). Irradiation of meats can make a significant contribution in the control of parasitic diseases as indicated by several studies. Schneider (1960) showed that cysts of *Entamoeba histolytica* can be inactivated by a dose of 0.25 kGy. Doses between 0.4 and 0.7 kGy effectively inactivated the cysts of *Toxoplasma gondii* (Dubey and others 1986, Dubey and others 1998, Dubey and Thayer 1994, Kuticic and Wikerhauser 1996, Murrell and Dubey 1993, Song and Yuan 1993, Wikerhauser and others 1992). Van kooy and Robijns (1968) observed that a dose of 3 kGy would be required for complete inactivation of *Cysticercus bovis* and a combined treatment of 3 kGy and storage for 7 days at 2°C postirradiation was fatal to cysts in beef. Verster and others (1977) stated that carcasses lightly infected with cysticerci could be rendered fit for human consumption by exposure to low doses of 0.2–0.6 kGy of γ-irradiation. However, in a controlled experiment conducted by Geerts (1990) using human volunteers showed that cysticerci irradiated at 0.2 kGy still developed into adult *Taenia saginata*, whereas cysticerci which received a dose of 0.6 kGy did not. Other studies concluded that 0.3 kGy can be the minimum effective dose to inhibit the development of larvae into adult *T. saginata* (Alabay and others 1993, Geerts and others 1993). With the objective of interrupting the life cycle of *Taenia solium*, Aluja and others (1993) observed that a dose of 7 kGy kill the metacestodes, whereas doses of 0.5 –0.7 kGy produce changes that do not seem to interfere with the viability of the larvae but suppress their infectivity. These authors suggested in another study that 0.3 kGy dose is sufficient to prevent infectivity of *T. solium* metacestodes (Aluja and others 1998). Investigations of the effectiveness of irradiation to inactivate *Trichinella spiralis* in pork were carried out. Irradiation at doses between 0.15 and 0.3 kGy prevented larval development and rendered pork trichina safe (Brake and others 1985, Murrell and Dubey 1993, Sivinski and Switzer 1984), neither the age of the encysted muscle larvae nor vacuum-packaging of the meat significantly affected the radiosensitivity of the larvae (Brake and others 1985). In another study, Kasprzak and others (1993) stated that, for most *T. spiralis*, irrespective of their hosts, higher doses of irradiation, over 0.4 kGy, were required for total destruction of encysted muscle larvae.

20.6.2 Shelf-Life Extension (Radurization)

Irradiation can be applied for reducing the number of spoilage microorganisms and attaining extended shelf life of meat and meat products during refrigerated storage (radurization). The effects of low irradiation doses on the properties of raw meats were studied. The results of Urbain and Giddings (1972) indicated that the counts of total aerobic mesophilic bacteria in vacuum-packaged beef steaks did not exceed 10^4/g during 21 days of storage at 4°C when irradiated at 2.5 kGy dose. Niemand and others (1981) concluded that a shelf life of up to 10 weeks at 4°C could be expected for sirloin steak that vacuum-packaged in oxygen-permeable laminated bags and exposed to 2 kGy irradiation dose at 25°C, twice that of the nonirradiated samples. It has been reported that the lactic acid bacterial group in nonirradiated beef

composed of *Lactobacillus* spp, *Leuconostoc* spp., and *Pediococcus* spp., while consisted mostly of lactobacillus in vacuum-packaged beef that irradiated at 2 kGy dose and had a doubled shelf life during storage at 4°C (Niemand and Holzapfel 1984). Beilken and others (1987) observed that irradiated vacuum-packaged lamb carcasses, 3 days postslaughter, at a mean dose of 2.43 kGy exhibited more purge, lower microbial number, and more constant acceptability during storage for several weeks at 0–2°C than did nonirradiated carcasses that showed a more rapid bacterial spoilage. Irradiation of vacuum-packaged pork loins at a dose of 3 kGy at ambient temperature extended their shelf life to more than 90 days at 2–4°C compared to 41 days for controls (Lebepe and others 1990). These authors showed that there was no evidence that the spoilage of the irradiated pork loins differed from that of the nonirradiated loins. Wong and Kitts (2002) illustrated that electron-beam irradiation of sirloin steaks at doses of 2, 3, and 4 kGy effectively reduced the counts of psychrotrophic bacteria to a maximum of 2 log cycles. Meanwhile, Chen and others (2007) observed that irradiation of Chinese yellow cattle at doses of 1.13, 2.09, and 3.17 kGy reduced the total bacterial count by 1.05, 1.44, and 1.66 log units for beef samples, respectively, compared with the control samples having 5.39 log cfu/g.

Extensive researches were conducted on extending the shelf life of ground meat by irradiation. Tiwari and Maxcy (1971) observed substantial reductions in the counts of total aerobic mesophilic bacteria in ground beef due to irradiation at 0.86 kGy dose and storage at either 2°C or 5°C for up to 6 days. At that time the count reached only 2.6×10^6/g in irradiated samples, whereas the control nonirradiated samples had a total count of 10^9/g in the same period. Niemand and others (1983) observed that the population of aerobic bacteria in vacuum-packaged minced beef irradiated at 2.5 kGy did not exceed 10^4/g during 21 days of storage at 4°C, while the count in the control samples reached 10^7/g in 4 days of storage. The yeast flora was examined by Johannsen and others (1984) in minced beef before and after irradiation at a dose of 2.5 kGy. The authors could not observe any reduction in the counts of yeasts due to irradiation and the counts of psychrotrophic yeasts increased rapidly in the radurized meat during storage at 4°C for 14 days. Irradiation of vacuum-packaged beef burgers at doses of 1.3 and 1.54 kGy at 3°C reduced their bacterial population by 82% and 92%, respectively, and pseudomonads (the main spoilage bacteria in aerobically stored meat) could not survive treatments. Beef burgers that were irradiated at 1.54 kGy did not exhibit off odors even after 15 days of storage at 3°C (Dempster and others 1985). A study by Holzapfel and Niemand (1985) showed that the shelf life of vacuum-packaged ground beef could be trebled by a radurization dose of 3 kGy and consecutive storage at 4°C. They also showed that, while most typical meat spoilage bacteria were effectively eliminated by radurization, several factors seem to favor the survival and growth of lactobacilli in vacuum-packaged radurized meat. The comparative survival of various bacteria in fresh, vacuum-packaged pork irradiated at 1 kGy (at 6–10°C) was examined during storage at 5°C or at 24–25°C for 48 h. A pronounced decrease in the counts of mesophilic, psychrotrophic, and anaerobic or facultative anaerobic bacteria was observed in the irradiated pork that had 30–40% longer shelf life at 5°C than the control nonirradiated samples (Ehioba and others 1987, 1988). The authors showed that spoilage in the irradiated meat did not differ from that in the nonirradiated meat because a large proportion of the surviving flora corresponded to lactic acid-producing bacteria and there was no reason to support the concern about potential unimpeded botulinal growth even when meat was exposed to sever mishandling at 24–25°C for 24–48 h. The better survival of lactic acid-producing bacteria in the irradiated minced beef was confirmed by Hastings and Holzapfel (1987). They showed that 113 Gram-positive, catalase-negative, rod-shaped bacteria that were isolated from minced beef irradiated at 5 kGy were identified as being homofermentative, aciduric bacteria belonging to the subgenus streptobacterium. Lefebvre and others (1992) stated that a shelf-life extension of ground beef was expected with radurization doses of 1–5 kGy due to the radiosensitivity of *Pseudomonas* spp. (mainly responsible for spoilage). They also found that the number of yeasts was proportionally decreased when samples of ground beef were exposed to 1 or 2 kGy dose, while yeasts and molds were not recovered until 7 days when a 5 kGy dose was administered which suggested that injuries due to irradiation were sublethal. Thayer and others (1993) could not detect any surviving bacteria in vacuum-packaged ground pork irradiated at doses above 1.91 kGy even after 35 days of storage at 35°C. In other study by Murano and others (1998), ground beef patties were packaged under air or vacuum and irradiated at a dose of 2 kGy. Immediately after irradiation of samples, there was a 2- to 3-log reduction in the total bacterial count, regardless of whether they were irradiated under air or vacuum. In this study, the total bacterial count in nonirradiated patties, with an initial bacterial load of

10^6 cells/g, reached 10^8 cells/g after 8 days of storage at 4°C, while irradiated samples had a total count of 10^6 after 55 days. Roberts and Weese (1998) observed that ground beef patties of excellent initial quality (having total aerobic count of $<10^2$ cfu/g) were microbiologically acceptable (having a total aerobic count of $<10^7$ cfu/g) up to 42 days of storage at 4°C when irradiated at a dose of 3 kGy, whereas the control samples were spoiled after 7 days only at 4°C. However, when the initial quality of the beef patties was lower (having a total aerobic count of $<10^4$ cfu/g), only samples that irradiated at 5 kGy or higher lasted for 42 days at 4°C without reaching the levels of spoilage. Ouattara and others (2002) observed that irradiation doses of 1, 2, and 3 kGy induced an immediate reduction of the total aerobic plate counts by 2-, 3-, and 4-log units in ground beef, respectively. Similarly, Duong and others (2008) found that irradiation at a dose of 2 kGy produced a 3-log bacterial reduction in ground beef.

The effectiveness of irradiation as a means of extending the shelf life of different processed meat products was documented by several researchers. Kiss and Zachariev (1981) observed that irradiation of minced pork meatballs at doses up to 2 kGy increased the shelf life of samples for 12–14 days at 4°C compared to 4–6 days in the nonirradiated controls. The counts of mesophilic bacteria were reduced by two- to threefolds, while psychrotrophic microorganisms were reduced by a factor of 3 due to irradiation treatment of the product. Kwon and others (1983) irradiated cooked cued ham at doses of 3–8 kGy and examined the shelf-life extension of samples during a storage period of 8 weeks at 30°C. They observed that nonirradiated cured ham had a total bacterial count of the order of 10^6/g after 2 weeks of storage, while similar counts were observed after 3–5 weeks of storage in the irradiated samples with the absence of coliforms. A study by Risvik (1986) showed that vacuum-packaged smoked ham continued to have low bacterial counts during storage at 4°C for 3 months after irradiation at a dose of 2.5 kGy. In another study with ground beef, irradiation doses of 1, 2, and 4 kGy decreased the bacterial counts in vacuum-packaged sliced corned beef by 1-, 2.5-, and 5-log cycles, respectively. The 2.5 kGy dose extended the shelf life of samples to 5 weeks at 5°C compared to 2–3 weeks for the nonirradiated samples, whereas further shelf-life extension was observed by irradiation at the highest dose (Wills and others 1987). Improvements in the quality and shelf life of meat products could be observed through irradiating one or more of their ingredients. Spices are known as an important source for microbial contamination in meat products. The use of irradiated spices in different uncooked sausages reduced the counts of viable bacterial spores by 99.7%. In addition, the shelf life of cooked sausages was extended considerably by using irradiated spices that had earlier reduced the heat-resistant bacterial spores in the added spices (Niemand and Fabian 1986). Fresh salted and semidried natural pork and lamb casings were washed and γ-irradiated at doses of 0, 3, and 5 kGy, then used for the manufacture of emulsion-type pork sausage which was refrigerated and stored at 4°C. The counts of total aerobic bacteria, enterococcus, and coliforms in the irradiated casing as well as in the resultant sausages were significantly decreased or eliminated as compared with the control samples (Byun and others 2001). In other study, Byun and others (2002) examined the effects of γ-irradiation on the processing properties in meat products and found that most contaminated bacteria in raw ground beef were killed by irradiation at a dose of 3 kGy and the sausage could be manufactured with an extended shelf life through the irradiation of the raw ground meat. Chouliara and others (2006) studied the changes in microbiological quality attributes resulting from the use of irradiation in the production of Greek dry fermented sausage as a function of fermentation/ripening time. They found that irradiating meat/fat trimmings at doses of 2 or 4 kGy prior to sausage production eliminated the natural contamination with *Listeria* spp. and *Pseudomonads*, while reduced the populations of Enterobacteriaceae, enterococci, and pathogenic staphylococci to levels below 1, 2, and 2 log cfu/g, respectively. The treatment improved the quality of the trimmings and did not affect the growth of the starter bacteria as well as the end of ripening period of the prepared sausage. In the aspect of the survival of lactic acid bacteria during fermentation and aging, irradiation of the raw material (roasts) at a dose of 2 kGy before the addition of starter culture could be considered beneficial in controlling undesirable microorganisms in the resultant sausages (Lim and others 2008).

20.6.3 Radiation Sterilization (Radappertization)

In the case of shelf-stable radappertized products, the radiation dose must be high enough to inactivate spores of *C. botulinum*, the most radiation-resistant pathogenic bacterium that can be present. A $12 \times D_{10}$

inactivation of type A and B spores of *C. botulinum* (the most resistant serotypes) has been accepted as the minimum dose requirement for radappertization (the same extent of inactivation is accepted as the public health requirement for other sterilizing processes such as canning of low-acid food). The necessary radiation doses have been well established by extensive inoculated-pack studies, mainly in the framework of the US Army's radappertization program, and they depend on the type of meat, the pH, the presence of curing salts, and irradiation conditions (Anellis and others 1979, Rowley and others 1983, Wierbicki and Heiligman 1980). The resulting radappertized products are free from food spoilage microorganisms and those of public health significance including viable botulinal spores, and they can be stored without refrigeration for years, the limiting factor being the integrity of the primary packaging material (IAEA 1995, WHO 1995).

The technological steps for radappertization of meat includes preparation of meats for shape, size, and appearance including the addition of sodium chloride and sodium tripolyphosphate to improve juiciness; thermal enzyme-inactivation (precooking to 65–80°C); vacuum-packaging in metal cans or flexible pouches to prevent lipid oxidation and provide protection against bacterial recontamination after irradiation; freezing at –20 to –40°C; irradiation (in the frozen state to prevent or reduce off-flavor formation or nutrient loss during irradiation) using sterilizing radiation doses; and defrosting for storage without refrigeration (Anellis and others 1979, Wierbicki 1980).

Several inoculated-pack studies have been performed to establish the dose required to ensure the microbiological safety of radiation-sterilized meats. Anellis and others (1965) stated that sliced cured bacon, which was vacuum-packed in cans and inoculated with approximately 10^6 spores of *C. botulinum* types A and B per can, was sterilized by a radiation dose of 45 kGy. They added that the minimal sterilizing dose was 20 kGy, while at doses below 20 kGy, viable *C. botulinum* spores could survive at least 8 months at 30°C without producing visible or toxic spoilage. Based on an inoculated-pack study with cans of enzyme-inactivated pork loin inoculated with 10^6 *C. botulinum* spores of 5 types of A and B strains, Anellis and others (1969) observed a sterilizing dose of 43.3 ± 1.7 kGy. Anellis and others (1975) carried out another inoculated-pack study with canned beef containing a mixture of 10^7 spores/can from types A and B *C. botulinum* and irradiated at –30°C ± 10°C. They found that the minimal experimental sterilizing dose based on nonswollen, nontoxic product was 22.26 kGy, while the 12D dose was estimated to be 37 kGy. Inoculated pack studies with irradiated pork to establish the 12D dose at –30°C ± 10°C, using inocula of 10^7 from a mixture of five types of A and B *C. botulinum* spores per can, showed that the minimal sterilizing dose based on flat nontoxic product was 30–32 kGy and the 12D value was 43 kGy (Anellis and others 1977). The dose necessary to reduce the number of viable spores of a single most resistant strain of *C. botulinum* by 12-log cycles in vacuum-canned enzyme-inactivated beef inoculated with 10^7 of a mixture of spores/can was evaluated by Anellis and others (1979). Based on their study, the 12D dose was 41.2 kGy when samples were irradiated at –30 ± 10°C. In a recent study, pouches containing a beef casserole were inoculated with a mixed spore suspension of *Clostridium sporogenes* and *C. botulinum* and irradiated at –40°C to doses of 10–40 kGy. The irradiated pouches were incubated at 30°C for 6 months until they were swollen. The results indicated that the experimental sterilizing dose was 26–30 kGy and the calculated minimum radiation dose would be in the region of 45 kGy (De Bruyn and De Villiers 2003). Narvaiz and others (2003) irradiated frozen (–20°C) enzyme-inactivated, vacuum-packaged beef burger at a dose of 45 kGy and found that neither pouch vacuum loss nor subsequent microbiological growth was observed in any sample during storage at room temperature for 7 days, confirming commercial sterility. The authors concluded that the treatment offered the possibility of sterilizing meats of good nutritive and sensory qualities and completely eliminating microbial risk to immumosuppressed patients.

The presence of nitrosamines in foods is one of the problems that has aroused a great deal of controversy. Nitrites and nitrates, which are used as food additives in processed meats, have been indicated as major factors responsible for aggravating the problem. From this viewpoint, researches with radiation sterilization showed that nitrites and nitrates could be eliminated or used at very low levels in meat preserved by ionizing radiation without any adverse changes. Wierbicki and Brynjolfsson (1979) found that irradiation at sterilizing doses allowed reduction or elimination of nitrite in cured meats. Their experiments showed that the added sodium nitrite in ham could be reduced from 156 to 25 mg/kg, and in corned beef to 50 mg/kg. The 25 mg/kg sodium nitrite would be required to prevent the color fading in

ham after irradiation. The results indicated that there was neither residual nitrite nor nitrosamines in the treated products. Wierbicki (1981) indicated that radiation sterilization of cured meats allowed complete elimination or drastic reduction of the added nitrite to levels needed only for keeping the characteristic of color and flavor of the meat product. The irradiated-cured products with low levels of added nitrite were free from residual nitrite and nitrosamines including nitrosopyrrolidine produced in fried bacon. Furthermore, irradiation also destroyed preformed nitrosamines in bacon. The effect of radiation sterilization dose of 30 kGy at −40°C on nitrosamine formation in bacon was investigated by Fiddler and others (1981). The authors also studied the production of low nitrite, nitrosamine-free bacon that provides protection against *C. botulinum*. Their results showed that bacon with a reduced residual nitrite prior to frying could be observed, thereby reduced nitrosamines after frying, while irradiation destroyed the volatile nitrosamines that could be present before irradiation. Irradiation of bacon, which had 20 ppm of sodium nitrite and 550 ppm of sodium ascorbate, produced concentrations of nitrosamines that were indistinguishable from those in nitrite-free bacon.

20.6.4 Effect of Combination of Irradiation and Other Treatments on Microbiological Quality of Meat and Meat Products

The possibility of using irradiation in combination with other food preservation treatments (either post- or preirradiation treatment) was studied and showed a great potential for improving the quality of the treated meat products through their synergistic antimicrobial effect.

20.6.4.1 Irradiation and Chemicals

Acidifying agents have been widely used in combination with irradiation to improve the microbiological quality of meat products. Niemand and others (1983) studied the effect of irradiation at 2.5 kGy, lactic acid, and the combination of lactic acid and irradiation on the quality of minced beef during storage at 4°C. They observed that the bacterial populations did not exceed 10^4/g during 21 days of storage in vacuum-packaged samples that had been treated with irradiation plus lactic acid, whereas they reached 10^7/g in 4 days of storage for the control vacuum-packaged samples. The effect of γ-irradiation dose of 2 kGy and acidulants, 0.1% (w/w) ascorbic acid or 0.5% (w/w) glucono-deltalactone, on the quality of vacuum-packaged minced meat prepared with pork and beef with spices and cereal fillings was investigated during storage at 0–2°C for 4 weeks or temperature abused at 10°C for 1 week. Acidulants delayed the growth of Enterobacteriaceae in the nonirradiated meat for 2 weeks at 0–2°C. However, combination of acidulant with irradiation completely prevented the growth of the Enterobacteriaceae group during refrigerated or temperature-abused storage (Farkas and Andrassy 1993). Bhide and others (2001) sprayed sheep carcasses with 1% propionic acid, 2% lactic acid, or 2% acetic acid at a pressure of 3 kg/cm^2 for 2 min, then the harvested meat from the carcasses were packaged and irradiated at doses of 1, 2, or 3 kGy. They found that all organic acids used in their study increased the sensitivity of *B. cereus* to irradiation; however, acetic acid (2%) combined with an irradiation dose of 3 kGy was most effective in reducing the total viable count of this pathogen during storage of samples at 5–7°C. Sommers and others (2003) observed that surface treatment of frankfurters with citric acid decreased the radio-resistance of *L. monocytogenes*. The D_{10}-values for *L. monocytogenes* were 0.61, 0.60, 0.54, and 0.53 kGy when frankfurters were dipped in 0%, 1%, 5%, or 10% (w/w) citric acid, respectively. Kim and others (2004) also studied the combined effects of organic acids (lactic, citric, and acetic) at 2% and electron-beam irradiation doses of 1, 2, and 3 kGy on the shelf life of pork loins. Combinations of organic acid and irradiation were more effective than each intervention used alone for controlling growth of total microbial population and coliforms in pork during storage for 14 days at 4°C. The possible antibacterial synergism between irradiation and some additives was studied. The combined effects of 1 kGy irradiation dose (at 10°C) and curing salts ($NaNO_2$ and NaCl) on the survival of *Salmonella* that inoculated in ground pork treated with no additives or with (1) 10 ppm nitrite, (2) 200 ppm nitrite, and (3) 3% NaCl + 200 ppm nitrite before irradiation were examined by Szczawinski and others (1985). Samples were examined during aerobic storage at 0–2°C for 20 days or at 20°C for 7 days. The authors found that irradiation at 1 kGy dose immediately reduced the counts of salmonellae by 1.2–2 log cycles and the

organisms continued to decline in all samples during postirradiation storage at 0–2°C. Combined treatment of irradiation and curing salts exhibited a synergistic effect in controlling the growth of salmonellae in samples. Ehioba and others (1987) found that treatment of fresh, high-quality, vacuum-packaged ground pork with 0.4% sodium acid pyrophosphate before being irradiated at 1 kGy dose showed no effect on the shelf life of samples during storage at 5°C. However, when meat was inoculated with 10^5 spoilage bacterial cells per gram before irradiation, phosphate-treated meat remained unspoiled 2 days longer than control meat samples without phosphate. Ouattara and others (2002) evaluated the combined effect of γ-irradiation and incorporation of naturally occurring antimicrobial compounds on microbial characteristics of ground beef patties. They concluded that combination of γ-irradiation and edible-coating film containing natural antimicrobial compounds significantly reduced the bacterial growth. Although lactic acid bacteria were more resistant, *S. aureus*, coliforms, enterobacteriaceae, and pseudomonas exhibited a greater sensitivity to irradiation. Chen and others (2004) demonstrated a synergistic effect between the bacteriocin pediocin and electron-beam irradiation for inhibiting *L. monocytogenes* in frankfurters. They showed that storage of frankfurters at 4°C enhanced the antibacterial effects of the combined treatment and little or no growth of the pathogen was occurred in packages of frankfurters during 12 weeks of storage. With the objective of extending ground beef retail display life using antioxidants, reductants, and/or trisodiumpolyphosphate treatments combined with electron-beam irradiation, Duong and others (2008) observed that by adding trisodiumpolyphosphate, antioxidants and/or reductants to ground beef before irradiation, a dose of 2 kGy decreased the bacterial population by 99.9%. The efficiency of spice essential oils to reduce the dose necessary to eliminate *E. coli* and *S. typhimurium* in medium fat (23% fat) ground beef when packed under air or under atmosphere rich in oxygen [modified atmosphere packaging (MAP)] was examined. Essential oils were added in ground beef at concentration that does not affect the taste and the meat was treated at a dose of 1.5 kGy, then the counts of total mesophilic aerobic, *E. coli*, *Salmonella*, total coliforms, lactic acid bacteria, and pseudomonas were determined during 28 days of storage. The combined treatment could eliminate *Salmonella* and *E. coli* when done under air, while under MAP, pseudomonas could be eliminated and shelf life of more than 28 days was observed (Lacroix and others 2009).

20.6.4.2 Irradiation and Heat

The combined use of irradiation and heat showed additive or synergistic antimicrobial characteristics. Barnhart (1977) showed that spores of *C. perfringens* that irradiated in ground beef gravy or ground beef were made more heat sensitive due to irradiation. Gel's fand and Nomerotskaya (1980) stated that beef and pork that were cooked to an internal temperature of 85°C along with spices, carrots, and onions, vacuum-packaged in a laminated film incorporating aluminum foil, and irradiated at a dose of 8 kGy could be stored for more than 6 months at 4–5°C without microbiological spoilage. Increased radiation resistance in vegetative cells of *Moraxella-Acinitobacter*, *Micrococcus radioduranse*, *E. Coli*, and *B. cereus* at subfreezing temperatures was demonstrated by Ma and Maxcy (1981). El-Wakeil and others (1983) conducted a study with patties formulated with 58% or 48% lean buffalo meat, 10% or 20% fat, 30% rehydrated soy flour, and 1.5% NaCl and cooked to an internal temperature of 75°C, then irradiated at dose of 3.75 kGy immediately. The authors concluded that the elimination of the psychrotrophic microbial flora (coliforms, psychrotrophic bacteria, and *S. aureus*) in the tested patties could have been a result only of the combination of treatments.

20.6.4.3 Irradiation and Packaging Atmosphere

The effect of various packaging atmospheres in combination with irradiation treatment on the microbiological quality of meats was also studied. Niemand and others (1981) vacuum-packaged sirloin steak in oxygen-permeable laminated bags and irradiated the samples at doses of 0 and 2 kGy (at 25°C), then all samples were stored at 4°C. The authors concluded that a shelf life of up to 10 weeks could be expected for irradiated samples, twice that of the nonirradiated controls. Lebepe and others (1990) irradiated vacuum-packaged pork loins at ambient temperature to 3 kGy dose and studied the effects of the treatment

on the microflora during storage at 2–4°C. They found that irradiation at 3 kGy extended the shelf life of the vacuum-packaged pork loins to more than 90 days compared to 41 days for the nonirradiated loins. They added that there was no evidence that the spoilage of the irradiated pork loins differed from that of the nonirradiated loins. In a study with minced pork that was packed in 25% CO_2 and 75% N_2 and then irradiated at a dose of 1.75 kGy, Grant and Patterson (1991a) concluded that the irradiated modified atmosphere-packaged pork was microbiologically safer than the nonirradiated MAP controls. In another study, the same authors investigated the combination effect of irradiation at a dose of 1.75 kGy with modified atmosphere packaging on the microbiological quality of pork during storage at 4°C. A mixture of 25% CO_2 and 75% N_2 was recommended by the authors to improve the microbiological and sensory quality of the pork chops (Grant and Patterson 1991b). Lambert and others (1991) stated that irradiation at a dose of 1 kGy with modified atmosphere packaging of 45–70% CO_2 delayed the production of *C. botulinum* toxin in fresh pork samples. Kanatt and others (2002) developed a shelf-stable intermediate moisture-spiced mutton, in which the water activity of meat was reduced to 0.85 by grilling or hot air drying and the meat was then vacuum-sealed and irradiated at doses of 0, 2.5, 5, and 10 kGy. No viable microorganisms were detected in meat treated with 10 kGy during storage at 30°C for 9 months.

20.7 Quality Characteristics of Meat and Meat Products as Affected by Irradiation Treatment

20.7.1 Physical Characteristics

Decreased water-holding capacity and appearance of increased purge or weep due to irradiating beef meat at doses above 5 kGy were observed by Nakai and others (1974), this could be attributed to decreasing the solubility of protein fraction as a result of irradiation treatment which was linear with increasing the applied dose. It was shown that mainly myosin was affected, while other proteins were largely unaffected, as indicated by electrophoresis of the phosphate buffer-soluble fraction. Velinov and Dimitrova (1977) observed with electron-microscopic examination a disruption of Z lines and hydrolysis of protofibrils in the A and I zones of individual sarcomeres in 3 kGy irradiated meat. *Longissimus dorsi* muscles of meat from Friesian steer that had been treated with tetracycline and irradiation at 5 kGy dose exhibited complete aging after 2 days of storage at 4°C, while the untreated, nonirradiated muscle required 8 days at the same temperature for complete aging (Soliman and others 1979). Grozdanov and others (1982) indicated that irradiation at 3 kGy under vacuum had no significant effect on beef *semimembranosus* muscle. Neither radiation-induced changes in postmortem metabolism nor acceleration of adenosine triphosphate breakdown was detected. However, irradiated muscle was deemed inferior to nonirradiated samples in terms of water-holding capacity, protein extractability, emulsifying capacity, and gelling ability after 20 days of refrigerated storage at 2°C. In another study, irradiation doses of 5 and 10 kGy had no effect on the structure of raw bovine *Longissimus dorsi* which packaged in pouches made from material having low oxygen permeability and irradiated 72 h postmortem. Increasing the irradiation dose to 30 or 50 kGy, however, substantially increased myofibril fragmentation and decreased the tensile strength, sheer value (Warner-Bratzler), and myosin content (Lakritz and others 1987). Lakritz and Maerker (1988) observed that irradiation of beef *semimembranosus* muscle at doses of 1, 5, and 10 kGy at a temperature of 0–4°C followed by storage for up to 28 days at 2°C reduced nonspecific proteolysis by the endogenous enzymes. Zhu and others (2004) stated that irradiation significantly increased centrifugation loss of water from pork loins that was partly reversed during refrigerated storage, which could be attributed to the hydrolysis of muscle proteins. In contrast, irradiation of raw beef sausage at doses of 1–3 kGy showed no significant effect on the water-holding capacity of samples as compared with the nonirradiated controls (Badr 2005).

20.7.2 Oxidation

Oxidation of lipids is one of the major concerns in irradiating meat and meat products. The chemical changes in irradiated meats are initiated by free radicals that produced during irradiation from water

molecules. Studies with irradiated meat products showed that lipid oxidation in meat as a result of irradiation was a significant problem under aerobic conditions. Irradiated (2 kGy) aerobically packaged ground beef produced higher amounts of thiobarbituric acid-reactive substances (TBARS) than nonirradiated samples (Luchsinger and others 1997a, Murano and others 1998). Studies with aerobically packaged sausages showed that the amounts of TBARS increased with increasing irradiation dose. Also the produced TBARS increased with increasing contents of polyunsaturated fatty acids in both aerobic and vacuum-packaged sausages (Ahn and others 1999). Under frozen conditions, irradiation increased the TBARS of pork patties, however, storage time had no effect on lipid oxidation even under aerobic packaging conditions (Ahn and others 2000a). Ahn and others (2001) examined the effect of irradiation and packaging conditions on the contents of cholesterol oxidation products and lipid oxidation in cooked beef during storage. Cooked beef samples were packaged either in oxygen-permeable or in oxygen-impermeable bags and irradiated at doses of 0 and 4 kGy, then samples were stored for 7 days at 4°C. Irradiation had no significant effect on the amounts of any of the cholesterol oxidation products in the cooked beef. In addition, the amounts of cholesterol oxidation products and lipid oxidation products were closely related to the proportion of polyunsaturated fatty acids. Also Nam and others (2003) observed that electron-beam irradiation at dose of 2.5 kGy increased TBARS in preaged ground beef, but the differences between irradiated and nonirradiated long-term aged beef were not significant. Irradiated ground beef and beef patties (2 and 4 kGy doses at room temperature) produced higher peroxide and TBARS values than the controls (Badr 2007). Also, irradiation of Chinese yellow cattle *semitendinous* muscles at doses of 1.13, 2.09, or 3.17 kGy increased the values of TBARS for samples (Chen and others 2007). Duong and others (2008) indicated that irradiation increased lipid oxidation of ground beef regardless of their aging time and storage period. With the objective to determine the effect of electron-beam irradiation before or after cooking on the chemical properties of ground beef and pork during storage at −40°C in oxygen permeable bags, Kwon and others (2008) showed that irradiation at 5 kGy dose had no effects on the amounts of TBARS, while cooking significantly increased the TBARS values whether it was done before or after irradiation and was most significant in pork. The effect of electron-beam irradiation (at 2, 5, and 8 kGy doses) and storage for 60 days on fatty acid and cholesterol oxidation was determined in three Italian, vacuum-packaged cured pork products (Salami, Milano, Coppa, and Pancetta). A significant increase in the degree of fatty acid oxidation was observed starting from 8 kGy dose. Unlike the nonirradiated samples, the vacuum storage was not sufficient to curb fatty acid oxidation in the irradiated pork products (Zanardi and others 2009). The authors added that cholesterol oxidation was affected by the highest dose and sometimes by storage in all irradiated pork products. The cholesterol oxide molecules were quantitatively similar in both irradiated (2–8 kGy dose) and nonirradiated pork products and the detected levels were about 100 times lower than the toxic level for *in vitro* and *in vivo* experiments. Park and others (2010) compared the effects of γ-rays and electron-beam irradiation on the quality of beef sausage patties during accelerated storage at 30°C and found that the effects of γ-rays irradiation was similar to electron-beam irradiation on lipid oxidation.

20.7.3 Color

The color changes in irradiated meat and meat products have been shown to be very significantly depending on various factors such as dose, packaging atmosphere, and animal species. It was found that oxygen reduced the red color of fresh meats during irradiation, whereas the opposite occurred in cooked muscle foods, in which reddening was caused by irradiation-induced reduction of heme iron in the brown pigment of the cooked meat (Kamarei and others 1979). Results from examination of sausage extract indicated that irradiation caused degradation of the nitrosoheme complex. In their study, Kume and others (1978) observed a discoloration of viennese sausages by irradiation, which was attributed to the presence of oxygen. The difference between color in irradiated and nonirradiated samples was significant at doses of 10 kGy in an atmosphere of nitrogen, at 5 kGy in air, and at 3 kGy in O_2. In another study, the color changes in fresh beef and pork and cured meat pigments as affected by radiation sterilization doses and the temperature during irradiation were investigated. It was shown that irradiation of the isolated myoglobin, metmyoglobin, and nitrosomyoglobin induced shifts in their peak characteristics, along with progressive decrease in the sort bands (375–450 nm) and these decreases were more apparent at 3°C than

at −30°C or −80°C. Moreover, irradiation of products not cured with sodium nitrite increased their color scores due to the formation of brighter red color, while the opposite was observed in nitrite-cured products, in which color scores were decreased by irradiation. Irradiation of smoked porcine *semimembranosus* muscle at 32 kGy dose (−40°C) showed the same phenomenon when studied with different levels of sodium nitrite, nitrate, and combinations (Kamarei and others 1979, 1981, Karel and others 1980). Packaging atmosphere has been found as an important factor influencing the color of stored irradiated meats. Irradiation increased the Hunter Lab *a* color, indicative of increased redness, for both aerobically and vacuum-packaged pork steaks; however, vacuum-packaged meat was redder than aerobically packaged ones and was stable during storage. Increasing the *a* color values was also observed in other meat products including pork loins, pork patties, and beef burgers (Chen and others 1999, Dempster and others 1985, Fu and others 1995a, Grant and Patterson 1991a, Lebepe and others 1990, Luchsinger and others 1996). Luchsinger and others (1997a) reported that irradiation at doses of 2 and 3.5 kGy initially darkened and reduced the redness of raw beef patties in aerobic or vacuum-packaging, but the color difference disappeared with display time. Similar findings were observed by Nanke and others (1998) when they studied the species dependence of meat color changes due to electron-beam irradiation. These authors found that irradiated pork (1.5–10.5 kGy at 0–2°C) showed increased *a* values indicative of a redder color, while the equivalent values for beef were decreased with the *b* values (yellowness) increased proportionally to radiation dose and storage time. They indicated that irradiation induced the appearance of an oxymyoglobin pigment in pork, while it developed oxy- and metmyoglobin in irradiated beef. A surface discoloration was observed for vacuum-packaged pork when irradiated at 4.5 kGy, but less so at higher irradiation doses. Under aerobic conditions, irradiation of the red meat changes the red color to brown or gray, and the color values were significantly affected by aging time. The color of ground beef changed from bright red to a greenish brown immediately after irradiation. During storage, the color *L* values of the irradiated ground beef increased with increasing time of storage (Nam and Ahn 2003a). It has been speculated that radicals such as hydroxyl or sulfuryl radicals produced by irradiation can react with the binding sites of myoglobin and form metmyoglobin and sulfmyoglobin leading to brown and green colors, respectively (Giroux and others 2001). The reduction of the color redness as a function of irradiation and storage was also observed in sausages (Kuo and Chen 2004) and beef jerky (Kim and others 2010).

20.7.4 Odor and Taste

Sensory attributes of different irradiated meat and meat products have been examined by many researchers. Rusz (1976) showed that heat-pasteurized canned hams had a very poor taste and odor scores when irradiated at doses of 10 and 20 kGy immediately after irradiation, while scores were markedly improved after 3 months of storage at ambient temperature when compared with the control-pasteurized and thermally sterilized samples. Irradiation odor was observed in ground beef when irradiated at doses of 2.5–10 kGy (Keskin 1977) and also in ground beef irradiated at 3 kGy as well as vacuum-packaged fresh beef cuts irradiated at 2 kGy (Niemand and others 1980). Kiss and Zachariev (1981) indicated that the sensory properties of minced pork meatballs were not affected by irradiation doses of 0.5–2 kGy. The quality of cooked cured ham as affected by irradiation at doses of 3–8 kGy were examined during storage at 30°C for 8 weeks by Kwon and others (1983). The authors reported that an irradiation odor was noted in ham samples that exposed to 8 kGy irradiation dose, while all sensory scores were highly acceptable when the applied dose was 5 kGy. Dempster and others (1985) discovered an irradiation odor in vacuum-packaged beef burgers irradiated at 1.03 and 1.54 kGy at 3°C when samples were first exposed to air. Mattison and others (1986) found that irradiated (1 kGy) samples of vacuum-packaged pork loins showed a minimal changes in their sensory characteristics when cooked to an internal temperature of 76°C and evaluated by the taste panel. They added that any sensory effects that irradiation may have caused in pork were short-lived or totally masked by cooking. In a study with vacuum-packaged beef and smoked bacon that irradiated at doses of 1–10 kGy and stored at 4°C for 3 months, Risvik (1986) observed higher intensities of off-taste, rancidity, metallic taste, and sweetness in samples irradiated at 1 kGy as compared to the nonirradiated samples and the observed off-taste as well as rancidity increased with increasing applied dose. However, Wills and others (1987) stated that, although detrimental changes

were induced in aroma and flavor of vacuum-packaged, sliced corned beef due to irradiation at 2 and 4 kGy, the trained taste panel rated such changes as only slight and slight to moderate. Luchsinger and others (1997b) evaluated the effects of irradiation on flavor and aroma of frozen raw and precooked ground beef patties with 10% and 22% fat packaged aerobically or in vacuum; frozen vacuum-packaged boneless beef steak; and chilled vacuum-packaged beef steaks that were repackaged in an oxygen-permeable film after 14 days of storage. They found that irradiation at doses up to 3.5 kGy had minimal effects on the studied properties of samples, as evaluated by a trained descriptive panel. Wheeler and others (1999) illustrated that nonirradiated patties had a more intensive ground beef aroma and less off-aroma than patties irradiated at doses of 3 and 4.5 kGy. In contrast, several studies showed that irradiation had no adverse effects on the odor and taste as well as acceptability of different evaluated ground beef patties (Fan and others 2004, Giroux and others 2001, Montgomery and others 2003, Vickers and Wang 2002), frankfurters (Chen and others 2004), and ground beef (Zienkewicz and Penner 2004). Park and others (2010) concluded that γ-irradiation up to 10 kGy had no adverse effect on the sensory quality of beef sausage patties including the taste of samples.

Studies have shown that volatiles from lipids accounted for only a small part of the off-odor in irradiated meat (Lee and Ahn 2003), while volatile sulfur compounds would be the major compounds responsible for the characteristic off-odor in irradiated meats (Ahn and others 2000b, Jo and others 1999, Jo and Ahn 2000, Nam and others 2002). The volatile sulfur compounds have been shown to be produced by two different ways that are direct radiolytic cleavage of the side chains of sulfur-containing amino acids, methionine, and cystein, and the other is a secondary reaction of primary sulfur compounds with surrounding compounds (Ahn and Lee 2002, Jo and Ahn 2000). Kim and others (2002) indicated that irradiated meats produced more volatiles than nonirradiated controls regardless of animal species and there were no distinct differences in preference scores between irradiated and control meats from the different meat species indicating that the effects of irradiation on the sensory properties of meat from different animal species are at least similar, if not the same. They added that the amounts of volatiles decreased during storage of the irradiated aerobically packaged meats, while those of the nonirradiated meats increased. Also, Kwon and others (2008) showed that the number of volatiles did not differ between beef and pork meats.

20.8 Control of Quality Changes in Irradiated Meat and Meat Products

20.8.1 Additives and Chemical Treatments

Several researchers showed that the possible quality changes in irradiated meat and meat products could be controlled by using different additives or antioxidants. Shults and others (1977) reported that samples of cooked pork rolls containing 0.75% sodium chloride and 0.3% sodium tripolyphosphate showed no decreases in their shear press values after irradiation at 30, 45, and 60 kGy at –30°C. Papadopoulos and others (1991) showed that addition of antimicrobial agents had a positive effect on improving the quality of irradiated meat products. They found that injection of sodium lactate into vacuum-packaged beef top rounds produced higher cooking yield and darker red color with less gray surface area. Moreover, the flavor notes associated with fresh beef were enhanced by this treatment, while the deterioration of flavor during storage was minimized. Zhao and Sebranek (1996) investigated the oxidation development in pork chops that dipped in solutions of 0.5% sodium tripolyphosphate, 550 ppm sodium ascorbate, or 0.1% potassium sorbate prior irradiation at 1 kGy. They indicated that preirradiation treatment with sodium tripolyphosphate (STPP) effectively reduced drip loss and provided lipid stability against radiation-induced oxidation. Experiments also have shown that preseasoning of beef sirloin steaks with ginseng decreased the formation of malondialdehyde due to electron-beam irradiation of samples (Wong and Kitts 2002). The effectiveness of phenolic antioxidants in reducing the oxidative reactions in irradiated pork meat, through scavenging free radicals, was reported by Nam and Ahn (2003a). The same authors showed in another study that the addition of ascorbic acid to ground beef maintained heme pigments in ferrous state and stabilized the color of irradiated beef (Nam and Ahn 2003b). On the other hand, ascorbic acid and sesamol + tochopherol lowered the amount of dimethyl disulfide in irradiated

ground beef (Nam and others 2003). Badr (2007) examined the antioxidant activity of carnocine in γ-irradiated ground beef and beef patties and found that addition of carnosine increased the oxidative stability of raw and cooked samples and reduced metmyoglobin formation in the raw samples. In addition, carnosine excreted significant efficacy in maintaining an acceptable visual red color for the tested samples postirradiation and during storage. The addition of rosemary extracts was effective in controlling the development of off-flavor and lipid oxidation during cold storage of electron-beam irradiated fermented sausage (Lim and others 2008). The potential application of rosemary and oregano extracts as a natural antioxidants in irradiated beef burgers for maintaining a low oxidation level and acceptable sensory quality was also illustrated by Trindade and others (2009, 2010).

20.8.2 Packaging Atmosphere

Irradiated meat odor and flavor varied with gas atmosphere in the packaged product. It is well established that the presence of oxygen during irradiation often results in radiolytic changes that precipitate oxidation and other unacceptable secondary breakdown products. By exclusion of oxygen, the detrimental effects of irradiation can be reduced. With vacuum-packaging and irradiation at dose of 2.5 kGy, smoked bacon had good sensory characteristics during postirradiation storage for 3 months at 4°C (Risvik 1986). Ahn and others (2000a) stated that irradiation had no effect on the odor preference of pork patties with vacuum-packaging. Incorporation of low levels of carbon monoxide (<1%) into modified atmosphere packaging greatly improved color and odor quality of irradiated fresh ground beef and reduced the oxidation of lipids (Kusmider and others 2002). The authors added that samples that irradiated at 4.5 kGy provided a very stable, cherry-red color as indicated by instrumental and sensory analysis. Irradiation at doses up to 4.5 kGy did not accelerate the oxidation of lipid in vacuum-packaged cooked pork sausages and their values of TBARS did not increase with storage (Jo and others 2003). The combined effect of irradiation and modified atmospheric packaging on color and nitrosoheme pigment in sausage during storage was also studied by Ahn and others (2004). They observed that vacuum or modified atmosphere packaging was effective in minimizing the loss of redness and effectively maintained the nitrosoheme pigments than aerobic packaging.

20.8.3 Combined Treatments

Combination of different treatments showed good results for maintaining the quality of irradiated meat products. Results arrived at by different researchers have shown that incorporation of ascorbic acid into ground beef and spice powder into edible coating film can stabilize lipid oxidation and production of –SH radicals in ground beef during postirradiation storage. This can be attributed to the ability of ascorbic acid and many compounds in spices to act as scavengers for oxygen and free radicals (Demos and others 1996, Mitsumoto and others 1991). Murano and others (1998) showed that samples of ground beef that were vacuum-packaged and irradiated at 2 kGy and then frozen at –25°C before cooking gained in texture and juiciness attributes and had less aftertaste 1 day after frozen precook storage. Concentrations of TBARS and free sulfhydryl were stabilized during postirradiation storage for samples of irradiated ground beef (1–3 kGy) containing ascorbic acid and coated with a protein-based coating containing spices (Ouattara and others 2002). Furthermore, the addition of α-tocopherol into ground beef combined with packaging with a charcoal pack was found to be a good method for effective elimination of an irradiation off-odor and retardation of the development of lipid oxidation in the irradiated ground beef (Sohn and others 2009).

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21

Slaughtering Operations and Equipment

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21.1 Introduction

Slaughtering, the first step in the transformation of muscle into edible meat, affects the quality and quantity of meat. This depends on postmortem biochemical changes, related to sanitation, as well as physicochemical and physical attributes (aroma, color, and texture among others). From the quantity point of view, carcass yield is related to pre- and postmortem handling. However, carcass handling after leaving the slaughter floor, also alters meat quality. A number of operations are included during transformation of muscle into meat, starting from slaughtering through refrigeration, wholesale storage, fabrication and distribution, and retail storage. Each one includes changes in physicochemical and microbial characteristics that finally affect quality. In any case, changes must be directed to improve quality and/or to extend shelf life.

21.2 Preslaughter Conditions Affecting Meat Quality

The main factors that affect meat quality are preslaughter stress and halothane genotype. Stress is the inevitable consequence of processes of transferring animals from farm to slaughter. The preslaughter phase includes the conditions and practices that apply during the period when the animals are moved. During this time, animals can be exposed to a range of challenging stimuli: handling and increased human contact, transport, unfamiliar environments, food and water deprivation, changes in social structure, and so on (Ferguson and Warner 2008). In glycolytic muscles, such as *Longissimus dorsi*, high physical and psychological stress levels associated with the immediate preslaughter period have a greater effect on meat water-holding capacity (WHC) and may promote pale, soft, and exudative development (Hambrecht and others

2004, 2005). These authors studied the physiological changes elicited in response to stress in immediate preslaughter periods and link them to pork quality characteristics. They concluded that a high preslaughter stress leads to impaired pork quality, with high muscle energy levels aggravating the negative effects of preslaughter stress. Monitoring stress level by blood lactate measurement in combination with strategies to control muscle energy during slaughtering may help improve meat quality. An increase in blood lactate concentration is associated with preslaughter stress, such as aggressive handling immediately prior to stunning; these actions show detrimental effects on pork quality and can be used as an indicator of differences in the overall handling experience of individual pigs (Edwards and others 2010).

A number of reports describe the modifications induced in horse behavior by commercial transports, all data underline that the response to stressors is related to type and length of the transport. Micera and others (2010) concluded that plasma cortisol, epinephrine, norepinephrine, and β-endorphin are main stress indicators in horses, suggesting that, under stress, the release of β-endorphin could be different from that of ACTH. There was no adverse effects in rabbits with respect to the loading method, the stress condition evidenced by hematological and biochemical parameters prior to slaughter, did not affect meat quality (Mazzone and others 2010).

Young and others (2009) found that only 1h of rest after stress by exercise was enough to reduce these effects, but interesting overshooting effect was noted when pigs were allowed to rest for 1–3 h before slaughter. They concluded that quality characteristics such as drip loss and toughness are not related to stress by exercise in pigs. Bertram and others (2010) applied nuclear magnetic resonance to study the effect of preslaughter exercise stress on plasma metabolite profile at the time of slaughter. It resulted in highly correlated muscle temperature until 1 min, mainly due to WHC. Lactate was found to be the most important metabolite in correlation to plasma metabolome and pH, temperature, and WHC.

Texture is one of the most important components of meat quality. In pigs, halothane susceptibility has been repeatedly shown to negatively affect tenderness. Monin and others (1999) studied the effect of three halothane genotype (NN, Nn, and nn HAL), slaughter weight, and the HAL × slaughter weight interaction. The nn allele of HAL gene unfavorably affects pork texture, this effect was maintained throughout for body weight between 101 and 127 kg. However, several methods have been developed to improve tenderness, such as electrical stimulation. It was originally developed to avoid cold shortening and toughening. The effect of high-voltage stimulation on the tenderness of steers and heifers with a range of known *B. indicus* and *B. taurus* genotypes enabled the contribution to the genotypes to final meat tenderness (Gursansky and others 2010).

21.3 Stunning

Stunning is the ability loss to experience physical experiences due to a mechanical, chemical, or electrical process that temporarily interrupts sensory perception (Rosmini 2006). In animals, stunning is applied in order to establish an unconscious state (Gregory and others 2009) and, in this way, to ensure it will not recover before drying by bleeding. At the same time, stunning ensures animal immobilization to avoid any damage to the operators during handling prior to the animal's death. The stunning methods must fulfill several characteristics (Rosmini 2006):

- Not affecting the meat shelf life, therefore the method must allow rapid bleeding avoiding contamination of internal tissues.
- Not affecting meat quality regarding its physical, chemical, and sanitary characteristics.
- To stun the animal preventing any unnecessary pain and suffering, but without causing its death.
- Easy to apply, requiring the minimum effort to the operator and avoiding difficulties in the use of stunning devices.
- Producing a rapid and efficient effect, adapting to the processing line and avoiding process repetitions.
- With no risks to the operator, either due to the direct application or to the animal's defensive movements.

Although sanitary legislations require animal stunning before slaughtering, in almost all countries several exceptions are considered due to religious or ethnic reasons. These are the cases of *kosher* (Regestein and Regestein 2010) and *halal* (Che Man and Qurni Sazili 2010) food-processing methods. Slaughtering without stunning is recognized as a religious prerogative, but there have been concerns that the method can comprise the welfare of the animals due to the time it takes to collapse, or for the prevalence of blood in the respiratory tract (Gregory and others 2009, 2010).

Stunning methods can be divided into three groups, according to the principle the main device uses: electricity, contusion, and gas narcosis.

21.3.1 Electric Stunning

Electricity promotes unconsciousness due to an epileptic shock caused by massive neurotransmitters (glutamate and aspartate) discharge to the brain; when efficiently applied, it presents three phases: tonic, clonic, and recovery (McKinestry and Anil 2004). Electric shock stunning is usually applied to pigs and sheep, although in some cases is also used in cattle. The epileptic shock depends on the voltage intensity passing through the brain. If electricity goes through the brain, the animal is only stunned, and bleeding must be carried out within 30 s because the animal can recover consciousness. The electrodes must be placed on each side of the head and intensity must not be <250 mA and 75 V during 10 s (Grandin 1980); they are kept moist in a 20% saline solution to assure electricity conduction. If bleeding is not carried out in a pig after 2–3 min of stunning, it will recover and in 5 min will be completely normal. If electricity goes through the heart, the shock is irreversible and kills the animal by electrocution. If electric shock is adequately applied, the animal will not feel any pain. Conversely, if amperage is not high enough, the animal may feel a painful shock. If applied to cattle, electrical stunning must be applied in two stages; in a first stage a 2.5 amp discharge is applied in the head, and in a second the current is passed through the entire body (Gregory and others 2010).

Commercially available electrical stunning equipments have the following characteristics (Rosmini 2006): alternate current; voltage from 34–40 to 1000 V (low: 35–90 V; medium: 100–300 V; high voltage: 400–1000 V), although low voltages are not efficient to cause unconsciousness. The most widely used conditions are 240 V, 1.25–1.3 amp. Most equipments use 50–150 Hz. The region where the electrodes are placed depends on the animal species.

The main advantage of electrical stunning is its high efficiency to cause unconsciousness when properly applied according to electric discharge, electrode placing, time, among others. In addition, it is noiseless therefore the animal is usually not affected by equipment vibrations. However, electric stunning causes hemorrhages more frequently than other stunning methods (Channon and others 2003). These are due to the rupture of small blood vessels due to muscle constriction and increase in blood pressure (Lawrie 1998) or to severe myofibrillar contraction (Channon and others 2002). Most large processing plants apply electric stunning restraining the animal in a confined area. The stunning area must be isolated and kept dry and the operator must wear rubber boots and stand on insulated ground.

21.3.2 Contusion Methods

The principle underlying the use of these equipments is the trauma caused in the brain by a blow or by penetrating the skull, causing total or partial unconsciousness. They include captive-bolt and pneumatic pistols. Stunning with a captive bolt pistol consists in projecting a metallic cylinder into the animal's skull, this penetration is combined with a blow caused by the pistol against the animal's head; the cylinder is then returned to their original position for the next shot. However, in order to achieve an efficient stunning, the anatomical location where the pistol is placed must be carefully chosen. In calves, swine, horses, and cattle (*Bos taurus*), the muzzle is applied to the forehead. In Cebu cattle (*Bos indicus*), sheep and in plants industrializing the brains, it is applied at the back of the head, due to thickness of the forehead in *Bos indicu*s and sheep, or to avoid brain destruction (De la Puente 2002, pers. commun).

Pneumatic pistols impact the animal's head without penetration, using compressed air at 160–180 psi; the blow permanently damages the brain cortex. Captive-bolt pistols are used in sheep and cattle; when this stunning method is used in pigs, the muzzle is applied just above the eye level, in the center of the

forehead. The main problem in using pneumatic pistols is that if compressed air is too high it could harm the operator's hands, back, or arms. In addition, it is used only when the slaughtering rate is <240 or more animals per hour because recharge is time consuming.

21.3.3 Carbon Dioxide

It was developed specially for pigs although it is also applied to sheep; the stunning action of carbon dioxide (CO_2) is accomplished by blockade of the animal's neural terminals, reducing the nervous impulses. CO_2 concentrations of 65–70% during 45 s work most efficiently (Velazco 2000). CO_2 stunning reduced hemorrhage incidence in the carcass and risks to the operators as animals defensive movements are also reduced (Rosmini 2006). It is reported that meat quality is improved (Channon and others 2003). However, Becerril-Herrera and others (2009), comparing the effect of two different stunning methods, electrical stunning and to exposure to CO_2, concluded that CO_2 stunning leads to a major imbalance because of mineral and acid–base gaseous interchange, compared to electric stunning, thus possibly compromising animal welfare. The main disadvantage of this method is that facilities building and maintenance costs are very high. Gas application must be carefully controlled; if the gas concentration is low, the animals are not adequately stunned. If it is too high, there is a tendency for stiffness, reflex muscular activity, and inadequate bleeding (Grandin 1991). If time of exposure is too long, skin congestion can occur and the carcass can take a bluish hue after scalding. CO_2 is heavier than air, so all devices operate on the basis of keeping the gas at low levels, making the animals descend to an area where suitable gas concentrations are fed, such as the oval tunnel (for slaughtering 120–240 animals/h). Vergara and others (2009) studied the effect of different gas stunning methods on light lamb meat quality in order to define the best CO_2 concentration (80% and 90% volume in air) and exposure time (60 and 90 s) to maximize the meat quality. The authors concluded that there were no significant differences among treatments with respect to color, a minimum of 90% CO_2 for 90 or 60 s was recommended; lower CO_2 concentrations (80%) could cause meat hardening.

21.4 Bleeding

During unconsciousness, the animal dies by massive bleeding. There are a number of reasons to promote the animal's death during this period, the main one is for humane considerations. In addition, biochemical reactions proceed to obtain the optimum meat quality when the animal is properly stunned and slaughtered. When stunned, the animal is still alive; therefore, bleeding is achieved when the heart and respiratory functions are still working. Bleeding is carried out by sectioning the carotid arteries and the jugular vein with a sticking knife (15–25 cm long). The point of the knife is inserted about 2 cm in front of the breastbone, an incision is made toward the jaw, penetrating 12–15 cm and sectioning the carotid artery and jugular veins. Care must be taken when introducing the knife to avoid puncturing the pleura, as this could cause the blood to flow into the chest cavity, increasing the risk of microbial contamination. In sheep, the sticking knife is inserted immediately below and behind the ear, severing the jugular vein. In beef, an incision is made just in front of the sternum, also cutting into the main blood vessels (De la Puente 1996). Because blood is a vehicle for microbial distribution throughout the animal body, microorganisms introduced during sticking can be found after a few hours in other parts of the carcass.

Dikeman and others (2003) studied the effects of postexsanguination vascular infusion of cattle with a solution of saccharides, sodium chloride, and phosphates or with calcium chloride on quality and sensory traits of steaks and ground beef, there were no distinct meat quality advantages for infusing cattle with a solution of saccharides, sodium chloride, and phosphates. Infusion with 0.3 M $CaCl_2$ increased dressing percentage, but caused severe muscle contraction during early postmortem, markedly decreased *Longissimus lumborum* tenderness and reduced flavor.

Room temperature must be reduced to 10°C during slaughtering, bleeding, evisceration, and cleaning, as carcass temperature affects microbial growth. However, bleeding also reduces microbial contamination

to a large extent. It also prevents the formation of "blood-splash" due to pressure built within the muscles, decreasing meat acceptability, and increasing hygienic risk (Palumbo and others 1996).

Blood is also of economic importance as it can be transformed into meal for animal consumption or used in pharmaceutical applications. Extracted blood, on an average, is 3.8–4% of live weight or 6.6–8% of carcass weight, although these percentages vary widely depending on the species: cattle (5–6%), horses (9–10%), pigs (3–3.5%), sheep (7%), and goats (8%) (Rosmini 2006). It is carried out in curbed-in areas with enough space to prevent blood from splashing on stunned animals or on carcasses; the carcass is hung in a rail head down to allow maximum blood drain. Rails 4.8 m high above the floor should be used for beef bleeding; 3.3 m for pig, sheep, and calf; 2.74 m for goats (Guerrero-Legarreta and Pérez-Chabela 2001). Bleeding must be carried within a minimum time from stunning to take advantage of the stunning effect and, at the same time, to avoid hormones freed by stress to distribute throughout the animal's body, affecting meat quality (Rosmini 2006).

21.5 Dressing, Evisceration, and Cleaning

After bleeding, the carcass undergoes cleaning operations by various methods, depending on the species. Cattle, sheep, horse, and goat hides are removed; viscera are also removed from the carcass of all species, and finally the carcass is trimmed and washed with pressurized water. The head, feet, and tail are left attached to the pig carcass, whereas head and hide are removed from beef carcasses.

21.5.1 Dehiding

Beef carcass dressing starts with dehiding making and incision on the neck centerline toward the head. The animal is then scalped, the horns chopped, and the head is skinned out. A cut is made across the larynx and the head is detached by cutting through the occipital joint. The front legs are cut, leaving the knucklebones on the carcass and the hind legs are cut through the tendons; the hide is then removed using a small saw. Legs in sheep carcasses are cut from the knuckle down the front legs; the skin is removed, starting from the neck up to the root of the tail; and the hind legs are cut.

21.5.2 Pork Carcass Hair Removal

For obvious reasons, pig hides are not removed; in this case, carcasses are scalded in tanks with water at 60–65°C heated by steam. The scalding vats are equipped with a cradle to remove the pig after scalding and before scraping. Scalding process of up to 9 min may be needed for adequate hair removal; however, carcass temperature may increase during extended scalding. Reducing the duration of scalding allows for earlier evisceration and entry into the cooler, which may minimize the protein denaturation due to prolonged exposure to high muscle temperatures. The carcass is then hung from the hind legs in a shaving rail equipped with a drained cabinet washer. The remaining hair is then burned off by using a blowtorch or by passing the carcass through a furnace, allowing the skin to become dry and hard. Gardner and others (2006) studied the specific effects of extending the interval between dwell time and duration of scalding on pork quality attributes. Treatments included extending the dwell duration from 5 to 10 min and extending the scald duration from 5 to 8 min. Lengthening the duration of dwell and scalding resulted in a more rapid postmortem pH decline. Reducing the duration of scalding may lead to increased time for manual removal of hair.

21.5.3 Evisceration and Trimming

The most frequently used edible by-products are blood, liver, heart, kidney, tongue, heart, stomach, and lungs. The specific by-product, organ or tissue, considered edible by a given human population group depends on facts such as tradition, religion, and culture (Guerrero-Legarreta 2010). Inedible viscera and by-products are also utilized in other industries such as animal feeds, fertilizers, or pharmacy: gall

bladder, bones, and hooves from beef, sheep, and pigs; horns and feet from sheep and beef; wool and skin from sheep. Beef hide and hair from pigs are inedible by-products. Scraps and condemned parts can also be used for meat meals. Pharmaceutical products are obtained from slaughter by-products: thymus and thyroid of beef, sheep, and pig; pig stomach lining; sheep prostate and intestine; beef and sheep pancreas and suprarenal glands; pig and beef ovaries and spleen; and beef pituitary and pineal glands, corpus luteum, parathyroid.

To remove the viscera, the abdominal cavity is opened by cutting behind the brisket; the liver is removed by cutting it from the diaphragm, which in turn is cut. The content of the chest cavity is removed and the aorta is trimmed. The body is then sawn, dividing the carcass into two sides. To prevent microbial contamination it is important to trim excessive tissue, the area where the sticking knife was introduced if this was the case, and the spinal cord. The carcass is then washed by high-pressure spraying (19 kg/cm^2). Inclusion of head, hide, and feet in beef and sheep carcass weight depends on commercial agreements in each country or region.

21.6 Storage

Owing to its composition making them a suitable substrate for pathogens and spoilage microorganisms, carcasses and viscera should be stored under refrigeration conditions. Refrigeration is any process that removes heat from a material (Chanona-Pérez and others 2006).

Once the carcass is clean, refrigeration is necessary to 8°C or below to delay the multiplication of psychrophilic organisms associated to spoilage or any microorganisms of public health significance (Manral and Bawa 2010). Refrigeration also restricts enzymatic activity, although if ripening (or aging) is desirable as in the case of added-value beef, storage at higher temperature (12–15°C) during the initial postslaughtering period is necessary with further reduction to 4–6°C. Therefore, the optimum cooling temperature and time vary with the species and desired product (Alarcón-Rojo and Pérez-Chabela 2006).

In commercial slaughtering facilities, the types of installed refrigerators are bulk storage rooms. In these, the air movement around the unwrapped carcasses should be the minimum required to maintain a constant temperature so as to minimize weight loss and appearance changes associated with desiccation. Air distribution must be as homogenous as possible (Manral and Bawa 2010).

In few cases, controlled atmosphere storage rooms ensure optimal quality due to the considerable reduction of aerobic microorganisms; these types of storage rooms must include gas-tight seals to keep atmospheres with low oxygen and high nitrogen and CO_2 concentrations, making these facilities expensive. Additional installations are necessary to control gas concentrations (Manral and Bawa 2010)

The average temperature of a cooler must be between 2°C and 6°C. A certain degree of "sweating" occurs when relative humidity is 70% or more. Required building material in coolers must be easy to clean; floors must have a slope to allow proper drain. Rails should be at least 3.3 m above the floor for halves of beef, 9 m for calves and hogs, and 2.25 m for quarters of beef; goat and sheep carcasses should be suspended 1.95 m to the hook. Carcasses must be hung 1 m from the walls and 0.60 m from the refrigeration equipment, with a separation among carcasses of 0.30 m (Guerrero-Legarreta and Pérez-Chabela 2001). Edible organs and offal should be placed in a separate cooler of the carcasses. Retained carcasses or parts should be located in a refrigerated separate compartment.

21.7 Slaughter Plant Facilities

It is recommended that slaughtering plants are situated far from areas where objectionable odors or particles are generated, such as dumps or chemical plants; they should also have accessibility; therefore, they should be connected to streets or highways but separated from other plants or buildings. Drinking water supply must be in good quantity because washing is a continuous operation throughout the plant. Carcasses are washed after dressing, so any bacterial contamination in the water supply will be passed to the meat substrate. As water contains high amount of grease, blood, hair, tissue, and bone particles,

water disposal must be carefully calculated. In addition, rumen and intestine content must be discarded according to local regulations, as this material is highly polluting. Building materials for walls, floors, drains, ceilings, and equipment are also subject to regulations. All these materials should provide easy cleaning. In general, these materials comprise concrete, ceramic floor tile, floor-glazed brick, glazed tiles, smooth surface Portland cement plaster, plastic, or Portland cement plaster for ceilings. Certain materials in particular are not acceptable, such as lead, porcelain, wood, leather, fabrics, or any material that undergoes chemical reactions.

Other construction characteristics must be considered, such as slopes in floors to avoid accumulation of effluents; ceilings not <3 m high or more in certain working areas, such as those for evisceration and cleaning and free of unnecessary structures; to avoid square angles or joints where material can accumulate; nonslippery floors; illumination (220 lux in the working areas, 540 lux in the inspection areas, and 110 lux in the coolers); pedal-operated hand-washing facilities and drinking fountains; insect and rodent barriers in all windows, doors, and other openings (Arthur 2010).

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22

A Review of Kosher Laws with an Emphasis on Meat and Meat Products[*†]

Joe M. Regenstein and Carrie E. Regenstein

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[*] Revised version of an original article by Regenstein, J.M., Chaudry, M.M., Regenstein, C.E. 2003. The kosher and halal food laws. *Comprehensive Reviews in Food Science and Food Safety* 2(3):111–127. Courtesy of Wiley-Blackwell (US/UK). It has been further revised from the version that appeared in the companion volume on poultry.

[†] *Preliminary Note*: The information in this article is as accurate as possible (as of September 1, 2010). However, the final decision on any application of the material rests with the religious authorities providing supervision. The ruling of the religious authorities may differ from the information presented here.

22.1 Introduction

The objective of this chapter is to describe the kosher laws as they apply in the food industry, with particular emphasis on red meat (mainly beef, sheep, and goat, although water buffalo, bison, deer, and elk may also be slaughtered as regularly as kosher). The focus is on practices in the United States. To understand the impact of kosher in the marketplace, one must have some understanding of how kosher foods are produced, and how important kosher compliance is to consumers. To best appreciate the scope of kosher and how it impacts on the food industry, topics beyond those specifically related to red meat are covered. Many people in the food industry move between different segments of the industry and it is worth having a more complete picture of kosher.

22.2 The Kosher Laws

We start by focusing on the religious significance of the dietary laws for Jews. The kosher (kashrus) dietary laws determine which foods are "fit or proper" for consumption by Jewish consumers who observe these laws. The laws are Biblical in origin, coming mainly from the original five books of the Holy Scriptures, the Torah, which have remained unchanged. At the same time that Moses received the Ten Commandments on Mount Sinai, Jewish tradition teaches that he also received the oral law, which was eventually written down many years later in the Talmud (the Mishnah). This oral law is as much a part of Biblical law as the written text, although other religions have only adopted the written Hebrew scriptures. Over the years, the meaning of the Biblical kosher laws has been interpreted and extended by the rabbis to protect the Jewish people from violating any of the fundamental laws, and to address new issues and technologies. The system of Jewish law is referred to as "halacha." The initial discussions of the law occur in the Talmud (the Talmudic commentaries known as the Gemmorrah, which contain the conflicting opinions of various rabbis who lived prior to the codification and which is written in a number of different languages). The most important relatively modern recodifications of the laws occurred in the 1600s, through a religious text called the Shulchan Aruch ("set table") by Rabbi Joseph Karo and was shortly followed by a commentary by Rabbi Moses Isserles, known as the Aruch Ha'Shulcan ("tablecloth"). These latter texts remain the primary basis of the current practice of the kosher laws. The text of Rabbi Isserles is followed by the Jews of Europe, the predominant sect of modern Judaism, especially in those cases where the recommendations differ from those of Rabbi Karo. However, Jews of African and Asian descent tend to follow the rulings of Rabbi Karo in all matters.

Why do Jews follow the kosher dietary laws? Many explanations have been given. The explanation by Rabbi I. Grunfeld given below summarizes the most widely held ideas about the subject (Grunfeld 1972) and serves to illustrate the fundamental importance of the kosher laws within the larger system of halacha.

The Kosher laws are viewed by the Jewish community as part of a group of laws given to the community without a need for explanation. Only in modern times have some people felt a need to try to justify them as health laws. For a discussion on why the kosher laws are *not* health laws, see J.M. Regenstein, 1994.

> "And ye shall be men of a holy calling unto Me, and ye shall not eat any meat that is torn in the field" (Exodus XXII:30). Holiness or self-sanctification is a moral term; it is identical with … moral freedom or moral autonomy. Its aim is the complete self-mastery of man.
>
> To the superficial observer it seems that men who do not obey the law are freer than law-abiding men, because they can follow their own inclinations. In reality, however, such men are subject to the most cruel bondage; they are slaves of their own instincts, impulses and desires. The first step towards emancipation from the tyranny of animal inclinations in man is, therefore, a voluntary submission to the moral law. The constraint of law is the beginning of human freedom …. Thus the fundamental idea of Jewish ethics, holiness, is inseparably connected with the idea of Law;

> and the dietary laws occupy a central position in that system of moral discipline which is the basis of all Jewish laws.
>
> The three strongest natural instincts in man are the impulses of food, sex, and acquisition. Judaism does not aim at the destruction of these impulses, but at their control and indeed their sanctification. It is the law which spiritualizes these instincts and transfigures them into legitimate joys of life (pp. 11–12).

22.3 The Kosher Market

Why are we concerned about kosher in the secular world? Because kosher is an important component of the food business, especially in the United States and also in many other countries. Most people, even those in the food industry who may deal with some aspects of kosher in their work, are not aware of the range of foods that are under religious supervision. This section provides background on the economic aspects that make it important for the food industry to have a better understanding of kosher.

The kosher market according to Integrated Marketing, an advertising agency specializing in the kosher food industry, comprises almost 150,000 products in the United States. In 2009, about $200 billion worth of finished products are estimated to have a kosher marking on them. However, the deliberate consumers of kosher food, that is, those who specifically look for the kosher mark (see below), are estimated to be around 10 million Americans and they purchase almost $20 billion worth kosher products. Less than 1/3 (possibly as low as 20%) of the kosher consumers are Jewish (approximately 1 million year-round consumers). However, Jewish consumers keeping kosher obviously purchase all of their food as kosher and therefore represent a larger percentage of the dollar value of kosher. Their overall purchases are also about twice the amount yearly as that of the average consumer, both because of the higher price of certain kosher products, particularly meat, and because of larger family sizes. Other consumers, who at times find kosher products helpful in meeting their dietary needs, include Muslims, Seventh Day Adventists, vegetarians, vegans, people with various types of allergies—particularly to dairy, grains, and legumes—and general consumers who value the quality of kosher products, even though there is rarely a one-to-one correlation between kosher and these consumers' needs. Hebrew National's slogan "We report to a higher authority" and "You don't have to be Jewish to love Levy's Rye Bread" are two of the more famous campaigns used to advertise kosher products to nonkosher consumers. *AdWeek Magazine* in the early 1990s called kosher "the Good Housekeeping Seal for the 90s." By undertaking kosher certification, companies can incrementally expand their market by opening up new markets. It should be noted that although many supermarkets define the kosher consumer in their scanning data as someone who only purchases products with kosher supervision symbols on the package, there are products that the rabbis accept that do not always need to have a supervision mark as we will describe. This chapter also includes information that might assist kosher supervision agencies in addressing the specific needs of these other consumer groups.

The Muslim population in the United States is developing a stronger marketplace presence each year. Over the past 30 years, many halal markets and ethnic stores have sprung up, mainly in the major metropolitan areas. Most of the 6–8 million Muslims in North America observe halal laws, particularly the avoidance of pork, but the food industry has, for the most part, ignored this consumer group. Although there are excellent opportunities to be realized in the North American halal market, even more compelling opportunities exist on a worldwide basis as the food industry moves to a more global business model. The number of Muslims in the world is over 1.3 billion people with another 0.2–0.3 billion people living in countries where much of the market is halal and trade in halal products is about $150 billion (Egan 2002). Many countries of South Asia, Southeast Asia, the Middle East, and Northern Africa have predominantly Muslim populations. Although only about 15% of India's population is Muslim, it is the second largest Muslim country in the world after Indonesia. Most meat in India is slaughtered by the Muslim community and is halal. In many countries halal certification has become necessary for products to be imported.

Although many Muslims purchase kosher food in the United States, these foods do not always meet the needs of Muslim consumers. The most common areas of concern for Muslim consumers when considering purchasing kosher products are the use of various questionable gelatins in products produced by more lenient kosher supervisions and the use of alcohol as a carrier for flavors, as well as a food ingredient. The details of both ideas are developed later in this chapter. The special issue of kosher and halal slaughter will be taken up as a separate discussion.

With the agreement of the client company, kosher supervisors can address the needs of the non-Jewish markets. A document establishing preliminary guidelines for making kosher appropriate for all the groups mentioned above without violating Jewish law has been prepared (J.M. Regenstein, personal communication) and serves as a basis for a multicultural kosher dining program at Cornell University (dining.cornell.edu/docs/multicultural_doc.pdf). Other universities have established or are also exploring kosher/halal and multicultural food options. Hopefully in the future, more kosher foods will pay more attention to the needs of other user groups.

Although limited market data are available, the most dramatic data illustrating the impact of kosher certification in the marketplace are that of the Coors Brewing Company. According to their market analysis, their share of market in the Philadelphia market went up 18% when the company went kosher. Somewhat less dramatic increases were observed in other cities in the Northeast. Dannon Yogurt experienced a growth in sales when it switched from a "lenient" kosher certification to one that was normative mainstream (see Section 22.7.5.1). A Northeast soda bottling company let its kosher certification lapse and as a result their sales dropped significantly. The company quickly got recertified!

In recent years, many of the large national companies have gone kosher. For some, the effort has been quite extensive. For example, when Nabisco took many of its cookie products kosher, the process of equipment kosherization (see Section 22.5.1) took over 3 years before its many bakeries around the country were kosher and all its kosher products could finally be marketed in the United States. To consider whether a company wants to participate in the kosher (or halal) market, its management needs to have some knowledge about the laws themselves to determine the potential profitability.

22.3.1 The Kosher Dietary Laws

The kosher dietary laws predominantly deal with three issues, all focused on the animal kingdom:

1. Allowed animals
2. Prohibition of blood
3. Prohibition of mixing of milk and meat

Additionally, for the week of Passover (in late March or April) restrictions on "chometz," the prohibited grains (wheat, rye, oats, barley, and spelt) in other than unleavened form—and the rabbinical extensions of this prohibition—lead to a whole new set of regulations, focused in this case on the plant kingdom.

Ninety-two percent of American Jews celebrate Passover in some way, making it the most observed holiday in the Jewish calendar. It also accounts for about 40% of the sales of kosher products to the Jewish community. Although only 20–33% of the kosher market in the United States is Jewish, these consumers account for over half of the total dollar volume of the kosher market, since they purchase kosher food more consistently.

In this chapter, we discuss additional laws dealing with special issues such as grape juice, wine, and alcohol derived from grape products; Jewish supervision of milk; Jewish cooking, cheese making, and baking; equipment kosherization; purchasing new equipment from non-Jews; and old and new flour. The role of slaughter and postslaughter handling of red meats will also be highlighted in more detail than has been covered in previous papers on kosher by the authors.

The kosher laws are an internally consistent logic system and have an implied "science" behind them—which may or may not agree with modern science. This system is the basis on which rabbis work through problems (by responding to questions) and come up with solutions (by writing a response,

a written document that sets out their position). Once their position is public, other rabbis have the right to accept or reject the position taken, which may be done simply by telling their followers or may be done in writing. Over time, the broader community accepts and rejects many of these responses to the point where a position becomes normative. In general, once that happens, the rejected responses are no longer considered as a basis for decision making. This sorting process may be relatively fast, but in other cases it can take many years. In those cases where the religious texts do not define the religious "science," modern science can be helpful. However, where it disagrees with the texts, the texts of accepted responses will often predominate.

22.3.2 Allowed Animals

Ruminants with split hoofs that chew their cud, the traditional domestic birds, and fish with fins and removable scales are generally permitted. Pigs, wild birds, sharks, dogfish, catfish, monkfish, and similar species are prohibited, as are all crustacean and molluscan shellfish. Almost all insects are prohibited such that carmine and cochineal, which are used as natural red pigments, are not permitted in kosher products by most rabbinical supervisors. However, honey and shellac (lac resin) are permitted as will be discussed later in this section.

Four classes of prohibited animals (mammals) are specifically described in the Torah. These are those animals that have one kosher characteristic but not both. For example, the rockbadger, the hare, and the camel chew their cud but do not have a split hoof; the pig has a split hoof but does not chew its cud. Neither category is more or less nonkosher nor are those animals with neither trait; quite simply, none of these animals are kosher, and these specific examples are listed specifically in the Torah only to clarify the text. In modern times, the prohibition of pork has often been the focus of both kosher and halal laws, since pork is such a major item of commerce, both in Western and Eastern societies. The ability to specifically detect pork and pork derivatives in other foods, particularly as an adulterant, has become a major research topic in Israel and many Muslim countries. Interestingly, giraffe is a true ruminant and has a split hoof rendering it kosher, with specific guidelines about its proper slaughtering procedures. However, in addition to the importance of the animal having both signs of a kosher animal, there is also in Jewish law the concept of the importance of there being a tradition of eating that animal within the Jewish community. Without such a tradition, some animals that might be considered kosher may be rejected. In recent years two rabbis (Rabbi Zivotofsky and Rabbi Greenspan) in Israel have made a point of having "game dinners" to ensure that the tradition of use of some less common animals and birds is maintained. Interestingly, what is accepted in Israel and America at these dinners has been different.

With respect to poultry, the traditional domestic birds, that is, chicken, turkey, squab, duck, and goose are kosher. Birds in the rattrie category (ostrich, emu, and rhea) are not kosher as the ostrich is specifically mentioned in the Bible (Lev. XI:16). However, it is not clear whether the animal of the Bible is the same animal we know today as an ostrich. This is a problem that is often encountered with ancient texts—the Hebrew words for animals are difficult to determine in modern times.

There is a set of criteria that are sometimes referred to in trying to determine if a bird is kosher. The kosher bird has a stomach (gizzard) lining that can be removed from the rest of the gizzard. It cannot be a bird of prey. Based on the concept of tradition, some rabbis do not accept wild turkey while others do not accept the domestic turkey. Some others do not accept the featherless chicken.

The only animals from the sea that are permitted are those with fins and scales. All fish with scales have fins, so the focus is on the scales. These must be visible to the human eye and must be removable from the fish skin without tearing the skin. Cycloid and ctenoid scales found on traditional fish are generally considered acceptable, but the ganoid and placoid scales of sharks, gar, and so on are generally not accepted. A few fish remain controversial, probably the most controversial being the swordfish, whose scales do not seem to belong to any of the biologists standard scale types. The Conservative movement permits swordfish and also permits sturgeon, which most Orthodox authorities consider both nonkosher. A recent publication that included Rabbi Zivotofsky and his brother who is a veterinarian, along with American scientists and veterinarians, has looked at the scalation of the swordfish (Govani et al. 2004). It is not clear if the juvenile scales are visible and removable without tearing the skin as required by

Jewish law. Thereafter, the scales descend into the skin and certainly become nonremovable. Although most Orthodox groups do not accept swordfish, the most recent game dinner (2010) in Israel discussed above included swordfish!

Most insects are not kosher. The exception includes a few types of grasshoppers, which are acceptable in the parts of the world where the tradition of eating them has not been lost. The edible insects are all in the "grasshopper" family identified as permitted in the Torah due to their unique "jumping" movement mechanism. Again, only visible insects are of concern; an insect that spends its entire life cycle inside a single food is not of concern. The recent development of exhaustive cleaning methods to prepare prepackaged salad vegetables eliminates a lot of insects that are sometimes visible, rendering the product kosher and, therefore, usable in kosher foodservice establishments and in the kosher home without requiring extensive special inspection procedures. Although companies in this arena go to a great deal of effort to produce insect-free products, some kosher supervision agencies remain unconvinced, and only certify those products (or particular production lots, e.g., one day the production may be acceptable and the next day it might not) that meet their more stringent requirements. Following a recent outbreak of locust in the Middle East, some rabbis formally declared that were actually a kosher (and halal) species.

The prohibition of insects focuses on the whole animal. If one's intent is to make a dish where the food will be chopped up in a food processor, then one may skip the elaborate inspection of fruits and vegetables for insects and assume that the presence of insect parts does not render the food nonkosher. There are guidebooks describing which fruits and vegetables in particular countries need inspection; recommended methods for doing this inspection are included. How well the procedures for removing insects work in practice has been a subject of controversy. Kosher consumers have appreciated the use of pesticides to keep products insect-free as well as the use of prepackaged vegetables that have been properly inspected. Modern integrated pest management programs that allow for an increased level of insect infestation in fruits and vegetables can cause problems for kosher consumers. Examples of problems with insects that one might not think about include insects under the "triangles" on the stalks of asparagus and under the "greens" of strawberries, and thrips on cabbage leaves. Kosher consumers and mashgichim, religious supervisors onsite, are trained to properly inspect those fruits and vegetables that need to be examined. Owing to the difficulty of properly inspecting them, many Orthodox consumers do not use brussel sprouts.

A complication with respect to insects has surfaced recently. Worms, which are found in fish (although the industry usually takes great effort to remove them before the consumer gets the product), have generally not been a problem because of the unique aspect that they appear visually in the fish flesh from previously microscopic stages of development that had migrated into the flesh. From a religious point of view, this makes them acceptable. However, the anasakis worms, commonly found in salmon, are very difficult to detect, and so they are not removed before purchase and the science suggests that they are visible in the intestinal tract and migrate as "visible" worms into the flesh. In salmon, it is very difficult to see them and the industry has not been successful in removing them, although all salmon sushi is previously frozen to assure that the animals are killed as anasakis does have a human pathology. Some Orthodox rabbis have ruled in the past few years that salmon and other fish species having such worms are not kosher. Some distinguish between wild and farmed ones and favor the farmed versions. If the fish were raised on land, this is even better.

Honey and other products from bees are covered by a unique set of laws that essentially permits honey and beeswax. Other bee-derived materials, for example, royal jelly, are more controversial. An article by Rabbi Z. Blech (2004) discusses this unique set of materials and the special laws surrounding bees and honey. Most rabbis extend this permission to the use of lac resin or shellac, which is used in candy and fruit coatings to provide a "shine."

22.3.3 Prohibition of Blood

Blood is considered as the life fluid. Therefore, the removal of blood through a specific set of practices is extremely important. Judaism also has many laws dealing with the importance of not harming an animal (Tzar Balay Chayim) and these laws are reflected in the importance of properly slaughtering an animal

so as to minimize pain and that it is to be done with respect for the animal. However, Judaism also makes it clear that humans have the right to use the resources of the world for their benefit, as long as they do so without wasting and within the restrictions of the law.

The importance of properly dealing with animals is first articulated in the Noahide Code. This is a covenant between all of the Earth's inhabitants and the holy one following the flood. The giving of the rainbow to humans as a sign that there will not be this level of catastrophic flooding in the future comes with a few broad rules now known as the Noahide Code, many of which are subsequently included in the Ten Commandments, which are laws that according to Jewish law are only required to be obeyed by Jews.

One of the seven Laws of Noah prohibits the tearing of the limb of a living animal (Ever Min HaChi). This requires that all people around the world must not mutilate an animal until it is dead, that is, after slaughter no further processing shall take place until it is dead. There are religious texts designed to be read by Jews and gentiles alike explaining the detailed requirements for meeting this law (Weiner 2008).

While mentioning the Noahide Code, it is worth noting that the animals on the ark were divided into two different categories—the clean animals (i.e., those that would eventually be permitted for kosher slaughter) that were brought onto the ark seven by seven and the unclean animals that were brought onto the ark two by two.

Modern evaluations of the ark suggest that it was more like a three story barge, with the middle level housing the animals, the bottom level handling waste management and the upper level being the living quarters for Noah's family and also for raising the plant materials that were needed to feed the animals. Not quite what is usually pictured in most children's books about the ark!

Ruminants and fowl must be slaughtered according to Jewish law by a specially trained religious slaughterman (shochet) using a special knife designed for the purpose (chalef). The knife must be extremely sharp and have a very straight blade that is at least twice the diameter of the neck of the animal to be slaughtered. A great deal of training goes into making sure the knife is razor sharp. The knife is checked by running its entire working blade along a finger nail. A trained shochet will find "nicks" that most secularly trained knife sharpeners will miss. This check occurs before and after each kill for large animals. If a nick is found after the slaughter, the slaughter is not acceptable for kosher.

Before a shochet is permitted to do slaughter on his own, he will be tested for both religious knowledge and for practical skills. The rabbi doing the training will then give the slaughterman a certificate attesting to his training so that he may then offer his services. Because of all this training, the shochet is a professional, who is expecting a professional salary and is in return expected to perform both competently and with full respect for the importance attached to religious slaughter.

Before the slaughter occurs, the shochet will quickly check the neck of the animal to be sure it is clean so that there is nothing on the neck that could harm the knife. If there is a problem, the neck of the animal needs to be washed and this slows the process. Therefore, it may be desirable in the future to arrange for animals destined for kosher slaughter to be washed just prior to entering the slaughter area so that time is not lost washing the neck. This will presumably be less stressful for the animal.

During the actual slaughter, the following five laws must be observed

1. No pausing (Shehiyyah): Can be multiple continuous strokes.
2. No pressure (Derasah): Concern that the head falls back on the knife.
3. No burrowing (Haladah): The knife has to be doing its job by cutting.
4. No deviating (Hagrama): There is a correct area for cutting. Work by Dr. Temple Grandin suggests that the upper limit of this allowed range is the best for overall animal welfare.
5. No tearing (Ikkur) If the neck is stretched too tight, tearing may occur before the cutting. If it is too loose, then pressure on the knife may occur. It is for this reason that Dr. Grandin has developed a special head holder for religious slaughter that is designed, and so the head holder is away from the cutting area on the neck, gives the right amount of tension, and allows the eyes to be observed as the eyes are the last organ in the head to become insensible.

This knife, ironically, is not kosher as it is used with both animals that will be declared kosher and some that are not. Also because of its constant contact with blood, it remains in a special category. It is the process itself, and the strict following of the law, that makes a product kosher, and not the presence or absence of a blessing over the food. However, prior to undertaking a period of slaughter the shochet makes a blessing. The animal is not stunned prior to slaughter, which is controversial in the Western world where the requirement for stunning has become a matter of secular faith. Many people simply cannot believe that a well-done religious slaughter may actually be better than current stunning systems where the most up-to-date standards for stunning permit 5% of the animals to be misstunned on the first try and the actual figures in the best slaughterhouses tend to be around 3% (T. Grandin, personal communication). If the slaughter is done in accordance with Jewish law and with the highest standards of modern animal-handling practices, the animal will die without showing any signs of stress. In 1958, the U.S. Congress declared kosher slaughter and similar systems (e.g., such as halal) to be humane, but included an exemption for preslaughter handling of the animal prior to kosher and halal slaughter. To deal with problems due to inappropriate preslaughter handling, the Food Marketing Institute, the trade association for many North American supermarkets, and the National Council of Chain Restaurants are developing a set of basic animal welfare-based kosher/halal standards for upright slaughter based on the American Meat Institutes' slaughter guidelines that have existed for a number of years. Those aspects dealing with kosher slaughter, specifically red meat slaughter, will be discussed in more detail.

Dr. Temple Grandin, a Professor of Animal Science at Colorado State University, has designed much of the special equipment used for religious slaughter. She has also summarized her attitude to religious slaughter in the following quote:

> Recently, I participated in a ritual kosher slaughter—in this ritual, the way it was meant to be done, I must say. This was at a plant where the management really understood the importance and significance of what they were doing, and communicated this to their employees—and to the animals as well, I believe. As each steer entered the kosher restraining box, I manipulated the controls to gently position the animal. After some practice, I learned that the animals would stand quietly and not resist being restrained if I eased the chin-lift up under the animal's chin. Jerking the controls or causing the apparatus to make sudden movements made the cattle jump. . . Some cattle were held so loosely by the head-holder and the rear pusher gate that they could easily have pulled away from the rabbi's knife. I was relieved and surprised to discover that the animals don't even feel the supersharp blade as it touches their skin. They made no attempt to pull away. I felt peaceful and calm. (Regenstein and Grandin 1992, pp. 156–157)

With respect to kosher, or kashrus, supervision, slaughtering is the only time a blessing is said over a food product in the commercial setting, mainly because of the mandatory participation of a Jewish religious official—and it is said before commencing slaughter. The slaughterman asks forgiveness for taking a life. The blessing is not said over each animal, an issue we will return to when discussing the Muslim concept of the meat of the "People of the Book." The rules for slaughter are very strict and the shochet checks the chalef before and after the slaughter of each animal. If any problem occurs with the knife, the animal becomes treife, that is, not kosher. The shochet also checks the cut on the animal's neck after each slaughter to make sure it was done correctly. Current educational efforts are focused on working with the shochtim to minimize any contact with the external cut surfaces during this inspection. If the initial cut is not done fully, certain additional cuts can be made by the non-Jewish helper to aid in the rapid removal of blood, that is, the blood vessels may need to be more completely cut.

The animal welfare issues associated with religious slaughter are controversial and it is often difficult to separate the impact of preslaughter handling from those aspects directly related to religious slaughter. Much of the current research on religious slaughter has been done on systems that are not fully described and which in many cases represent systems that need to be upgraded. In many cases, generalizations about religious slaughter are made from very specifically poorly operating facilities. A paper describing some of the key components of reporting about kosher and halal slaughter needs to be prepared. A paper by Barnett et al. (2007) reviews a poultry slaughter operation and is unfortunately unique in that it does so with a very complete description of what happens during slaughter at the particular plant being studied.

Slaughtered animals are subsequently inspected for visible internal organ defects by rabbinically trained inspectors called a "Bodek." If an animal is found to have a defect, the animal is deemed unacceptable and becomes treife. There is no "trimming" of defective portions as generally permitted under secular law. The general rule is that a defect is religiously important if it would lead to a situation where the animal could be expected to die within a year. Some rabbis invoke these rules in dealing with issues related to veterinary practices, for example, injections into certain parts of the animal's anatomy such as the neck of a chicken. Again, the concept of a religious ability to live for 12 months more is not always consistent with modern practice as in this case where the whole point of the veterinary actions is to extend the animal's life. But such actions are described in the religious texts in other contexts, and as mentioned previously, the text almost always trumps the modern science.

Consumer desire for more stringent kosher meat inspection requirements in the United States, especially when slaughter began to go to higher speeds, has led to the development of a standard for kosher meat, mainly focusing on cattle, that meets a stricter inspection requirement, mainly with respect to the condition of the animal's lungs. As the major site of halachic (Jewish religious law) defects, the lungs of mammals, but not birds, must always be inspected. This inspection is done in two parts. Initially during the initial evisceration of the animal, the heart and lungs are left in the animal. The bodek then examines the lungs by moving his hands around the lungs—if he cannot get fully around the lung, he knows he will need to very carefully further inspect that lung. After his inspection, the lung is removed and treated as described below. Other organs are spot-checked or examined when a potential problem is observed. Meat that meets this stricter standard is referred to as "glatt kosher," referring to the fact that the animal's lungs do not have any adhesions (sirkas). The word "glatt" means smooth, referring to the absence of sirkas (Figures 22.1 and 22.2) on the lungs. The bodek, or the inspector of the internal organs, is trained to look for lung adhesions in the animal both before and after its lungs are removed from the lung cavity and has studied some very detailed religious texts that describe many features of the various organs, particularly the lungs, to help determine what is and what is not acceptable according to kosher law. To test a lung, the bodek first removes all sirkas according to a prescribed method and examines the lungs for any other religious concerns. Finally, he blows up the lung using normal human air pressure or a bike pump! The lung is then put into a water tank and the bodek looks for air bubbles. If the lung is still intact, it is kosher. In the United States, a glatt kosher cow's lungs generally have fewer than two adhesions, which permit the inspection tasks to be done carefully in the limited time available in large plants. In some of these plants, those cattle that are not glatt are "reinspected" to determine if they are kosher.

Some Jewish groups—particularly Jews who originated from countries under Muslim rule during the "dark ages" (i.e., Sephardim)—require a total absence of adhesions even in adult large animals, that is, cattle. Such meat is referred to as "Beit Yosef" meat. If a plant is doing Beit Yosef, it might

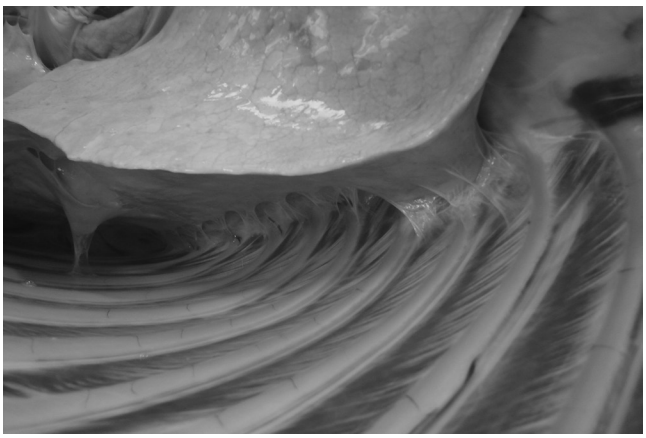

FIGURE 22.1 Sirchas (lung adhesions). (Pictures courtesy of Judy Moses, Spirit of Humane, Boyceville, WI, www.spiritofhumane.com.)

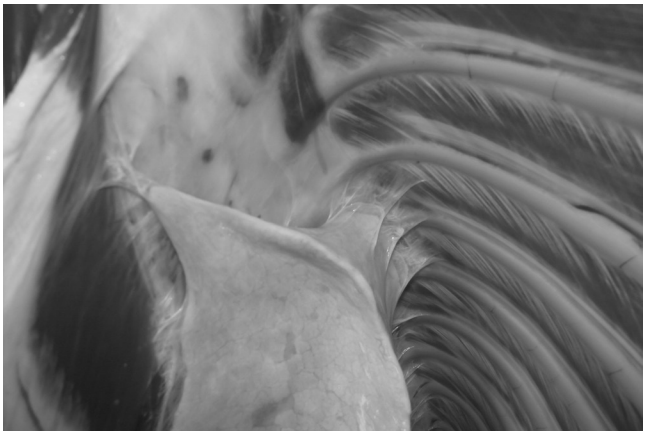

FIGURE 22.2 Sirchas (lung adhesions). (Pictures courtesy of Judy Moses, Spirit of Humane, Boyceville, WI, www.spiritofhumane.com.)

actually be set up to have two subsequent inspections, one for glatt and a final one for nonglatt. Note that young (e.g., veal calves) and small (e.g., all sheep and goats) red meat animals must always be without adhesions according to the customs of all major Orthodox groups. At this time, we do not have a full understanding of what animal-handling practices lead to higher incidences of lung adhesions, although pneumonia in the calf is certainly one consideration. Preliminary research using ultrasound to look at the lungs of the living animal is being done to determine if it is possible to increase the success rate for kosher slaughter and to improve the economics of kosher slaughter by screening out animals that would not pass the lung inspection. Currently, the preliminary results do suggest that this should be possible. The same methodology will also help determine the management practices that lead to problems and allow for better animal health protocols to be developed. Studying how to improve the lungs of animals for kosher slaughter may be beneficial for all animals. Lung inspections are not required for poultry.

The use of the word "glatt" for any other kosher product, including poultry, is only meant to convey the message that a higher standard is being used. It would be more accurate to use the word "Mehadrin"—meaning a stricter standard—and both of these words are used on some U.S. products and in other countries. However, there is also a company in America whose brand name is "Mehadrin," leading to some confusion. Nonglatt meat and nonmehadrin poultry products encompass a larger percentage of the Kosher marketplace (by volume) although the "glatt" community is probably the most strictly observant.

In modern times, to decrease the amount of blood splash (capillary rupture in the meat, particularly in the hindquarter), it has been determined that a postslaughter nonpenetrating captive bolt stun will greatly reduce blood splash. The part of the Orthodox community that accepts nonglatt meat has generally accepted this practice. The Conservative movement also accepts this process.

Meat and poultry must be further prepared by properly removing certain veins, arteries, prohibited fats, blood, and the sciatic nerve. The parts of the veins and arteries removed are high in blood, the prohibited fats are those mainly in the belly cavity that were used for sacrifices in ancient times, and the sciatic nerve commemorates Jacobs bought with the angel, where he then limped the rest of life. This process is called "nikkur" in Hebrew and "treiboring" in Yiddish. The person who is specifically trained to do this is called a "Menacker." In practical terms, this means that only the front quarter cuts of kosher red meat are used in the United States and most Western countries because it is difficult to remove the siatic nerve and the meat obtained ends up in the form of a lot of small pieces. Although it is very difficult and time consuming to remove an animal's sciatic nerve, necessity demanded that this deveining be done in parts of the world where the hindquarter was needed in the kosher food supply. In some animals (e.g., deer), it is relatively easy to devein the hindquarter. However, if there is no tradition of eating any hindquarter meat within a community, some rabbis have rejected the deer

hindquarters for their community. This is an example of an issue where both sides of the argument are currently still accepted by various rabbis as the process of determining a final stance on this issue has not been reached.

Thus, about 70% of the meat slaughtered in a kosher slaughter house is sold in the regular nonkosher market. Plants doing kosher must be able to totally segregate the kosher and nonkosher meat.

At times, there have been attempts to have such nonkosher meat specifically marked when sold in the general marketplace with the implication that it is less acceptable. This is, we believe, more political than scientific. The fact is that one poultry producer (Murray's Chickens, South Fallsburg, NY) proudly announces on his natural product, "Traditionally hand slaughtered"! Does the meat industry really want to tell how each animal was slaughtered? At this time (2010), the European Union proposed that all meat from nonstunned animals be specifically labeled. However, it appears that such a regulation would be a violation of the World Trade Organization standards.

Somewhat tongue in cheek we would propose the following labeling scheme:

Slaughtered by smashing the animal over the head to crack its skull: Nonpenetrating stunner.

Slaughtered by smashing through the skull: penetrating stunner.

Slaughtered by electrocuting the animal: electrical stunning

Slaughtered by using a gas chamber: gas stunning.

Traditionally hand slaughtered with respect for the animal: religious slaughter.

To further remove the prohibited blood, red meat and poultry must then be soaked and salted (melicha) within 72 h of slaughter. If this is not possible, then nonglatt meat is specially washed (begissing) and this wash procedure may be repeated for up to two more times, each time within 72 h of the previous washing. The soaking is done for half an hour in cool water; thereafter, the salting is done for 1 h with all surfaces, including cut surfaces being covered with ample amounts of salt. The salted meat is then rinsed well three times. The salted meat must be able to drain throughout the hour and all the blood being removed must flow away freely. Shorter soaking and salting times are sometimes permitted, for example, when there is not enough time before the Sabbath or a holiday to complete the process. In poultry, this step is often done between the prechilling (which most rabbis permit to double as the soak period) and the chiller. The chiller is then considered the third rinse and helps in removing the salt.

The animal's heart must be cut open and the congealed blood removed before beginning the overall soaking and salting process. Once the meat is properly koshered, any remaining "red liquid" is no longer considered "blood" according to halacha and the meat can be used without further concern for these issues, including raw as steak tartare.

The salt used for koshering must be of crystal size that is large enough that the crystals will not dissolve within the hour of salting and must be small enough to permit complete coverage of the meat. The salt industry refers to this size crystal as "kosher" salt. Although most salt is religiously kosher, the term "kosher" in this case is referring to the grain size. The specific process of salting and soaking meat to make it ready for use is also referred to as "koshering" meat. The grain size and shape of salt from different salt companies will vary and different rabbis will have different salt preferences.

Because of its high blood content, liver cannot be soaked and salted, but must instead be broiled to at least over half-cooked using special equipment reserved for this purpose. The liver is then rinsed, after which the liver can be used in any way the user wishes. Enough salt must be sprinkled on the liver to cover it. Koshering liver is one of the most complicated tasks to do in institutional settings. In theory, any meat can be broiled instead of soaking and salting. However, this has not been done by many people for so many years that some rabbis no longer accept this alternative.

Some concern has been raised about the salt level in kosher meat. Note that only the surfaces are salted, generally using primal cuts, that is, 20–40 lb pieces of meat, and that the penetration of the salt is less than half a centimeter in red meat (NY Department of Agriculture and Markets, personal communication). Many pieces of meat, as consumed, have therefore not been directly subjected to the salt treatment. If salt content in a diet is a very important consideration, then one should cut off all surfaces

(or learn to recognize those surfaces that have been salted) and not use any of the drippings that come out of the meat or poultry during cooking. Much of the salt that goes into the meat at the surface is lost during the cooking process.

Another issue that can arise when meat has not been soaked and salted is that of "kavoush." For example, if meat trimmings remain in the blood released by meat for more than 24 h, the meat is considered to be pickled, and cannot subsequently be soaked and salted. This meat is therefore not kosher. When large totes are used for shipping meat, it is almost impossible to prevent kavoush. These totes should only be used if the meat will be removed within 24 h or the meat has already been koshered.

Any ingredients or materials, like tallow, that might be derived from animal sources are generally prohibited because of the difficulty of obtaining them exclusively from kosher animals. This includes many products that might be used in foods and dietary supplement, such as emulsifiers, stabilizers, and surfactants, particularly those materials that are fat derived. Very careful rabbinical supervision would be necessary to assure that no animal-derived ingredients are included in kosher food products. Almost all such materials are available in a kosher form derived from plant oils. A possible practical and important exception to this is that no animal ingredients might be a normative mainstream gelatin, which is now being produced from glatt kosher beef hides (see Section 22.7.1). Also some rennet, the cheese-coagulating enzyme, is obtained from the dried fourth stomach of a kosher-slaughtered milk-fed calf, although most cheese, including kosher cheese, is currently using a biotechnology-derived chymosin (see Section 22.7.2).

There are a few concepts in Jewish laws that permit materials to alter their status. The first is "Dvar Hadash," or a new entity. If something undergoes a sufficient transformation, as defined rabbinically, it may become a new entity and not retain the identity of its source materials. Exactly what needs to be done to invoke this concept is quite controversial. Another concept that may help create flexibility for food manufacturers is the concept of "dry as wood" where the "drying" is defined as natural drying for over a year. The concept is used in part to justify the use of the natural calf rennet discussed above: the extraction of a chemical from such a material permits its use when it would not otherwise be permitted. Finally, there is the concept of "not fit for either a person or, less critically, for a dog." If a material is unacceptable and would not even be eaten by a dog, then the source is not considered a food, which means that anything derived from it could be kosher. Note, however, that some rabbis argue that if an identifiable object, for example, a bone, is placed in such a mixture and is then recovered, the item was not necessarily ever unfit for a dog.

22.3.4 Prohibition of Mixing of Milk and Meat

> "Thou shalt not seeth the kid in its mother's milk." (Exodus XXIII:19, Exodus XXXIV:26, Deuteronomy XIV:21).

This passage appears three times in the Torah and is therefore considered a very serious admonition. As a result, the law cannot be violated even for nonfood use of such prohibited mixtures, for example, in uses such as pet food. Neither can one derive benefits from such a mixture. Therefore, one cannot own a cheeseburger business. The meat side of the equation has been rabbinically extended to include poultry (but not fish) as both meat and poultry are warm-blooded animals and need to be slaughtered, inspected, deveined, salted, and soaked. The basis for this extension is found in the concept of not being seen to do something wrong (i.e., maris ayin in Hebrew). Poultry, especially dark meat, could easily be confused for lighter forms of red meat, for example, veal. The dairy side of this prohibition includes all milk derivatives, or at least those that have not been declared a new entity.

Keeping meat and milk separate in accordance with kosher law requires that the processing and handling (including the status the processing and handling equipment) of all materials and products fall into one of the following three categories:

1. A meat product
2. A dairy product

3. A neutral product called "pareve," "parve," or "parev." Note that for words that are transliterations of Hebrew—like "pareve"—multiple English spellings are acceptable. (All we usually ask is that the spelling be consistent within a single document!)

The pareve category includes all products that are not classified religiously as meat or dairy. Secular classifications may be defined differently. All plant products are pareve along with eggs, fish, honey, and lac resin (shellac). These pareve foods or ingredients can be used with either meat products or dairy products. However, if they are mixed with meat or dairy they take on the identity of the product they are mixed with, that is, an egg in a cheese soufflé becomes dairy. In the home or a foodservice kitchen, spices in containers that are added to foods while they are cooking may adsorb some of the steam and volatiles and would then take on the characteristics of the product. So a serious kosher kitchen will have separate spice containers for meat and dairy.

A special set of rules applies to fish. Fish can be eaten at the same meal at which meat is eaten, but it cannot be mixed directly with the meat. The dishes used with the fish are generally kept separate and rinsed (but not subject to the ritual of equipment kosherization) before they are used with meat, or vice versa. The original law in the Talmud speaks of a specific concern that one particular type of fish caused people to get sick when they mixed that fish with meat. Since we do not know what fish that is and have no modern evidence that such a problem exists, this rabbinical health concern is no longer valid or necessary according to the Conservative Jewish movement, while the Orthodox rabbis extended the prohibition to all fish and meat mixtures. This is a very specific exception to the generalization that kosher laws are not health laws. Another exception with respect to handling fish: one of the very traditional Chassidic Orthodox groups, Lubavitch or Chabad, also has a tradition of not mixing milk with fish, for example, so they would not permit a kosher fish gelatin to be used in yogurt. Most Orthodox communities do not follow this later strictness.

To assure the complete separation of milk and meat, all equipment, utensils, pipes, steam, and so on must be of the properly designated category. If plant materials, such as fruit juices, are run through a dairy plant, they would be considered a dairy product under kosher law. Some kosher supervision agencies, but not others, would permit such a product to be listed as "dairy equipment (DE)" rather than "dairy." The DE tells the consumer that it does not contain any intentionally added dairy ingredients, but that it was made on DE (see Section 22.6). If a product with no meat ingredients is made in a meat plant, like a vegetarian vegetable soup, it may be marked "meat equipment (ME)." Although one may need to "wash" the dishes (much like the fish and meat separation discussed above) before and after use, the DE food can be eaten on meat dishes and the ME food on dairy dishes. For actual dairy and meat products, a significant wait is normally required to use a product with dairy ingredients after one has eaten meat. This can range from 3 to 6 h depending on the customs (minhag) of the area from which the husband of each family came. With the DE listing, the consumer can use the DE product intentionally immediately before or after a meat meal but not intentionally with a meat meal. Following the eating of dairy, the wait before eating meat is much less, usually ranging from a "rinse of the mouth" with water to a 1 h wait. However, certain dairy foods do require the full wait of 3–6 h, that is, when a hard cheese is eaten, the wait for dairy to meat is the same as that for meat to dairy. A hard cheese is defined either as a cheese that has been aged for over 6 months or one that is particularly dry and hard like many of the Italian cheeses. Thus, most companies producing cheese for the kosher market usually age their cheese for <6 months although with proper package marking indicating a longer aging period, the quality of the cheese might be improved and many kosher consumers might be happy to purchase these products. Traditionally, most Jews had dairy for breakfast and lunch, and meat for supper. Now it is more likely that supper will sometimes be dairy, especially if high-quality cheese is available! With the advent of a more upscale-market of Jewish consumer, products with aged and/or hard cheese are more common. Some products are now being marked specifically with a statement along the lines of "Use of this dairy product, requires a wait equivalent to what you normally use after meat."

If one wants to make an ingredient or product truly pareve, the plant equipment must undergo a process of equipment kosherization (see Section 22.5.1). From a marketing standpoint, a pareve designation is most desirable since the product has most number of uses, both for the kosher and for the nonkosher consumer.

22.4 Kosher: Special Foods

22.4.1 Grape Products

To be kosher, all grape-juice-based products can only be handled by sabbath-observing Jews from grape-pressing to final processing. In manufacturing kosher grape juice then harvesting cannot occur on Saturday and only Jewish workers can press the grapes. If the juice is pasteurized (heated or "mevushal" in Hebrew), then it can be handled by any worker, as an ordinary kosher ingredient.

The actual pasteurization temperature is debated and different rabbinical groups use different temperatures. Some wineries do not pasteurize the product, preferring to hire only Jews to handle the wine, which then does not require any heating and is preferred by some Jewish consumers on religious grounds. The traditional Jewish religious wines that are still often used for religious ceremonies were historically very sweet, often made from Concord grapes. Initially, these were actually made from raisins, in some circumstances.

If a liquid bottling line, for example, a soda line, uses a product with nonkosher grape juice, the line would have to be cleaned (rinsed) out before proceeding to make kosher products. The normal scheduling of light to dark products in many bottling plants during the course of the day, which is done so that the carry-over from one product to the next is not observed by consumers, may need to be adjusted so that all grape-juice-containing products are run at the end of the day. (Note: this practice of continuous production of certain types of products with changes in the composition is something people with allergies should also be aware of.)

One controversial issue has been the status of marc alcohol. After the grapes are pressed, hot water containing cane or beet sugar is added to the pomace and a second press juice is obtained. This is then fermented and a commercial (marc) alcohol is obtained, whose kosher status remains controversial. Marc alcohol must be distilled before use as it would otherwise contain unacceptable levels of methanol, an issue that may be relevant to the adjudication of its kosher status.

22.4.2 Jewish Cheese (Gevinas Yisroel)

Similar to the laws concerning kosher wine production, most kosher supervision organizations require the supervising rabbi or a Sabbath-observing Jew to add the coagulating agent, that is, the agent that makes the cheese form a curd, into the vat to ensure that the cheese is kosher. Some rabbis argue that the Sabbath-observing Jew must be there to observe the coagulant being added but does not have to do it himself. This would make it possible for a cheese plant to maintain its Gevinas Yisroel status while operating on the Jewish Sabbath or holidays, that is, a Jew can be present on these occasions but cannot do any prohibited work. Any cheese that does not meet this requirement is unacceptable. This participation is required because a ripened cheese is considered a product fit for the table of nobility.

Kosher whey can be created more easily. If all the ingredients and equipment used during cheese making are kosher, the whey will be kosher as long as the curds and whey have not been heated above 120°F (49°C) before the whey is drained off. This is true even if a Sabbath observer has not added the coagulant. The necessity for Jewish participation in cheese-making is that the cheese is a product "fit for a king." Clearly, whey, which until recently was dumped into streams, does not fit into this category. Therefore, there is much more kosher whey and whey products available in the United States than kosher cheese.

Increasingly, the dairy industry is seeking to sell more whey and whey products to other food companies. Since many of these companies are kosher, there has been growing interest in assuring the kosher status of whey. For example, several manufacturers of Swiss cheese, which has a most desirable white whey, have reduced the temperature at which they work the curds under the whey. Instead of using the traditional 125–127°F (52–53°C), they are using a temperature under 120°F (49°C) to work the curds and to obtain kosher whey. Without these changes most of the Swiss cheese whey and similar products actually end up being used as a feed ingredient rather than as human food. So sometimes the kosher status of a material, particularly an ingredient, can be very strongly affected by its kosher status. Obviously, all of these products cannot be used with meat and poultry.

There are also other challenges to overcome. Much of the whey is produced in spray driers, which are among the most difficult pieces of equipment to kosherize. The process of cleaning out the entire system is quite time consuming. Some spray driers also have an automatic shut-off device that does not permit hot water at 190°F (88°C) or hotter to be run through the system, which is what the rabbis require.

Another dairy problem deals with whey cream. Any cream that is separated from cheese at above 120°F (49°C) is subject to the restrictions that come with cheese and is generally not considered kosher. These types of creams have recently been used to produce butter, which is therefore not considered kosher. Most rabbis had traditionally accepted butter as kosher without supervision as is still the case for most rabbis with respect to fluid milk. The transition to requiring kosher supervision of butter has been difficult as it happened in a short time and the butter industry did not always understand what was happening to it. A more detailed article on this, and closely related kosher dairy issues, has been published in 2002 (Regenstein and Regenstein, 2002a–c).

22.4.3 Cholev Yisroel

Kosher-observant Jews are concerned about possible adulteration of milk with the milk of nonkosher animals, such as mare's milk or camel's milk (which is beginning to appear in the American market), and, therefore, the tradition requires that the kosher milk be watched from the time of milking. This "Cholev Yisroel" (Jewish milk) milk is required by some of the stricter kosher supervision agencies for all dairy ingredients. Rabbis who accept non-"Cholev Yisroel" milk in the United States and other Western countries are able to do so for two reasons. First, they believe that the laws in the United States and many other countries are strong enough to assure that adulteration of milk by milk from prohibited species does not occur. Second, the nonkosher milks are usually worth more money than kosher milks, and so there is no economic incentive to add nonkosher milk to the milk of kosher species.

Farms producing Cholev Yisroel milk would have a Sabbath-observing Jew on the farm whenever milking is taking place, including the Sabbath and all holidays. This essentially requires that the Jewish supervisors live on or very near the farm, and often requires them to forgo communal celebration of the Sabbath and the holidays. The milk tanks on the farm and the tanker truck taking the milk to market would both be sealed by the onsite religious supervisor, and then the seal would be broken by the receiving religious supervisor at the milk plant.

22.4.4 Yashon (Old) and Chodesh (New) Flour

On the second day of Passover, Jews traditionally brought a grain offering to the Temple in Jerusalem. This served to bless all of the flour that was "growing" or had already been harvested on that day. Such flour has attained the status of "yashon" (old) flour. All wheat for flour that has not started to grow by the second day of Passover is considered "chodesh" (new) and should not be used until the next Passover. For all intents and purposes, the new grain would have been planted more than 14 days before the second day of Passover, the minimum time assumed necessary religiously for the seeds to germinate. All winter wheat from the Northern Hemisphere is automatically considered yashon as it would be planted in the fall. It is more difficult to assure the yashon status of spring wheat, which generally is harvested in August. Manufacturers may receive inquiries from consumers about the source and timing of their wheat and other grain purchases, particularly between August and the next Passover. The extent of observance of this law is increasing, so processors, including kosher meat and poultry processors, may be asked about the status of the grains used, for example, in batters and breading.

22.4.5 Early Fruit (Orleh)

Another kosher law concerning plants is the requirement that tree fruits should not be harvested for human benefit until the fourth year. This has been particularly problematic with respect to papaya, a tree fruit that is often grown commercially for <4 years! Discussion and disagreement about what are "tree" fruits subject to this ruling remain at this time.

22.4.6 Passover

The Passover holiday occurs in spring and requires Sabbath-observant Jews to avoid eating the usual products made from five prohibited grains: wheat, rye, oats, barley, and spelt (Hebrew: chometz) except in the limit form of matzos (or unleavened bread). Those observing kosher laws can only eat the specially supervised unleavened bread from wheat (Hebrew: matzos) that were prepared especially for the holiday. Once again, some matzos, that is, schmura matzos are made to a stricter standard than the normative product available in most Western countries, with rabbinical inspection beginning in the field at the time of harvest, especially to assure that the grain being harvest is so dry that no fermentation can take place. For other Passover matzo, the rabbinical supervision does not start until the wheat is about to be milled into flour. Matzo made from oats and spelt is now available for consumers with allergies, but are not supposed to be used by people without a health requirement for these products.

Special care is taken to assure that the matzo does not have any time or opportunity to "rise." In some cases, this literally means that products are made in cycles of <18 min, that is, the entire process stops, everything is cleaned, and the 18 min cycle starts over again. This is likely to be the case for hand-made schmura matzo. In continuous large-scale operations, the equipment is constantly vibrating so that there is no opportunity for the dough to rise and this permits a more continuous production.

Why 18 min? Note that the word for "life" is the two letter Hebrew word "Chai." Since the Hebrew alphabet is "mapped" to numbers (e.g., Aleph = 1, Bet = 2), the word "Chai" equals the number 18! Thus fermentation, "life" is considered to require 18 min to occur. Anything made in <18 min has not fermented and has, therefore, not violated the prohibitions of Passover. Also, the drinking toast among Jews is "L'Chaim," to life.

In the Middle Ages, the rabbis of Europe also made products derived from corn, rice, legumes, mustard seed, buckwheat, and some other plants (Hebrew: kitnyos) prohibited for Passover. In addition to the actual "flours" of these materials, many contemporary rabbis also prohibit derivatives such as corn syrup, cornstarch, and cornstarch derivatives such as citric acid. Some rabbis will permit the "citric acid" and similar materials to meet the new entity status. A small number of rabbis permit the oil from kitnyos materials, or liquid kitnyos products and their derivatives such as corn syrup. The major source of sweeteners and starches used for production of "sweet" Passover items are either real sugar or potato- or tapioca-derived products such as potato syrup.

Rabbis are also concerned with other foodstuffs that are being raised in areas where wheat and other Passover grains are grown. Because of possible cross-contamination between these crops and the prohibited grains, some crops such as fennel and fenugreek are also prohibited for Passover. An interesting product is Quinoa, a grain-like material grown in the Andes of Bolivia and Peru. This product is new to the rabbinate and many rabbis have ruled that it is acceptable. However, because it would normally be handled in a plant handling other grains and grain-like materials, the strictest rabbis require that the product's handling and packaging be supervised before permitting it for Passover.

During the Dark Ages, the Jewish communities within Christian countries did not have regular contact with Jews living in Muslim countries. The laws governing these two communities began to drift apart. As a result, today's European, or Ashkenazic Jewish community has significantly different laws and customs from the Sephardic Jewish communities, which included Spain, North Africa, and the Middle East. Sephardic custom, which is the default in Israel, includes among other rules, no ban on all or some of the kitnyos materials such as rice, a "beit yosef" meat standard of absolutely no lung adhesions on animals, and a willingness to use hindquarter that has been correctly subject to nikkur or deveining. With a few exceptions, however, Passover foods in the United States are processed to Ashkenazic standards. Products from Israel may be specifically marked as "acceptable for those who use kitnyos," so American consumers sometimes need to check new and unusual products for Passover before purchasing them rather than just assume that they are kitnyos free.

Passover is a time of large family gatherings. The requirement for two separate sets of dishes specifically for Passover, that is, one meat set and one dairy set, adds another element of resource usage and activity in providing hospitality. In previous generations, some kosher consumers limited themselves to meat products for the entire week of Passover, but this is less common today. Overall, 40% of kosher sales for the traditional "kosher" companies such as Manischewitz, Rokeach, and Kedem occur for the

week of Passover. Stores generally begin to make Passover products available to consumers between 4 and 6 weeks prior to Passover. Consumers who regularly use products such as dietary supplements, and nonlife-threatening drugs will be concerned about obtaining a version of their favorite and/or required product that is acceptable at Passover. For drugs, the prohibition of chometz is of special concern since many Jews do not want any manner of chometz in their home, including drugs, pet feeds, and nonfood items such as rubbing alcohol.

A violation of the laws of Passover is considered on Biblical grounds for being "separated from the community." This is generally the highest level of prohibition and has led to extra strictness with respect to the production of kosher for Passover products. Thus, the decision to produce Passover products will lead a company into a more complex manufacturing territory.

The most stringent kosher consumers eat only "whole" unbroken matzos on the first 7 days of Passover, the 7 days observed by Jews everywhere including Israel. Thus, any prepared food for those 7 days (the Biblically commanded time) may need to be made without the use of any matzo meal or matzo flour, that is, no gebruckts (no broken matzos). However, on the eighth day—which is a rabbinical extension of Passover outside of the land of Israel—these people will also eat products made with less than whole matzos, including the traditional Jewish matzo ball soup.

With all the limitations of Passover, it is a challenge to make Passover food products that are tasty and have a decent texture. The kosher community welcomes the assistance of the food scientist and the food industry to develop more and better Passover products.

22.5 Kosher: Other Processing Issues

22.5.1 Equipment Kosherization

There are generally three ways to make equipment kosher or to change its status back to pareve from dairy or meat. Rabbis generally frown on intentionally going from meat to dairy or vice versa directly, that is, after some time as pareve, it might be possible to go to meat with a piece of equipment that was dairy way back when. Most conversions are from dairy to pareve or from treife to one of the categories of kosher. There are a range of process procedures to be considered, depending on the equipment's prior production history.

After a plant, or a processing line, has been used to produce kosher pareve products, it can be switched to either kosher dairy or kosher meat without a special equipment kosherization step. It can also subsequently be used for halal production (from pareve or dairy lines, but not always from meat lines), and then, finally, for nonkosher products. In many cases, a mashgiach, that is, the rabbinically approved kosher supervisor, is needed onsite for equipment kosherization, so it normally is beneficial to minimize the number of changeovers from one status to another.

The most common ways to kosherize equipment are:

1. The simplest equipment kosherization occurs with equipment that has only been handled cold. This requires a good liquid caustic/soap cleaning, that is, the type of cleaning done normally in most food plants. Some plants do not normally do a wet cleanup between runs, for example, a dry powder packing plant or a chocolate line, and these would need to seek specific rabbinical guidance for the change-over.

Materials such as ceramics, rubber, earthenware, and porcelain cannot be koshered because they are considered not "capable" of releasing the flavors trapped within them during the equipment kosherization process. If these materials are found in a processing plant, new materials may be required for production.

2. Most food processing equipment is operated at cooking temperatures, generally above 120°F (49°C), the temperature that is usually rabbinically defined as "cooking." However, the exact temperature for "cooking" depends on the individual rabbi, in that it is the temperature at which he must immediately remove his hand when he puts it into hot water. Recently, through an agreement by the four largest mainstream American kosher certifying agencies, most normative kosher supervision agencies in the United

States have settled on 120°F (49°C) as the temperature at which foods are cooked and this figure is used throughout this chapter. (See Section 22.7.5.1)

Equipment that has been used with cooked products must be thoroughly cleaned with liquid caustic/soap before being kosherized. The equipment must then be left idle for 24 h, after which it is "flooded" with boiling water: being defined as water between 190°F (88°C) and 212°F (100°C), in the presence of a kosher supervisor. The details depend on the equipment being kosherized. In some cases, particularly with foodservice establishments, a "pogem" (bittering agent, oftentimes ammonia) is used in the boiling water in lieu of the 24 h wait. The absolutely clean equipment, for example, silverware, is put into the ammonia-containing boiling water to pick up a "bad" flavor. This bad flavor is removed by a second boiling with clean water. The 24 h wait accomplishes the same thing as the ammonia, that is, it turns any desirable flavors attached to the equipment into undesirable flavors.

The principles concerning koshering by hagalah (boiling water) or irui (boiling water poured over a surface) are based on an ancient understanding of the movement of "taam" (flavor) in and out of solid materials. The concepts of taam and its movement between products are also used to analyze the many possible combinations of kosher meat, kosher dairy, and/or nonkosher products interacting accidentally, that is, for analysis "after the fact" ("b'de-eved"). For real accidents, the rabbis are able to be more lenient than they might be for things that are done intentionally ("l'chatchilla," i.e., planned ahead of time). In modern times, because kosher supervision in the United States is active, that is, the rabbis are operating with a contractual agreement and ongoing inspections, there is less room to work with some of these after the fact leniencies. In Europe, where rabbis sometimes only make informal visits to plants and report on their visits to their congregants and the greater Jewish community, the rules with respect to "after the fact" issues are sometimes used more freely—since the rabbi cannot control, nor is he responsible for any changes the processing plant may make once he has left the plant.

3. In the case of ovens or other equipment that uses "fire," or dry heat, kosherization involves heating the metal until it glows. Again, the supervising rabbi is generally present while this process is taking place. In the case of ovens, particularly large commercial ovens, issues related to religiously defined "odor/vapors" and "steam" must also be considered. Sometimes the same oven can be used sequentially for alternating pareve and dairy baking. The details are beyond the scope of this chapter and require a sophisticated rabbinical analysis to determine which ovens can be used for more than one status (at separate times) without requiring kosherization.

The procedures that must be followed for equipment kosherization, especially for hot equipment, can be quite extensive and time consuming—so the fewer the status conversions, the better. Careful formulating of products and good production planning can minimize the inconvenience. If a conversion involving hot foods using "wet" heat is needed, it is often scheduled for early Monday morning, before the production week starts. Since rabbis observe the Sabbath on Saturday, they are available to travel to food plants all around the country on Sunday to start work on Monday morning at 3, 4, or 5 a.m.

22.5.2 Jewish Cooking and Jewish Baking

In cases where it is necessary for rabbis to "do" the cooking (Bishul Yisroel), their contribution to the process must remain independent of the company's activities. Often this means turning on the pilot light. As long as the pilot light remains lit, the rabbi does not have to be present; if it goes out, he must return. With electrical equipment and appliances, it is possible to keep electricity on all the time, using the lowest setting when actual heating is not taking place. The most difficult situation for kosher operations is a gas stove with an electrical starter. Care in selecting equipment can minimize a number of problems.

Baking generally requires Jewish participation, Pas Yisroel, that is, the Jew must start the ovens. In addition, if the owner of the bakery is a Jew, there may be a requirement for "taking challah," a portion of the dough that is removed and needs to be specially handled. Again, the details need to be worked out with the supervising rabbi.

Note that a company having more than 50% Jewish management or Jewish ownership is usually subject to these stricter rules, for example, the taking of challah and the need to observe the Sabbath and other Jewish holidays. To be accountable for the rules that apply to everyone, some owners sell their business to a gentile for the period of concern, even a single day each week. This is a legally binding contract

and, in theory, the gentile owner can renege on his or her informal agreement to legally sell it back at the end of Shabbos or the end of the holiday. On Passover, the need to do this can be more critical: Any chometz in the possession of a Jew during Passover is forever prohibited in a kosher home, that is, if a "Jewish" grocery store receives a shipment of bread during Passover, that bread, even if marked as kosher, though obviously non-Passover, can never be used by an observant kosher-observing Jew.

22.5.3 "Toveling" (Immersing Equipment Purchased from a Gentile)

When a Jewish company purchases or takes new or used equipment from gentiles, the equipment must be bathed in a ritual bath ("mikvah") prior to undergoing equipment kosherized. Equipment from metal and glass requires a blessing; complex items that contain glass or metal may need to be toveled (i.e., immersed), but may not need a blessing. A mashgiach needs to be present for this activity. A natural body of water can be used instead of the indoor mikvah, which may be very helpful with large equipment.

22.5.4 Tithing and Other Israeli Agricultural Laws

In ancient times, products from Israel were subject to special rules concerning tithing for the priests, their helpers, the poor, and so on. These are complex laws that only affect products from Israel. There is a rabbinical process for doing the tithing that does not require some of the actual product to be removed from the lot. The land of Israel is also subject to the Sabbath (sabbatical) years, that is, crops from certain years cannot be used. These additional requirements challenge kosher consumers in the United States who are interested in purchasing and trying Israeli products. Rabbis in Israel arrange for companies to tithe when the products are destined for sale in Israel, but rarely for exports. In 2002, at least one major U.S. kosher supervision agency has begun to arrange for tithing before the product is offered to the consumer in the United States. The details of this process are beyond the scope of this chapter.

22.6 Kosher and Allergies

Many consumers use the kosher markings as a guideline to determine whether food products might meet their special needs including those with allergies. There are, however, limitations that the particularly sensitive allergic consumer needs to keep in mind.

1. When equipment is kosherized or converted from one status into another, the procedure may not yield 100% removal of previous materials run on the equipment. This became an issue some years ago when rabbis discovered that the special procedures being used to convert a dairy chocolate line to a pareve chocolate line led to enough dairy contamination that consumers who were very sensitive to dairy allergens were having problems. These lines are koshered without water: either a hot oil or "pareve" chocolate is run through the line in a quantity sufficient to remove any "dairy" residual as calculated by the supervising rabbi. The problem, however, may be the dust from the handling of the nonfat dry milk used for milk chocolate. Airborne dust is not of any religious concern.

Both Islam and Judaism do not permit practices that will endanger life to occur. As a result, rabbis decided that none of the current religiously acceptable methods for equipment kosherization of chocolate are effective enough to move between dairy and pareve production, therefore, mainstream kosher supervision agencies no longer permit this conversion in chocolate plants.

2. Kosher law does permit certain ex-post-facto (after the fact) errors to be negated. Trace amounts of materials accidentally added to a food can be nullified if the amount of "offending" material is <1/60 by volume under very specific conditions, that is, truly added by accident. However, some items can never be negated, for example, strong flavor compounds that make a significant impact on the product even at <1/60. In deference to their industrial client company's desire to minimize negative publicity when problems arise, many kosher supervision agencies do *not* announce when they have used this nullification procedure to make a product acceptable. When there is a concern about allergic reactions, however, many rabbis are more willing in more recent years to alert the public as soon as possible for health and safety reasons and they have definitely become more sensitive about this issue over time.

Products that might be made in a dairy plant—for example, pareve substitutes for dairy products and some other liquids such as teas and fruit juices—may be produced in plants that have been kosherized, but may not meet a very critical allergy standard. Care in consuming such products is recommended.

3. Labels that say Dairy and Meat Equipment: These are used on products, when there are no intentionally added dairy or meat ingredients, but the product is produced on a dairy or meat line without any equipment kosherization. The product is considered pareve with some use restrictions on how and when it might be used in a kosher home, but the rules for these products are more lenient than for actual meat and dairy products. Again, the more sensitive the allergy, the more caution is advised.

4. In a few instances where pareve or dairy products contain small amounts of fish, such as anchovies in Worcestershire sauce, this ingredient *may* be marked as part of the kosher supervision symbol. Many certifications do not specifically mark this if the fish in the initial material, that is, in the Worcestershire sauce itself, is <1/60. Someone who is allergic should always read the ingredient label.

5. At Passover, there is some dispute about "derivatives" of kitnyos materials, the nongrain materials that are also prohibited for Ashkenazic Jews. A few rabbis permit some of these items such as corn syrup, soybean oil, peanut oil, and materials from these liquid products. The "proteinaeous" part of these materials is generally not used even by the more lenient rabbis. Consumers with allergies to these items can therefore purchase these special Passover products from supervision agencies that do *not* permit "kitnyos" derivatives. With respect to "equipment kosherization": supervising rabbis tend to be very strict about the cleanup of the prohibited grains (wheat, rye, oats, barley, and spelt), and so these Passover products come the closest to meeting potential allergy concerns; this may not be the case with respect to the extended kitnyos prohibitions.

Consumers should not assume that kosher markings ensure the absence of trace amounts of the ingredient to which they are allergic. It is a useful first screen, but products should be carefully tested before assuming everything is acceptable, that is, the allergic person should eat a small portion of the product, and increase the amount consumed slowly, over time, to assure no adverse reaction. People with allergies should get into the habit of checking lot numbers on products and purchasing stable goods with a single lot number in sufficient quantity to meet anticipated needs within the shelf-life expectations of the goods. Every packaged product has a lot number, representing some unit of production. Some companies change lot numbers a few times a day, while others change it once a day. In any case, the same lot number represents a production run that can usually be expected to be more consistent than runs produced at different times with different lot numbers.

How thoroughly are dairy ingredients kept out of a pareve line? The current standard for kosher may not meet the needs of allergic consumers since the dairy powder dust in the air may be sufficient to cause allergy problems? A company might choose to use a special marking on kosher pareve chocolates produced in plants that also produce dairy products to indicate that these are religiously pareve, but may not be sufficiently devoid of dairy allergens for very allergic consumers. Furthermore, they may also want to consider checking the chocolate using one of the modern antibody or similar types of tests. For example, regular M&Ms are marked as containing "peanuts" to alert people who are very allergic to peanuts. The product does not contain peanuts, but common equipment (cleaned between product runs) is used for both products. And peanut dust may be in the air. More products including kosher products are marked with information concerning other products produced on the same line or in the same plant; so it is becoming more common to find a kosher product made it in a plant that also handles seafood. Again, equipment kosherization, inspections, and record keeping are all used to keep any such product out of the actual kosher product, but again not at the level that might be carried in the dust in the food plant.*

There has been much discussion and controversy among Muslim consumers as well as Islamic Scholars about the permissibility of consuming meat of animals killed by the Ahl-al-Kitab or people of the book, meaning, among certain other faith communities, Jews, and Christians. The issue focuses on whether meat prepared in the manner practiced by either faith would be permitted for Muslims.

In the Holy Quran, this issue is presented only once in Sura V, verse 5, in the following words:

> This day all good things are made lawful for you. The food of those who have received the Scripture is lawful for you, and your food is lawful for them.

* Meat of Animals Killed by an Ahl-al-Kitab. (The contribution of Dr. Muhammad Munir Chaudry, President of IFANCA, to this section is acknowledged with thanks.)

This verse addresses the Muslims and seems to establish a social context where Muslims, Jews, and Christians could interact with each other. It points toward two sides of the issue, first, "the food of the people of the book is lawful for you" and second, "your food is lawful for them."

In most discussions, scholars try to deal with the first part (food of Ahl-al-Kitab) and ignore the second part (food of Muslims) altogether, leaving that decision to the people of the book.

As far as the first part of the ruling is concerned, Muslims are allowed to eat the food of the Jews and Christians as long as it does not violate the first part of this verse, "this day all good and wholesome things have been made lawful for you" (*Quran*, Sura V, Verse 6).

The majority of Islamic scholars are of the opinion that the food of the Ahl-al-Kitab must meet the criteria established for halal and for wholesome food including proper slaughter of animals. They believe that the following verse establishes a strict requirement for Muslims.

> And eat not of that whereupon Allah's name hath not been mentioned, for lo! It is abomination. [Quran VI:121]

However, some Islamic scholars are of the opinion that the above verse does not apply to the food of Ahl-al-Kitab and there is no need to mention the name of God at the time of slaughtering (Al-Qaradawi 1984). It is up to the regulatory agencies in the halal food-importing countries, halal certifiers for export or domestic consumption, or the individual Muslim consumers to decide how to interpret these verses. However, for clarity in understanding the modern day practices of Ahl-al-Kitab, we would like to offer the following analysis:

1. Christians do not follow a strict food code.
2. Jews are divided into three major groups:
   i. Orthodox Jews who slaughter animals (ruminants and poultry) in their prescribed manner and prepare all "kosher" meat currently being marketed commercially in the United States and most other Western countries.
   ii. Conservative Jews who follow the kosher guidelines based on Jewish law, but who tend to be more lenient than Orthodox Jews, but who are not usually involved in slaughter.
   iii. Reform Jews who do not generally consider kosher laws an essential concern for modern Jewish practice.
3. Orthodox Jewish slaughterers say a blessing at the beginning of a slaughter session but do not pronounce the name of God at the actual time of slaying of each animal. However, in recent years, the Orthodox rabbinate has ruled that the saying of the Muslim takbir, that is, the blessing "Allah is great" in Arabic by the Jewish slaughterman, that is, the shochet, is permitted. At least one sheep and goat slaughter of a truckload of animals occurred in 2007 with the shochet saying the takbir out loud in the presence of a Muslim witness.

For the Muslims who want to strictly follow the requirements of verse VI:121, meat (red meat and poultry) of the Ahl-al-Kitab may not meet halal standards. In addition, as discussed elsewhere in this chapter, dairy and pareve kosher products may contain alcohol (e.g., in flavors) and some more lenient kosher supervisions as defined above will permit products that contain animal-based ingredients that may also be unacceptable to the halal-observing consumer.

22.7 Science

22.7.1 Gelatin

Important in many food products, gelatin is probably the most controversial of all modern kosher and halal ingredients. Gelatin can be derived from pork skin, beef bones, or beef skin. These were originally the only sources permitted in Food Chemical Codex. However, the definition has been extended to include other animal products including sheep, goat, poultry, and fish. In recent years, some gelatins

from fish skins have also entered the market. The first author is currently involved in research in this area. As a food ingredient, fish gelatin has many similarities to beef and pork gelatin, that is, it can have a similar range of bloom strengths and viscosities. Bloom is the number of grams of force needed to drive a specific probe under very specific conditions, 4 mm into a gelatin gel. However, depending on the species from which the fish skins are obtained, its melting point can vary over a much wider range of melting points than beef or pork gelatin. This may offer some unique opportunities to the food industry, especially for ice cream, yogurt, dessert gels, confections, and imitation margarine. Fish gelatins can be produced as kosher and/or halal with proper supervision, and would be acceptable to almost all the mainstream religious supervision organizations.

Most currently available gelatins—even if called "kosher"—are not acceptable to the mainstream U.S. kosher supervision organizations and to Islamic scholars. Many are, in fact, totally unacceptable to halal consumers because they may be pork-based gelatin.

A recent development has been the manufacture of kosher gelatin from the hides of kosher (glatt)-slaughtered cattle. It has been available in limited supply at great expense, and this gelatin has been accepted by the mainstream and even some of the stricter kosher supervision agencies. The plant produces gelatins of different bloom strength, and both soft and hard capsules of various sizes have been prepared from this gelatin. This is an important new development that should be of interest to the neutraceutical and drug markets. Vegetarian capsules are also available, made with starch, cellulose, or other vegetable ingredients. They are reported to be more difficult to work with. There are reports of efforts to prepare gelatin in a plant using biotechnology. This has been done successfully in the laboratory but has not yet been commercialized.

One finds a wide range of attitudes toward gelatin among the lenient kosher supervision agencies. The most liberal view holds that gelatin, being made from bones and skin, is not being made from a food (flesh). Further, the process used to make the product goes through a stage where the product is so "unfit" that it is not edible by man or dog, and as such becomes a new entity ("D'var Hadash). Rabbis holding this view may accept pork gelatin. Most water gelatin desserts with a generic "K" on the package follow this ruling.

Other rabbis permit only gelatin from beef bones and hides, and not pork. Still other rabbis only accept "India dry bones" as a source of beef gelatin. These bones, found naturally in India, are obtained from the animals that fell and die in the fields, because of the Hindu custom of not killing the cows, are aged for over a year and are "dry as wood"; additional religious laws exist for permitting these materials. Again, *none* of these products is accepted by the "mainstream" kosher or halal supervisions, and are therefore not accepted by a significant part of the kosher and halal community.

22.7.2 Biotechnology

Rabbis currently accept products made by simple genetic engineering, for example, chymosin (rennin) was accepted by the rabbis about half a year before it was accepted by the FDA. The basis for this decision involves the fact that the gene isolated from a nonkosher source is far below "visible." Subsequently, it is copied many times *in vitro* and then eventually injected into a host that is then reproduced many times. Thus, the original source of the "gene" is essentially totally lost by the time the food product appears. The production conditions in the fermenters must still be kosher, that is, the ingredients and the fermenter, and any subsequent processing must use kosher or halal equipment and ingredients of the appropriate status. A product produced in a dairy medium, for example, extracted directly from cow's milk, would be dairy. Mainstream rabbis may approve porcine lipase made through biotechnology when it becomes available, if all the other conditions are kosher. The rabbis have not yet determined the status of more complex genetic manipulations; such a discussion is therefore premature.

22.7.3 Pet Food

Jews who observe the kosher laws can feed their domestic animals pet food that contains pork or other prohibited meats. They cannot feed their animals products that contain a mixture of milk and meat. The meat component refers only to potentially kosher meats, that is, one cannot serve beef and milk, but one

can serve an animal pork and milk. On Passover, the pet food can contain kitnyos, but not chometz, as one cannot have chometz in one's home or possession.

22.7.4 Health

As described above, many people believe that the kosher laws are considered to be among the laws that were given for people's benefit, but this is not the case. One of the few exceptions is the rule concerning the mixing of meat and fish, which was rabbinically instituted to avoid a problem with a particular fish, which when eaten with meat, made people sick. Because this is one of the few laws that are health laws, the Conservative movement recently saw fit to rule that it is no longer valid since modern scholars cannot identify the fish nor have any evidence currently of such a problem.

The most common "health" aspect of the kosher laws that is cited is the prevention of trichinosis in pork. This argument has three weaknesses. First, all flesh products can be a source of pathogens. The thorough cooking of flesh foods that is traditional in the Jewish community gives better pathogen control. There seems to be no religious law or custom (minhag) that mandates this practice. Second, the presence of trichinosis in mummified pork has not been demonstrated. And third, the incubation period for trichinosis, that is, 10–14 days makes it unlikely that the ancient Israelites would have figured out the correlation at that time.

22.7.5 Regulatory

22.7.5.1 Dealing with Kosher and Halal Supervision Agencies

In practical terms, the food industry works with kosher supervision agencies to obtain permission to use the supervision agency's trademark symbol (or in a few cases the ethical right to use the generic K, see below) on their products. In this way, the industry can make claims in the marketplace that are legal and more importantly, credible to those intentionally purchasing these products. This decision can provide a significant potential marketing opportunity.

Kosher supervision is taken on by a company to expand its markets. It is a business investment that, like any other investment, must be examined critically in this era of Total Quality Management, Just-in-Time Production, Strategic Suppliers, and so on.

What criteria should a company use to select a supervision agency? Supervision fees must be taken into account, and the agency's name recognition is another consideration. Other important considerations include (1) the responsiveness of the supervision agency in handling paperwork, in providing mashgichim at the plants as needed on a timely basis, and in doing routine inspections at a defined frequency during the year, anywhere from twice a year to everyday (including continuous) depending on the nature of the production; (2) the willingness to work with the company on problem solving; (3) the ability to clearly explain their kosher standards and their fee structure; and, of course, one should consider (4) if the "personal" chemistry is right; and (5) if their religious standards meet the company's needs in the marketplace.

One of the hardest issues for the food industry to deal with in day-to-day kosher activities is the existence of so many different kosher supervision agencies. How does this impact the food companies? How do the Jewish kosher consumers perceive these different groups? Because there has not been a central ruling religious authority for many years, different rabbis follow different traditions with respect to their dietary standards. Some authorities tend to follow the more lenient standards, while others follow more stringent standards. The trend in the mainstream kosher community today is toward a more stringent standard since some of the previous leniencies were considered less than ideal but were tolerated when fewer alternatives were available.

One can generally divide the kosher supervision agencies into three broad categories. First there are the large organizations that dominate the supervision of larger food companies, that is, the OU (Union of Orthodox Jewish Congregations, Manhattan), the OK (Organized Kashrus Laboratories, Brooklyn), the Star-K (Baltimore), the Kof-K (Teaneck, NJ), and the Chicago Rabbinical Council, all five of which are nationwide and "mainstream."

A quick digression to explain the concept of "normative mainstream kosher supervision": The concept of a normative mainstream U.S. kosher standard was the outcome of surveys of kosher foods in the supermarket by a food science class on Kosher and Halal Food Regulations taught each year at Cornell University. Over 40% of the grocery products in the supermarket have a kosher certification and almost all of these reflect the same "normative" U.S. standard. This de facto kosher standard in the United States is represented by the major national supervision agencies, for example, the OU, the OK, the Kof-K, the Star-K, and the CRC. The only other kosher supervision agency routinely found in the marketplace is the Kosher Overseers ("Half Moon K" out of Los Angeles), which had been working its way toward mainstream status and was recently acquired by the OU (yes, there are mergers and acquisitions in the world of religious supervision). Many of the smaller kosher supervision agencies also use this same standard. There are numerous trademarked kosher symbols; over 1000 at last count (Kashrus Magazine) are used around the world to identify the kosher supervision agencies and, indirectly, their different, and sometimes controversial, standards of kosher supervision. Some are more lenient than the "normative" standard, while others are stricter. The letter "K" cannot be trademarked; any person or company can put a "K" on a product for any reason. However, a number of the more lenient supervisions use it. A few normative mainstream products may also have the "generic" K (e.g., Pepsi, Kellogg's). Symbol look-alikes sometimes occur both as kosher markings and as symbols used for other purposes, for example, the circle-K of a convenience store chain.

Three of these major agencies, the OU, the Star-K, and the CRC, are communal organizations, that is, they are part of a larger community religious organization. This provides them with a wide base of support, but also means that the organizations are potentially subject to the other priorities and needs of the greater organization. On the other hand, the Kof-K and the OK are private companies, as is the Half-Moon K. Their only function is to provide kosher supervision. Although they do not answer directly to the community, like all kosher supervision agencies, their reputation depends on community support.

In addition to these national supervision agencies, there are smaller private organizations and many local community organizations that provide equivalent religious standards of supervision. As such, products accepted by any of the normative mainstream organizations will, with an occasional exception, be accepted by other similar organizations. The local organizations may have a bigger stake in the local community. They may be more accessible and easier to work with. Although often having less technical expertise, they may be backed up by one of the national organizations. For a company marketing nationally, a limitation may be whether consumers elsewhere in the United States, and more recently around the world as the food industry becomes globalized, know and recognize the local kosher symbol. With the advent of *KASHRUS Magazine*, and its yearly review of symbols, this has become somewhat less of a problem. However, *KASHRUS Magazine* does not try to "evaluate" the standards of the various kosher supervision agencies, but simply "reports" their existence. It is the responsibility of the local congregational rabbi to inform the congregation of one's standards. Local rabbis who do not know enough about the "far-away" organization may be uncomfortable recommending an agency without calling one of the national agencies for advice.

The second category of kosher supervision (more stringent than normative mainstream) includes individual rabbis, generally associated with the "Hassidic" communities, that is, groups with standards beyond the normative Orthodox standard. These groups are often affiliated with the fervently Orthodox communities of Williamsburg and Borough Park in Brooklyn, Monsey, and Monroe, NY, and Lakewood, NJ. There are special food brands that cater specifically to these needs, for example, Hadar and Liebers. Many of the products used by these communities require continuous rabbinical supervision rather than the occasional supervision used by the mainstream organizations for production-line factory-produced products.

Note: for local food processing, for example, bakery, deli, restaurant, butcher shop, and so on either continuous, that is, with a mashigiach on the premise when it is operating, or fairly regular supervision is the norm—often with a local rabbi visiting almost every day. The symbols of the kosher supervisory agencies representing these local groups are not as widely recognized beyond these communities as those of the major mainstream agencies in the kosher world.

The rabbis for the stricter agencies will often do special continuous supervisions of products using a facility that is normally under mainstream supervision—often without any changes, but sometimes with special requirements for their custom production.

The third level is mainly composed of individual rabbis who are more "lenient" than the mainstream standard. Many of these rabbis are Orthodox; some may be Conservative. Their standards are based on their interpretation of the kosher laws and related religious rulings. Employing a more lenient rabbi means that the food processor cuts out more of the "mainstream" customers and the stricter markets; this is a retail marketing decision that each company must make for itself. More lenient supervisions are sometimes the only ones that will certify a product with a special problem that causes other supervisions to reject it. For example, since fish blocks, which are used for fish sticks and portions, are produced around the world, it is difficult to get proper onsite supervision to assure that all fillets in the block are really the species on the label. As a result, only a lenient rabbi will accept such blocks based on a rule of the majority and the assumption that governmental authorities are also monitoring this situation. Many consumers then make their buy or no-buy decisions based on the specific supervision, while others are comfortable buying a product with kosher supervision without worrying about the details of the standard.

Some companies have used the "Generic K," that is, the letter "K," which cannot be trademarked. The Generic K is not trusted by many educated kosher consumers. They realize that the symbol is generally used by one of the more lenient supervisions. A few large, national brands have used the Generic K for many years even though they have normative mainstream supervisions. Most kosher consumers are aware of these few companies, for example, Pepsi and Kellogg's. Although these companies do not seem to lose market share because of this decision, it is still viewed suspiciously by some consumers.

In recent years, we see products that have dual halal and kosher certification. The first were the military meals ready-to-eat (MRE) meals, particularly those made by My Own Meals (Deerfield, IL), but the market has since expanded to other industrial (ingredients) and consumer products. Some of the civilian versions of the MREs are available in long-term shelf-stable (nonrefrigerated) form that makes them convenient for use (Jackson 2000). Meat products, however, are not dual certified and are either glatt kosher or dhabiha halal, while the pareve and dairy products have the dual certification.

Ingredient companies should be particularly careful in selecting a supervision agency. They should try to use a "mainstream" kosher or halal supervision agency because most kosher or halal food manufacturing companies will require such supervision. The ability to sell the ingredients to as many customers as possible requires a broadly acceptable standard. Unless an ingredient is acceptable to the mainstream, it is almost impossible to gain the benefit of having a kosher ingredient for sale. Ingredient companies also need to pay attention to the status of the kosher product, that is, a pareve product is preferred over a dairy product because it has broader potential use. A joint venture to make lactic acid from whey failed, in part, because the major users of kosher food-grade lactic acid, pickle, and olive manufacturers were all kosher-pareve; given that their products are often used at a meat meal, the use of a dairy lactic acid would have been counterproductive.

Food companies will have to pay increasingly more attention to halal standards. In many cases a few changes make it possible to permit kosher products to serve the halal community as well, for example, the true absence of animal products and the care to assure that any residual alcohol in products is below 0.1%. Again a supervision standard acceptable in all or most Muslim countries is desirable.

Note that the 0.1% alcohol in finished product standard is the standard used by IFANCA and seems to be acceptable to the leadership of most halal communities. However, many halal consumers are not familiar with this standard at this time, so further education will be necessary. On the other hand, at least one major Muslim country seems to be currently examining permitting a higher alcohol level in finished product, which if accepted widely would provide additional flexibility in the creation of halal products.

There is some amount of interchangeability of products between kosher supervision agencies. A system of certification letters is used to provide information from the certifying rabbi concerning the products he has approved. The supervising rabbi certifies that a particular facility produces kosher products, or that only products with certain labels or codes are kosher under his supervision. To prevent fraud, it is helpful if these letters are renewed every year and dated with both a starting and ending date. These letters are the mainstay of how food companies and other kosher supervision agencies establish the kosher status of ingredients as ingredients move in commerce. These letters need to be very specific with respect to identifying the products covered by the certification. Consumers may also ask to see such letters. Obviously, a kosher supervision agency will "accept" letters only from agencies they find acceptable. That decision depends on two components: the actual kosher standards of the other agency

and an assessment of how well they operate and enforce their supervision, that is, both theory and practice have to be appropriate.

There are, of course, periodic "recalls" of specific products for various kosher defects that would prevent their use. *KASHRUS Magazine*, or its web site http://www.kashrusmagazine.com, and http://www.kashrut.com, a consumer alerting service, both try to provide up-to-date listings of products with problems, both of consumer items and industrial ingredients working across many different kosher supervision agencies. Most of the major kosher supervision agencies have their own sites and some even offer email announcements of any kosher problems.

The kosher symbol of the certifying agency or individual doing the certification may appear on the packaging. In some industrial situations, where kosher and nonkosher products are similar, some sort of color coding of product labels and packages may also be used. Most of these symbols are "trademarks," that is, they are duly registered and misuse of the symbol can result in secular legal action. In a few cases, multiple rabbis have used the same kosher symbol, causing consumer confusion. (Unfortunately, the trademark owner in one such case is not willing to pursue secular or religious legal action. There are functioning Jewish religious courts in most countries for matters arising within the Jewish community even though their "enforcement" powers are obviously limited in the absence of secular enforcement.)

Two additional notes about kosher markings on products:

1. To ensure that labels are marked properly, it is the responsibility of the food company to show its labels to its certifying agency prior to printing. This responsibility includes both the agency symbol and the documentation establishing its kosher status, for example, meat, dairy, or pareve. It is the responsibility of the kosher supervision agency to review these labels carefully. Many kosher supervision agencies currently do not require that "pareve" be marked on products; others do not use the "dairy" marking. This causes consumer confusion, which could be avoided if every kosher product had their status marked. In addition to providing the proper information, having each product marked with its status would challenge everyone to pay more attention to properly marking products, avoiding recalls or announcements of mislabeled products. The letter "P/p" has been used for both Passover and pareve. We suggest using the letter "N" for pareve, that is, for "neutral"—consistent with "D" for dairy and "M" for meat.
2. The labels for private label products with specific agency symbols on their labels should not be moved between plants and cannot be used if supervision changes. This is why some companies, both private label and branded, use the generic "K." Thus, if the kosher supervision agency changes, the label can still be used. The sophisticated kosher consumer, however, is more and more uncomfortable with this symbol. A major concern is that the labels may be too easily moved between plants, including plants that are not kosher.

The Kashrus Council of Toronto requires that each label have a plant number on it. This prevents the movement of labels between plants even of the same company. They are the only agency that currently requires this additional safeguard although the Kof-K appears to be introducing this requirement at least in some facilities.

In particular, if a company uses the generic "K," the customer service and sales departments of the company—and those people representing the company at trade shows—need to know who the certifying rabbi is. But it is always a good idea for all of the sales and marketing staff to be comfortable with providing information about the kosher and halal status of their products.

22.7.5.2 Federal and State Regulations

Making a claim of kosher on a product was a "legal" claim in the United States until fairly recently. The Code of Federal Regulations (21CFR101.29) had a paragraph indicating that such a claim must be appropriate. This clause was removed from the CFR a few years ago as part of a general cleanup of the federal

code. Approximately 20 states, some U.S. counties, and some cities have laws specifically regulating the claim of "kosher." Many of these laws refer to "Orthodox Hebrew Practice" or some variant of this term, for example, reference to specific Jewish documents, and their legality is subject to further court interpretation.

New York State probably has the most extensive set of state kosher laws. These laws, however, were declared unconstitutional a few years ago by the Federal District Court for Eastern New York. The verdict was upheld by the Federal Court of Appeals for the Second District. The appeal to the entire Second District for "en banc" review was denied. Subsequently, the Supreme Court of the United States refused to hear an appeal, so the State of New York has developed a new law that will be constitutional. The original law includes a requirement to register kosher products with the Kosher Enforcement Bureau of the Department of Agriculture and Markets (55 Hanson Pl., Brooklyn, NY 11217) and this has been retained in the new law, which are modeled on the New Jersey law.

The state of New Jersey also has relatively new kosher laws because the state's original kosher laws were declared unconstitutional by the New Jersey State Supreme Court. It has the same problem as that of the state of New York, that is, requiring an "Orthodox" standard. The new laws focus specifically on "consumer right to know issues" and "truth in labeling." They avoid having the State of New Jersey define kosher. Rather the food producer defines its terms and is held to that standard. Rabbis (or anyone else) providing supervision are asked to declare the information consumers need to know to make an informed decision. The state will enforce the accuracy of the statements, that is, if the rabbi and the company claim to do something, then the failure to do so is a violation. But it puts more onus on the buyer to ensure that the product meets his or her standard.

22.7.6 Animal Welfare

The largest fast food chains in the United States are seeking to develop a set of animal welfare standards that permit them to purchase products in the United States and in many other markets that then meet these animal welfare standards. As it became clear that it was not ideal to have each supermarket chain and each chain restaurant come up with its own standards, the Food Marketing Institute (FMI, the trade association for many of the supermarkets in North America) and the National Council of Chain Restaurants (NCCR) appointed an animal welfare technical committee to come up with a single national animal welfare standard for each species as well as for animal slaughter and poultry slaughter. It is anticipated that these standards will be predominantly based on the animal welfare guidelines developed by the trade associations of the major production agriculture groups and of the American Meat Institute, representing the meat-processing industry. However, the FMI/NCCR effort has in some instances required the trade associations to strengthen their guidelines. The establishment of these standards in the marketplace will have a major impact on animal agriculture throughout the United States and eventually around the world. These standards generally raise the bar in the United States for animal welfare, but are less aggressive than those currently being applied in Europe. The committee is trying to create conditions for the significant improvement of how ALL animals are raised and slaughtered. Initially, the effort has focused on each of the trade associations associated with the major animals of production agriculture (beef, dairy, chicken, turkey, egg layers, and pigs), and with the slaughter process for these animals. The religious aspects are only a very small component of that effort. Many other issues are being negotiated. For instance, the egg-laying industry is committed to major increases in the space per bird for cage layers, currently suggesting a reduction of approximately 15% of installed capacity nationwide, with the changes in this case to be phased in over a 10-year period.

Once the work is completed on these large-volume commodities, the FMI/NCCR committee is likely to review standards for other animals, including sheep and goats, fish and shellfish, both wild caught and aquacultured, farmed game animals and game birds, ducks, honey bees, and rattries (ostrich, emu, rhea, etc.).

Animal welfare issues that arise in religious slaughter are incorporated into the FMI/NCCR committee work. A discussion on issues relevant to religious slaughter appears in Regenstein and Grandin (2002) along with recommendations for auditable standards that will be used by the FMI/NCCR auditors. These standards are consistent with the American Meat Institute requirements. Religious slaughter should

ideally be done with the animals in an upright position (for mammals), although the need to use an upside down pen when religiously required is recognized. The standard shackling line is also permitted for poultry religious slaughter, that is, the animals can be shackled prior to slaughter, although most kosher slaughter is done with the animals being held by the slaughterman or his helper. Subsequently, the animals are then put on the shackles or into a bleeding cone. Appendix I in this volume contains a discussion paper providing a great deal of information about issues related to religious and regular slaughter practices. Also, for more information, see the AMI web site at http://www.meatami.org.

ACKNOWLEDGMENTS

We specifically thank Dr. Muhammad Chaudry of the Islamic Food and Nutrition Council of America (IFANCA) for his extensive input into this chapter, and especially for the sections dealing with Muslim issues. We thank Cornell University students Catherine E. Savage, Peng Zhou, and Andrew Rosenberg for assistance with the abstract and Dr. Shawkat Toorawa of Cornell University's Near Eastern Studies Department for critical comments.

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Part V

Secondary Processing: Principles and Applications

23

Meat Emulsions

Violeta Ugalde-Benítez

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23.1 Introduction

A large amount of processed foods are emulsions. The wide diversity in physicochemical and sensory characteristics of food emulsions is due to the variety of ingredients and processing conditions. Emulsified meat products, also called meat batters, are complex systems in which fat is emulsified into a viscous fluid mainly composed of solubilized myofibrillar proteins previously extracted from meat from different animal species. The importance of these products is based on their wide consumption throughout the world in a range of food items, from highly valued liver sausages and pâtés up to low-cost sausages and bologna.

23.2 Emulsions

Emulsions are colloidal two-phase systems, in which a liquid is dispersed in another liquid of different composition, the second liquid forming the continuous phase. The dispersed phase is also called internal phase, and the continuous phase is referred to as the external phase. In many emulsions, there is an aqueous phase and a hydrocarbon or oil phase (Schramm 2005).

McClements (2005) defines an emulsion as a system formed by two immiscible liquids (usually oil and water) in which one is dispersed as small spherical particles into the other. The mean particle diameter

in most food emulsions is between 0.1 and 100 μm. Schramm (2005) reports that oil droplets of milk fat usually have a mean diameter bigger than 0.2 μm, and even can be higher than 50 μm. In general, there are two types of emulsions, depending on the continuous and dispersed phase composition: oil-in-water emulsions (O/W) in which oil globules are dispersed in water; and water-in-oil emulsions (W/O) if water droplets are dispersed in oil (Schramm 2005). Examples of oil-in-water emulsions are milk, dairy cream, salad dressings, mayonnaise, fortified drinks, soups, and sauces. Butter and margarine are water-in-oil emulsions (McClements 2005).

In addition to O/W and W/O, other emulsion forms are possible. Multiple, or double, emulsions are complex dispersed systems characterized by low thermodynamic stability. These are "emulsions of emulsions," such as water droplets in oil-in-water (water-in-oil-in-water, W/O/W), or oil globules in water droplets in oil (O/W/O). The use of multiple emulsions has been reported for controlled liberation of the internal phase to the external phase, mainly for medical, pharmaceutical, cosmetic, and industrial purposes. This type of systems also have potential application in food processing to encapsulate or protect food components, active or sensitive, from environmental factors such as oxidation; to control aroma and flavor component release, or for low-lipid content food production. Multiple emulsion stability is mainly affected by emulsion composition and emulsifying conditions (Muschiolik 2007).

The operation of converting two immiscible phases into an emulsion, or reducing oil globule average size in a previously formed emulsion is called homogenization (McClements 2005). Globule resistance to deformation and breakage is due to Laplace pressure, which increases when average globule diameter decreases. As a result, a considerable amount of energy is necessary for emulsion formation. This energy is supplied by vigorous agitation, generated by a sufficiently intense shear force in high-viscosity continuous phase; this is often the case when preparing W/O emulsions. As a result, droplet diameter is reduced to a few micrometers. In an O/WE emulsion, the continuous phase viscosity tends to be low; therefore, to disrupt oil globules the force is generated by rapid and intense pressure fluctuations of a turbulent flow regime (Walstra 1996).

In the food industry, this process is carried out using equipments known as homogenizers, usually subjecting the liquid to intense mechanical agitation, such as high-speed stirrers, high-pressure homogenizers, and colloid mills (McClements 2005). High-pressure homogenizers, designed for milk homogenization, are the most widely used because fine emulsions with texture and high stability can be obtained. This type of equipment operates by forcing a coarse emulsion, previously obtained in a high-speed stirrer, through a narrow valve. The combination of high shear force, cavitation, and turbulent flow in the valve promote oil globule disruption. Globule diameter reduction decreases creaming and increases emulsion stability (Desrumaux and Marcand 2002).

23.2.1 Emulsion Stability

An emulsion is formed when pure oil and pure water are homogenized. However, these two phases rapidly separate into a system made of an oily layer in the upper part (low density) and an aqueous layer at the bottom (high density). This is due to the tendency of the oil globules to merge with neighboring globules as soon as they collapse; eventually, this leads to a complete phase separation. This happens because the contact between oil and water molecules is thermodynamically unfavorable (McClements 2005). Therefore, many emulsions are thermodynamically unstable systems, although some emulsions can be stable and resist emulsion-destabilizing treatments; these emulsions can also be stable for considerably long time. Metastable emulsions (kinetically stable) found in practice are made of oil, water, and an emulsifier (or stabilizer) usually a surfactant, that is, macromolecules or a finely divided solid (Schramm 2005).

The ability of an emulsion to resist changes in its properties throughout time is known as emulsion stability. The more stable the emulsion, the slower the changes occurring in its properties. An emulsion can become unstable due to different physical and chemical processes. Physical instability is observed as spatial distribution or alteration of the molecular structure organization; chemical instability occurs in the molecules forming the emulsion (McClements 2005).

The factors favoring emulsion stability are (a) low interfacial tension, a low interfacial free energy facilitates keeping a large interfacial area; (b) high surface viscosity and/or mechanically strong interfacial film, acting as a barrier against coalescence; adsorption of small solids improves this property;

(c) large electric double layer and/or steric repulsion that prevents collisions and aggregation, hence, coalescence does not occur; (d) small dispersion attractive force decreasing aggregation and coalescence velocity; (e) small volume of the dispersed phase, reducing collision and aggregation frequency; (f) small-size globules, in case they have electrostatic or steric interactions; (g) small density difference between phases, reducing creaming and sedimentation velocity, therefore reducing collisions and aggregation; and (h) high viscosity, reducing creaming and coalescence velocity (Schramm 2005).

23.2.2 Emulsion-Destabilization Mechanisms

The velocity at which an emulsion loses its stability, as well as the mechanisms leading to this breakdown, depends on the emulsion composition and microstructure and environmental factors to which it is exposed, such as temperature, mechanical stirring, and other storage conditions (McClements 2005). The main emulsion-destabilization mechanisms are creaming, flocculation, and coalescence. Structural changes occur in the emulsion during flocculation and creaming, but globule size distribution is not altered; conversely, during coalescence globule size distribution changes with time (Tcholakova and others 2006).

23.2.2.1 Creaming

This is a gravitational separation phenomenon occurring in emulsions when globules of lower density than the surrounding liquid phase are displaced to the emulsion upper part. The density of most oils in the liquid state is lower than water density; due to this, the oil tends to accumulate in the top and the water in the bottom (McClements 2005). Therefore, globules in an O/W emulsion tend to cream. Creaming velocity follows Stokes Law, it is directly proportional to the dispersed phase globule size, and inversely to the continuous or dispersing phase viscosity. In the case of meat emulsions, the smaller the fat globule size, the more stable is the formed emulsion (Nawar 1993).

23.2.2.2 Flocculation

Flocculation is the process in which two or more globules approach each other to form an aggregate, but the globules keep their individual integrity. It is the result of electric charge removal and subsequent inhibition of electrostatic repulsions. Globules merge but remain separated for a thin layer of continuous phase. The number of globule aggregates increases as flocculation proceeds, and, therefore sedimentation rate also increases. The globules move as a group and not individually. Flocculation implies disruption of the interfacial film surrounding the globule; therefore, changes in the original globule size are not expected (McClements 2005).

23.2.2.3 Coalescence

It is induced by disruption of the thin film that separates neighboring globules. This is a random process, leading to several consequences: (a) coalescence probability, when occurring, is proportional to the time the globules remain next to each other; for this reason, creaming is more likely to occur in aggregates or in clogged milk; (b) it is a first-order rate process, conversely to aggregation which is, in principle, a second-order rate process with respect to time and concentration; and (c) film disruption probability is proportional to its area; this means that as the area increases, the globules flatten as they approach each other, promoting coalescence. Fat globules generally present in food emulsions do not become flat as the Laplace pressure is too high. Coalescence is less probable in the following situations:

- With the smallest droplets. These have a very small area, therefore, the probability of disruption is also low. Higher coalescence is necessary to produce larger droplets; cream removal velocity decreases. In practice, the leading variable usually is the average globule size.
- With thick film separating globules. A thick film requires more intense repulsive forces or forces with more effect extension that provide better stability against coalescence. Steric repulsion is specifically efficient to prevent coalescence as it keeps globules relatively distant.

- With high interfacial tension (γ). It could be a paradox; to formulate an emulsion, a surfactant is necessary, and surfactants reduce the interfacial tension. In addition, small γ values mean lower surface free energy. However, the key matter is how much activation free energy to disrupt is present; this increases with high γ. High γ values make film deformation difficult, and film deformation leads to disruption.

These principles suggest that proteins are the most suitable compounds to prevent coalescence; this is confirmed by experience. Proteins do not greatly decrease γ and frequently promote considerable electric and steric repulsions (Walstra 1996).

Although many of the factors involved in emulsion stability are of thermodynamic and chemical nature, meat emulsion stability can be reached by increasing the continuous phase viscosity by food-grade emulsifier addition and adjusting the average particle size of the dispersed phase (McClements 2005). Van Ruth and others (2002) reported that the better stability of meat is a function of colloidal factors, such as ionic strength, particle form, particle surface charge, and particle size distribution.

23.2.3 Emulsifiers

An emulsifier is a surface-active molecule that adsorbs to the surface of globules recently formed during homogenization; it forms a membrane that protects the globules against aggregation when they come close to each other (McClements 2005). In a number of cases, emulsifiers are necessary to facilitate emulsion formation (Schramm 2005). Many emulsifiers are amphiphilic molecules, with polar and nonpolar regions within the same molecule (McClements 2005). The ability of fat emulsifier to form an emulsion is related to how easily it adsorbs to the water-in-oil interface. Emulsifiers reduce surface tension and work necessary to create new surfaces (Zhang and others 2009). They are not only necessary for emulsion formation, but also to stabilize the emulsions already prepared. It is important to distinguish between these two basic functions, as they are not interrelated. An emulsifier can be suitable for small globule formation but not to prevent coalescence for a given time, or vice versa (Walstra 1996).

Some of the emulsifiers commonly used in the food industry are small surfactant molecules, phospholipids, proteins, or polysaccharides (McClements 2005). Natural or modified soy lecithin is an example of phospholipids used as food stabilizer and emulsifier, giving products with acceptable attributes (Pand and others 2004). For food O/W, the most widely used emulsifiers are proteins (Walstra 1996) as the main functional property of these molecules is the ability to disperse in the aqueous phase (Mu and others 2009), they are edible, tensoactive, and provide resistance against coalescence. Emulsified oil globules are stabilized by protein accumulation in the surface, forming a protective barrier against coalescence and subsequent emulsion breakdown.

An emulsifier success also depends on its ability to maintain the emulsion structure in further processing steps, such as cooking and canning. However, in low-protein concentration or low protein/oil ratios, protein is not enough to cover the interface formed during emulsification; the resulting emulsion is highly unstable and flocculation is likely to occur.

The degree of emulsion flocculation is a function of the adsorbed film structure, as well as thermodynamic characteristics of the solvent. The factors affecting this phenomenon are: dispersed and continuous phase viscosity, globule deformability and size, interglobular forces, surface tension, and mobility of the adsorbed film. If the interfacial oil/water film is disrupted, coalescence occurs and oil globules combine to form a larger spherical globule (Eleousa and Doxastakis 2006). Proteins cannot be applied in W/O emulsions due to their low solubility in the oily phase (Walstra 1996).

23.3 Proteins as Emulsifiers

Many proteins, including casein, soy, muscle, and egg proteins are used in several foods as emulsifiers (Mine and others 1991). Walstra (1996) pointed out that proteins vary in their emulsification efficiency, mainly due to their molecular mass. Low-molecular mass proteins should be more efficient emulsifiers, however the application of these proteins is not always a good choice as very small peptides can be

formed, leading to rapid coalescence. It must be taken into consideration that different protein preparations, mainly at industrial levels, contain molecular aggregates of various sizes that considerably increase the effective molecular mass and decrease the emulsification efficiency. Protein solutions difficult to dissolve are not good emulsifiers. As a general rule, highly soluble proteins facilitate emulsion formation (or globule formation) almost to the same extent if the concentration is too low.

Another important variable is the protein surface charge. If the protein is highly charged, more concentration will be necessary to obtain a stable emulsion. A protein surface charge also depends on how the emulsion is prepared.

23.3.1 Animal Proteins

Egg white proteins are in fact composed of different chemical entities; they are of great importance for the food industry, possessing highly desirable characteristics for the preparation of a wide variety of foods. Ovalbumin, the most abundant protein in egg white, is highly functional, with properties related to emulsification, foaming, and stabilization (Mine and others 1991). This protein is composed of 385 residues (44 kDa), a carbohydrate chain, none to two phosphoryl groups, four free sulfhydryl groups, and one disulfide linkage (–S–S–) (Galazka and others 2000). Mine and others (1991) concluded that the emulsifying activity of ovalbumin in soy oil as the dispersed phase was higher at acidic pH than at neutral pH due to its high surface hydrophobicity and molecular flexibility when exposed to acidic conditions. In addition to egg white proteins, hen's egg yolk is an excellent food emulsifier for bakery products, sauces, and dressings. Fresh egg yolk contains around 40–50% dry matter, 80% water soluble and 20% formed by insoluble granules. The plasma is composed of 85% low-density lipoproteins (LDL) and globular glycoproteins, known as a, b, and g-livetins. LDL apoproteins are known as lipovitelins, these are the main egg yolk components, close to 68% total dry matter (Daimer 2009). Guilmineau and Kulozik (2006a,b) showed that egg yolk emulsification behavior is highly dependent on the environmental conditions (pH and ionic strength); thermal treatment can improve these properties. Partially denatured proteins are adsorbed in the oil/water interface and stabilize the emulsion more efficiently than egg yolk native proteins. In addition, emulsions prepared with heat-treated egg yolk are less sensitive to pH and ionic strength variations. This positive effect could be due to an increase in steric repulsion between oil globules when denatured protein aggregates cover the oil/water interface. Bovine serum globin has been known to be a good emulsifier and a stabilizer in acidic conditions (pH 3–6), where this protein is highly soluble (Bizzotto and others 2005). Milk whey proteins and concentrate are an important source of food ingredients due to their nutritional, sensory, and functional properties. Among others are their abilities to absorb water, form gels, to be used as emulsifiers and as foaming agents.

23.3.2 Plant Proteins

Several plant proteins are also excellent emulsifiers. Adebowale and Adebowale (2008) reported that protein isolates obtained from Mucuna bean play an important role in emulsification, as they favor W/O emulsion formation and stabilize the emulsion once formed. Being surface-active compounds, these protein isolates displace oil/water interfaces, decreasing the surface tension and facilitating emulsion formation. The high solubility within a wide pH range of Mucama bean protein isolates and the seed flour shows that this bean can be applied in protein-enriched soft drinks, sausages, salad dressings, mayonnaise, and other foods. Lima bean protein concentrates were studied by Akintayo and others (1998); these authors pointed out that the obtained material was a better emulsifier than pigeon pea protein concentrates; it can be an option for low-viscosity emulsion formulation; African yam bean protein concentrate is another option of a good emulsifier that can be applied in high-viscosity emulsions.

23.3.3 Enzymatically Modified Proteins

Enzymatic treatments of several proteins increase their functional properties even further. Enzymatic hydrolysis of the milk whey fatty fraction considerably improves its interfacial properties; air/water

interfacial adsorption rate is increased, thereby decreasing the equilibrium between surface tension and increasing the compressibility modulus of films formed at the water surface. The overall result improves the emulsion properties; the enzymatically treated whey can also be used to improve the food texture (Blecker and others 1997).

Pancreatine hydrolysis of soy protein alters the molecular weight of an isolate, increasing surface hydrophobicity and improving the functional properties, especially solubility and emulsifying activity. The resulting hydrolysis products can also be used as emulsifiers in meat products (Qi and others 1997).

23.3.4 Muscle Proteins

The various technological properties of meat proteins play an important role in emulsified meat products. Among the various proteins of this type, those in the myofibrils have the best emulsifying properties. Their high solubility and interactions with other compounds positively affect the oil-binding ability, water-holding capacity, stability, viscosity, density, and other emulsion characteristics. Myofibrillar proteins also take part in gel formation promoted by heat treatment; gelling contributes to texture development and stabilized water/oil systems in the emulsified meat products (Zorba 2006). On the other hand, the effect of sarcoplasmic proteins on emulsification is relatively low, as reported by Zorba (2006); the effect of these proteins in oil binding and water holding occurs when ionic strength is below 0.4 M, but decreases even further above this value. The effect of connective tissue proteins in emulsification is negligible.

Meat emulsion formation includes the activation of most of the proteins present in the muscle by disrupting the sarcolemma to release myosin and actin, subsequently solubilized by salts and phosphates. Myofibrillar proteins, with fibrous structures, turn into a viscous fluid during protein activation. This fluid is responsible for fat emulsification and immobilization of added water. Changing fibrous proteins into a viscous fluid is relatively easy with pork and chicken meat, but more difficult with beef and lamb (Feiner 2006). This is because different animal species may present a wide variety of protein characteristics, probably due to interaction effects (Zorba 2006). These differences in functional properties can also derive from intrinsic factors such as protein structure, molecular mass, and amino acid composition (Liu and others 2008).

According to Feiner (2006) meat hardness, as a result of fiber thickness variation among meat type and cuts, is also related to protein solubility variation within the same animal species. Westphalen and others (2006) agree in this respect and also report that the final characteristics of an emulsified meat product are due to muscle type. This is observed from the fact that myosin extracted from a red muscle consistently produces weak gels, as compared to proteins obtained from white muscles. It is also reported that gels made with myofibrillar proteins from hen's leg (red) have lower storage moduli, as compared to gels made with proteins from chicken breast; this variation in storage moduli was independent of pH, ionic strength, or antioxidant content.

Fish myofibrillar proteins have excellent functional properties, including emulsion formation. In fact, several marine products are preferred as raw materials due to their myofibrillar protein functionality, as well as for religious and health reasons. However, fish myofibrillar proteins are less stable to thermal and chemical treatments as compared to proteins from other vertebrates. Combinations of these proteins with other chemical species such as alginate oligosaccharides have shown to improve the functional properties, in particular, emulsion formation (Sato and others 2003). Proteins from other marine sources, such as invertebrates, are also of interest in food product development. The thick filaments in mollusks are formed by nuclei of paramyosin, surrounded by a cortical layer of myosin; paramyosin is the protein responsible of the considerable texture alterations in emulsified muscle of these marine animals (Mignino and Paredi 2006). Physicochemical and functional property analysis of myofibrillar proteins of scallops, mollusks, and squid by these authors showed that paramyosin in the muscles of these marine animals is different to similar proteins in vertebrates. Mollusk actomyosin has considerably higher viscosity, and lower hydrophobicity and water retention than other species, whereas actomyosin functionality varies depending on the anatomical regions of origin; the highest values were observed in the squid mantle muscle.

23.4 Meat Emulsions

Basically, a meat emulsion is a biphasic system composed of fat globules (dispersed phase) suspended in a matrix made of proteins solubilized in a salt solution (continuous phase). The droplet or fat globule diameter in a true emulsion is between 0.1 and 100 μm (McClements 2005); the fat globule diameter of the dispersed phase in a meat emulsion is larger than 100 μm; therefore, meat emulsions such as finely comminuted sausages or pâté are not considered true emulsions, but pastes or batters. Insoluble proteins, connective tissue, meat particles, and other material are also dispersed in the continuous phase (Rust 1994) although this is, in fact, a viscous fluid or exudate (Feiner 2006). Fat globules are suspended in the liquid bulk due to the formation of a protein film. The protein hydrophilic groups are oriented toward the aqueous phase and the hydrophobic groups, toward the fat (or lipid) phase, hence stabilizing the suspension.

As indicated before, myofibrillar as well as sarcoplasmic proteins can act as emulsifiers. However, myofibrillar proteins are preferably adsorbed to the water/fat interface, in particular to free myosin. Once the fat globules are surrounded by protein, the emulsion is formed; on further processing, the system is stabilized by protein denaturation caused by heat treatment. Myofibrillar proteins produce a strong gel; conversely, sarcoplasmic proteins do not contribute to the stabilization of the product because the gel they produce is very weak. Water retention is, therefore, a fundamental characteristic that must be promoted in meat aimed for emulsified product production. The amount of water bound depends, to a large extent, on the spatial arrangement of actin and myosin fibers, which in turn is due to the pH. At pH above and below the isoelectric point an increase in water retention occurs, resulting in higher water holding. Addition of sodium chloride solution modifies the muscle electric charge, increasing the water retention. The positive charges of sodium binds weakly to the protein's negative charges; whereas the negative chloride ions strongly bind to the positive charges of the protein. The net result is a shift in the isoelectric point, and more water molecules to interact with the proteins (Feiner 2006).

In the context of this chapter, emulsified meat products can be classified into two groups: sliceable products, such as wieners, frankfurters, mortadella, and bologna; and spreadable products, such as pâté, terrine, galantine, and roulade. Pork and beef are extensively used in emulsified meat products; although poultry meat has also been processed into emulsions due to its nutritional characteristics (Zorba 2006).

23.4.1 Sliceable Products

Sliceable sausages are emulsified cooked meat products of finely comminuted pork, beef, or poultry, mixed with fat, water and ice, and additives (salt, nitrate, phosphate seasonings, flavorings, and so on). The meat is emulsified in a cutter (or homogenizer), myofibrillar proteins acting as emulsifiers, although additives such as skimmed milk to increase the cohesiveness and sliceability are generally added. The added salt extracts the muscle proteins that emulsify the fat, forming a gel; this is further stabilized by heat treatment, changing into a solid material. Fat content in an emulsified sausage depends on regional legislation; it can range from 15% to 30%. After mixing the ingredients, the meat is stuffed into natural or synthetic casings. Finally, the ground ingredients are heated in an oven, or smoked/cooked in smokehouses. After cooking/smoking, sausages are cooled down in showers or dipped in a slush tank. For some sausage fabrication, such as wieners or hot dog frankfurters, the synthetic casing is removed. In others, such as Polish and breakfast sausages, edible hog casings are used and remain in the product. The basic textural characteristic of sliceable sausages is cohesiveness; it is measured by a two-cycle compression test using a texturometer or compressimeter (Rosenthal 2010).

23.4.2 Spreadable Products

In addition to traditional emulsified meat products, such as frankfurters or wieners, spreadable sausages are emulsion-type sausages fabricated in many parts of the world. Spreadable sausages are available in a wide variety of products, according to seasonings and adjuncts used in their formulations, such as spices, herbs, and other materials. Liver sausages are usually commercialized in

packages; pâté de foie is packed in a crust or croûte, and or molded or terrine; these are made of different components and various structures, including meat chunks or a pureé-like structure such as terrine de foie de volaille. Although many of these products are made with liver, other pâtés are formulated only by meat and fat. In general, the ingredients for liver sausages and pâté (meat and fat) are precooked; liver acts as an emulsifier. Basic ingredients for pâte can vary, although in general it is made of beef, pork, poultry liver, or shellfish, venison, rabbit, or even vegetables. The most widely commercialized liver pâtés are pork liver followed by duck liver pâtés (Totosaus and Pérez-Chabela 2005; Feiner 2006). For liver pâté fabrication, a meat paste similar to an emulsion is formed; hot water and hot liquid fat is mixed to the meat paste (Feiner 2006). Different pâté products are high calorie-containing foods, in addition to saturated fats, sodium, and cholesterol. Therefore, oxidation is one of the main problems in shelf-life reduction in this type of products.

Fabrication of a spreadable emulsified meat product starts with cooking coarsely ground or chopped ingredients. Previously scalded fat (45–50% total formulation) is then added, or fat substitutes are included in the formulation. The meat block is then homogenized with hot broth (Totosaus and Pérez-Chabela 2005). Nonmeat proteins, such as skim milk powder or soy protein concentrates are included in the formulation as stabilizers. The paste is then homogenized together with the liver, if included in the formulation, as well as spices, herbs, and other additives. Although the main emulsifiers are liver proteins, in commercial operations, other emulsifiers and plasticizers are added to improve spreadability. The batter is finally stuffed into casings or canned, avoiding air trapping, as air reduces heat transference and promotes lipid and pigment oxidation. Heating changes the gel into a spreadable emulsion, which, together with the high fat content accounts for the characteristic of this product (Guerrero-Legarreta 2010). Some attempts have been made to change these product formulations. Martin and others (2008) prepared pork liver pâté partially replacing pork fat by conjugated linoleic acid and olive oil. The authors reported that the products were stable to lipid oxidation for 71 days in refrigerated storage. Pâté and similar products are semisolid foods, therefore they are consumed as that is expected to be consumed as a spread; spreadability is the most important physicochemical characteristic. It is a subjective texture characteristic related to the material yield stress, that is, the minimum shear stress required to initiate flow (σ_0) (Guerrero-Legarreta 2010).

23.4.3 Low-Fat Products

In low-fat products, the dispersed phase has been totally or partially replaced by other material that contributes to the formation of a two-phase system similar to an emulsion. Fat substitutes are starches, hydrocolloids, skimmed dried milk, gums (pectin, carrageenan, gelan, xanthan), and plant proteins. Other carbohydrates, such as pregelatinized starch, develop instantaneous stable viscosity. These ingredients also provide gelling properties and texture, bind liquids, control syneresis, improve slicing, and increase product yield. Cogelling ingredients, such as protein–polysaccharides also allow fat reduction in formulations. Kaack and others (2006) studied the effect of high cellulose-containing fiber in reducing fat in liver pâté; they found that the product had better flavor and texture as compared to original pâté.

One of the main problems in developing low-fat spreadable meat batters is to keep good extensibility characteristics. Patel and Gupta (2006) described a spreadable product formulation including soy and skimmed dried milk, as well as sodium citrate to reduce oil release in the final product and to increase the flowing ability. Carrageenan and guar gum effectively increase extensibility, plasticizers such as sorbitol and glycerol favor viscosity, flavor, and texture, and annatto and β-carotene are used as colorants. The paste had improved extensibility properties and kept its quality for close to 3 months under refrigeration conditions. An American patent (5693350, Fernández and others 1997) describes a process to fabricate low-fat meat pâté; the patent refers to a product with <10% fat as a low-fat pâté; the formulation includes 40–80% lean meat (3–8% fat), up to 15% fat substitute, 15–50% added water, 1.2–2.4% nitrate salts, and up to 0.3% phosphate. The authors reported that the meat emulsion is treated for proteolytic digestion and further cooking; and hydrostatic pressure (>400,000 kPa) for enough time to obtain <4.5 protease units/g pâté. The product had better extensibility and spreadability than traditional pâtés.

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24

Mechanical Deboning

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24.1 Introduction

In recent decades, the amount of meat coproducts available from slaughterhouses, meat processors, and wholesalers has increased considerably. At the same time, traditional markets for edible meat by-products have gradually disappeared due to changing food habits and new health concerns among consumers in Western Europe (Darine and others 2010).

The meat, poultry, and fishery industries are always searching for alternatives to utilize low-value coproducts (cuts and trimmings) by transforming them into marketable products with added value (Castro-Briones and others 2009). When highly priced muscles from animals, poultry, or fish are removed, relatively hard or tough components, such as bone, gristle, tendons, and so on remain as the carcass. Mechanically deboned meat (MDM), mechanically recovered meat (MRM), or mechanically separated meat (MSM) are synonyms used to mark the material, obtained by application of mechanical force (pressure and/or shear) to animal bones (sheep, goat, pork, beef) or poultry carcasses (chicken, duck, turkey) from which the bulk of the meat has been manually removed (Püssa and others 2009). The deboning process can be applied to whole carcasses, necks, backs and in particular, to residual meat left on the bones after the completion of manual deboning operations (Barbut 2002).

The mechanical process of removing meat from the bone causes cell breakage, protein denaturation, and an increase in lipids and heme groups and poorer mechanical properties. MSM is characterized by a pasty texture. This texture is due to the high proportion of pulverized muscle fiber residue, and the presence of a significant quantity of partly destructured muscle fibers. The term we use for this loss or modification of muscle fiber structure is "destructuration" (Sifre and others 2009). MDM has a jelly-like consistency and high fat levels, so that it is mainly used in the preparation of emulsion products

(Guerra Daros and others 2005). However, meat recovered from bones or carcass parts by mechanical procedures is generally considered to be of poor nutritional and microbiological quality and is therefore subjected to strict regulations concerning its use as a binding agent or as a source of meat proteins in minced meat products.

Generally, MDM is used in the formulation of comminuted meat products due to its fine consistency and relatively low cost. This is of great importance, especially in underdeveloped countries, because the price of the product into which it is incorporated can be kept low. The incorporation of MDM into emulsified meat products and, in lower proportions, into nonemulsified meat products has opened up additional markets for this type of meat (Mielnik and others 2002).

24.2 Equipment

From an engineering point of view, the recovery machines used to separate the residual meat from the bone can be divided into three main types: those which separate the meat from the bones by means of a sieve screen, those which use a stripper disk and those using centrifugal force (Crosland and others 1995). Depending on the operating conditions and the type of machine selected, the chemical composition of the MDM obtained will be different.

24.3 Chemical Composition of Mechanically Deboned Meat

The chemical composition of MDM is inherently variable due to the natural variation within and between animal species, cuts of meat, previous treatment of the bones (trimming, freezing, and so on) and the machine type and operating conditions used in the recovery process (Day and Brown 2001). Studies on the most diversified characteristics of these meats (Al-Najdawi and Abdullah 2002; de Azevedo Gomes and others 2003a; Serdaroúlu and others 2005; Püssa and others 2008; Püssa and others 2009) have been carried out into the contents and quality of proteins, lipids and minerals, bone content, lipid and pigment stability, pathogenic and spoilage bacteria, among others, in order to demonstrate that their consumption represents a risk to human health and to prevent the wastage of large amounts of minerals, lipids, and animal protein of potential nutrition value (Trindade and others 2004).

24.3.1 Protein Content

The protein content of MDM varies from 11.4% to 20.6% depending on the material being deboned (e.g., backs, necks, wing, legs) and the animal (beef, pork, lamb, chicken, turkey, duck) (Navarro and others 2010). Thus, the protein content is higher in MSMs than in manually separated meats. Table 24.1 shows the protein content of MDM of some animal species.

One source of proteins in MDM is collagen; Table 24.2 shows the collagen content in some samples of MDM. Collagen exists in several forms and constitutes an integral part of both muscles and bones. The fact that currently applied methods of measuring collagen in meat are not type specific, that collagen is not included in consumer concepts of meat, that collagen contains lower content of essential amino acids in comparison to muscle and thus makes it discarded from a nutritional point of view, justify all efforts to reduce the levels of collagen in minced meat and minced meat products (Henckel and others 2004).

A high collagen content in any meat product can negatively influence its technological and nutritional characteristics, since it is a protein with inferior functionality and low nutritional value because of its poor balance of amino acids (Trindade and others 2004). Abdullah and Al-Najdawi (2005) reported that the collagen content of MDM is considered detrimental to its emulsifying capacity, although, no effects were observed upon the water-holding capacity (WHC).

As mentioned earlier, the animal parts and the method used may influence the collagen content of the samples of MDM. Thus, Henckel and others (2004) analyzed the collagen contents in back and breast parts of broilers observing no differences between manually and MDMs in either part. However, when

TABLE 24.1

Protein Content of MDM of Some Animal Species

Animal	Raw Material	Protein Content (%)	Reference
Chicken	Dorsal part of the carcass, neck and skin	11.00	Negrão and others (2005)
Chicken	Skinless breasts	13.60	Perlo and others (2006)
Chicken	Skinned carcasses	20.35	Al-Najdawi and Abdullah (2002)
Chicken	Dorsal part of carcasses	12.20	Stangierski and others (2002)
Chicken	Skinned carcasses	20.45	Abdullaha and Al-Najdawi (2005)
Turkey	Neck	17.20	Radomyski (2000)
Turkey	—	13.20	Liang and Hultin (2003)
Hen	Carcasses	14.72	USDA (1999)
Pork	Hams	11.52	Jimenez-Colmenero and Garcia (1982)
Pork	Chines	15.00	Crosland and others (1995)
Beef	Bones from extremities and ribs	7.60	Skarpeid and others (2001)
Beef	Neck bones	17.49	Mayer and others (2007)
Lamb	Neck and ribs	15.62	Crosland and others (1995)
Rabit	Carcasses	17.10	Mcnitt and Negatu (2003)

Al-Najdawi and Abdullah (2002) evaluated the collagen content of manually and MDMs of whole and skinned hens, they observed more collagen in the MDM than in the meats of manually deboned hens.

24.3.2 Lipid Content

Mechanical deboning of meat affects the lipid composition of the resulting meat, which normally has a higher lipid content than manually deboned meats. These extra lipids may originate from subcutaneous fat, the skin or abdominal fat (depending on the animal species and method used) but mainly come from bone marrow and bone tissue (Trindade and others 2004). The fat present in MDM is rich in polyunsaturated fatty acids due to the presence of phospholipids from the fraction of bone and accompanying spinal marrow (de Azevedo Gomes and others 2003a). The marrow contains varying amounts of fatty acids, ranging between 7% and 48%, depending on the animal species and even type of bone since the marrow from leg bones of adult animals can contain up to 90–95% fat (Field and others 1980). The reason why MDM has been regarded as having a potential health risk is the high concentration of various polyunsaturated fatty acids (PUFA), which may have adverse physiological effects via their (per)oxidation products (Püssa and others 2009). Table 24.3 shows the fat content and the fatty acid profile of MDM from several animal species. Amongst the fatty acid profile of meats, the high levels of unsaturated fatty acids are usually associated with MDM. Unsaturated fatty acids are regarded as beneficial to human health but are more prone to oxidation, causing losses in the sensorial quality of meats during storage (Trindade and others 2004).

TABLE 24.2

Collagen Content in Some Samples of MDM

Animal	Raw Material	Collagen Content (%)	Reference
Chicken	Half frames	1.49	Rivera and others (2000)
Chicken	Whole carcasses	3.45	Al-Najdawi and Abdullah (2002)
Chicken	Skinned carcasses	3.00	Al-Najdawi and Abdullah (2002)
Turkey	—	1.09	Liang and Hultin (2003)
Broilers	Breast	1.11	Henckel and others (2004)
Broilers	Back	1.36	Henckel and others (2004)
Pork	Shoulder blade	1.20	Crosland and others (1995)
Beef	Aitch bone	7.60	Crosland and others (1995)

TABLE 24.3

Fat Content and the Fatty Acid Profile of MDM of Some Animal Species

Raw Material	Fat Content (%)	SFA (%)	MSFA (%)	PSFA (%)	Reference
Broiler	14.7	29.7	48.6	21.5	Püssa and others (2008)
Turkey	20.3	31.2	47.2	21.3	Püssa and others (2009)
Turkey	14.0	36.28	29.83	33.89	Serdaroúlu and others (2005)
Turkey	13.8–17.2	37	43.5	19.6	Mielnik and others (2003)
Beef	31.8	55.84	41.22	2.94	Serdaroúlu and others (2005)
Pork	25.0	40.4	45.2	13.7	Püssa and others (2009)

Notes: MSFA: Monosaturated fatty acids; PSFA: Polysaturated fatty acids; SFA: Saturated fatty acids.

24.3.3 Cholesterol Content

One potential aspect of concern for consumers is the cholesterol content, which is higher in MSMs than in manually separated meats. Cholesterol released from the bone marrow by mechanical deboning increases cholesterol concentrations in MDM (Al-Najdawi and Abdullah 2002). The major factors affecting cholesterol level in MDM are: bone marrow, body fat, and skin (Demos and Mandigo 1995). Ang and Hamm (1982) reported higher levels of cholesterol in MDM broiler meat than in the corresponding hand deboned meat. Serdaroúlu and others (2005) reported higher values of cholesterol in beef and turkey meat after mechanical deboning: 89.6 mg/100 g in mechanically deboned beef meat and 63.6 mg/100g in mechanically deboned turkey meat (MDTM), compared with 50.3 and 56.9 mg/100 g in the manually deboned meats, respectively. Similarly, Al-Najdawi and Abdullah (2002) found that mechanically deboned chicken with skin contained more cholesterol than hand deboned chicken with skin.

24.3.4 Bone Content

During the mechanical deboning of meat, it is inevitable that some bone particles pass into the MDM. These particles contain high levels of calcium. In MDM, bone content and thus also calcium content are elevated compared to fresh meat (Mayer and others 2007). However, it is methodologically problematic to define MDM as regards a threshold for the calcium content of meat products. Calcium represents 37% of the ash contents of bones but both ashes and calcium increase during calcification processes, so different conversion factors would be necessary to estimate the content of bones through calcium content or ash. The original calcium content of bones varies with bone type (trabecular or compact) as well as with species, feeding, or age of the slaughtered animals (Stenzel and Hildebrandt 2006). Field (2000) conducted an extensive review on the use of ash and calcium as indicators of the presence of bones in MSM and they concluded, based on revised data which could be recommended by a factor of 4.5 for conversion of round bones of cattle, sheep, pigs and chickens, and a factor of 5 for bones of younger animals (calves, chickens), and for all flat bones. The use of the calcium content as an indicator of bone material is reflected by the provisional formulations of EC regulations 853/2004 and 2074/2005, which allow the use of MSM in food production. The size of bone particles is determined primarily by (i) the deboning machine, (ii) operation, and (iii) the size of filter used. Poor assembly or adjustment could lead to obtaining unacceptable particle size, affecting the quality of products which use the MDM, although, in general, the presence of bones is not a problem (Newman 1981). Thus, Koolmees and others (1986) concluded that in general MDM produced by pressure force machine presents more cartilage and less calcium (less bone) than sieve screen machines.

24.4 Functional Properties of Mechanically Deboned Meat

Since MDM is used in the manufacture of emulsion products, the emulsifying capacity (EC) is an important property of the raw material (Field 1988). EC has been defined as the amount of oil that can be

emulsified by the material prior to reversion or collapse of the emulsion (Kato and others 1985). The proteins present in MDM serve as the natural emulsifying agent in a meat (Álvarez and others 2007). Myosin is the most important of the proteins for fat emulsification-processed meats. These proteins may bridge the oil–water interface during the emulsification steps (Xiong 2000); however, sarcoplasmic and stromal proteins have a low fat-emulsifying capacity (Ordoñez and others 1998). Proteins absorb as an excess layer at the oil–water interface and lower the interfacial tension between the phases. The excess layer of protein on the surface of droplets may generate sufficient steric and electrostatic repulsion interactions to result in the formation of a stable emulsion (O'Regan and Mulvihill 2010). The presence of skin in MDM is considered detrimental to EC, because of its collagen content (Abdullah and Al-Najdawi 2005) while other factors that affect the stability of meat emulsions are the size of the fat globule, viscosity, pH, and temperature (Perlo 2000; Zogbi and Benejam 2010).

WHC is a very important property in meat and meat products as it is related which juiciness and tenderness (Totosaus and Guerrero-Legarreta 2006). This property, which refers to the ability to retain water within the matrix, is an important property of MDM from both a physiological and technological point of view (Navarro and others 2010). It is highly important in the formulation, processing, cooking, and freezing of meat products, because it is related with weight loss and the ultimate quality of the finished product (Field 1988). Factors affecting WHC are pH, the presence of iron, copper, calcium, and magnesium from bone, the skin and collagen contents and the cooking and freezing processes used (Abdullah and Al-Najdawi 2005).

Kumar and Wismer-Pedersen (1984) reported that the application of propyl-galate and tripolyphosphate in MDM samples improved its WHC and emulsifying properties, reducing fat separation and jelly product compared with untreated meat. Abdullah and Al-Najdawi (2005) analyzed the functional properties of mechanically deboned chicken meat (MDCM) with and without skin. These authors mentioned that the removal of the skin prior to deboning led to significantly higher EC values, while WHC values were not significantly affected. Smith and Brekke (1985) mentioned that the partial proteolysis of mechanically deboned fowl myofibrillar proteins with an acid protease improved protein solubility, emulsifying capacity, and gelation compared with a myofibrillar protein control.

24.5 Applications of Mechanically Deboned Meat

The use of MDM, principally poultry meat, has increased in the food industry because of the strong tendency to replace red meat by healthier white meat in industrialized countries, and the lower price of the latter compared with other kinds of meat (Guerra-Daros and others 2005). MDM is widely used in the formulation of comminuted meat products, owing to its fine consistency and lower cost (Ozkececi and others 2008). The incorporation of MDM into emulsified as well as nonemulsified meat products in relatively low proportions has provided extra markets for slaughterhouse coproducts (Mielnik and others 2002). The main applications of MDM are in products which do not require a fibrous texture but demand emulsion stability, natural color, and relatively low cost (Barbut 2002). However, a major problem for products manufactured with MDM is the rapid onset of oxidative rancidity, which results in off-flavors and off-odors (Lee and others 1975). Table 24.4 shows the application of MDM to different meat products.

The mechanically recovered meat used to elaborate meat products may cause various changes in the chemical, physicochemical, and sensory properties of these products. Thus, Trindade and others (2005) reported that the application of different levels (20%, 40%, 60%, 80%, and 100%) of mechanically separated layer hen meat (MSLM) to replacement levels of beef and pork backfat in *mortadellas* sausage had no influence on lipid oxidation. Likewise, the use of up to 100% MSLM in *mortadella* did not affect the microbial quality of the products. However, the increase in MSLM reduced the intensity of the red color and also the consistency and sliceability of the *mortadella*.

Perlo and others (2006) analyzed the effects of different proportions (0%, 10%, 20%, 30%, 40%) of washed mechanically deboned chicken meat (WM) as a substitute for hand deboned chicken meat, on the physicochemical and sensory characteristics of chicken nuggets. The addition of WM increased the fat content whereas the protein content was significantly reduced. The addition of WM did not affect the sensory attributes of chicken nuggets.

TABLE 24.4

Application of MDM to Different Meat Products

Product	MDM	% Addition	Reference
Bologna sausage	Hen Layer	20, 40, 60, 80, 100	Trindade and others (2005)
Nuggets	Chicken	40	Chinprahast and others (1997)
Nuggets	Chicken	10, 20, 30, 40	Perlo and others (2006)
Nuggets	Chicken	10, 20, 30, 40	Bonato and others (2006)
Pork sausage	Turkey	35	Li and Wick (2001)
Snack sausages	Beef	20	Smith and others (2001)
Snack sausages	Pork	10, 20, 30	Kramer and Sebranek (1990)
Hamburger	Pork	10, 20, 40, 60	Santos and others (2002)
Sausage	Chicken	20, 40, 60, 80, 100	Guerra-Daros and others (2005)
Patties	Beef	5, 10, 15, 20, 25, 30	Cross and others (2006)
Patties	Chicken	1, 2, 4	Püssa and others (2008)
Sausages	Turkey	100	Mielnik and others (2002)
Smoked sausages	Poultry	10, 30,50, 70	Jantawat and Carpenter (1989)
Frankfurters	Pork	10, 20, 30, 40, 50	McMillin and others (1980)
Frankfurters	Chicken	50, 60, 70, 80	Babji and others (1998)
Mortadella sausage	Hen	100	Trindade and others (2006)

Chia and others (1999) studied the possible changes in the quality of chicken sticks elaborated with MDCM (0–50%) mixed with chicken breast. The protein content and hardness of the sticks decreased as the proportion of MDCM concentration increased; however, the fat content and the losses due to cooking increased. Sensorially, when between 30% and 50% of MDM was used, the general acceptance improved, although the product was considered softer than the control.

Smith and others (1991) evaluated fermented beef snack sausages containing partially defatted chopped beef (PDCB), finding that the chemical and sensory properties were not affected by the incorporation of up to 20% PDCB.

Chinprahast and others (1997) compared the quality of nuggets prepared solely from muscle meat with nuggets prepared with a combination of MDM and chicken breast meat. The best results were obtained with a 40:60 combination of MSM:breast which showed no significant differences from the 100% breast nuggets; while at higher levels of MSM the sensory characteristics fell. In the product with 100% MSM, the strength and adhesiveness significantly decreased and the product presented a darker color. Cross and others (2006) characterized the palatability of cooked patties made from seven formulations of ground beef containing 0–30% of added mechanically deboned beef. The mean panel ratings for overall acceptability were greater for patties containing 5%, 10%, 15%, and 20% mechanically deboned beef than for the control. However, patties containing 25% and 30% mechanically deboned beef had significantly lower flavor and acceptability ratings than the control. As the percentages of added mechanically deboned beef increased, panel ratings for tenderness and juiciness increased. The amount of detectable connective tissue decreased significantly as the percentage of added mechanically deboned beef increased.

24.6 Mechanically Deboned Meat Deterioration

Many of the functional, organoleptic, and sensorial properties of food products diminish with time. In MDM, this may be attributed to a large number of factors such as chemical composition, comminute structure, and so on although the main factors are the action of lipid oxidation (provoked by oxygen, light, and temperature) and microorganisms (bacteria, moulds, and yeasts) (Viuda-Martos and others 2007). Lipid oxidation is a complex process whereby unsaturated fatty acids react with molecular oxygen via free radicals to form peroxides or other primary products of oxidation (Kapich and others 2010). Chemical and structural alterations during mechanical separation, the development of undesirable aro-

mas (rancidity) and the loss of its characteristic red color because of lipid and pigment oxidation, respectively, mean that MSMs lose stability during storage (Trindade and others 2006). Dawson and Garner (1983) have reported that extreme mechanical stress and extraction of important amounts of fats and heme components from the bone marrow, as well as aeration during the mechanical deboning process are responsible for the high oxidative potential of MDM. This process (mechanical deboning) may lead to heme pigments affecting the intramuscular fat and thus causing flavor problems (Froning 1981). Also, metal ions from the deboning machinery itself and calcium and phosphorus ions from bone may act as catalysts for heme oxidation (Field 1988). These transition metal ions can stimulate lipid peroxidation in two ways: (i) by participating in the generation of initiating species or (ii) by accelerating peroxidation, decomposing lipid hydroperoxides into other components which are able to abstract hydrogen, thus perpetuating the chain of reaction of lipid peroxidation (Deshpande and others 1995).

Quality loss may also result from the action of microorganisms. MDM is usually heavily contaminated with microorganisms, which are introduced during processing mainly as a result of poor hygienic measures (environment, handlers, and equipment) and improper holding temperature during production and storage phases (Yuste and others 2002). Several aspects of the mechanical recovery process, especially the small particle size and large surface area, the release of nutrient-rich cellular fluids due to tissue maceration, the heat generated during mechanical deboning and extensive handling, may enhance bacterial growth, all the above factors making MDM highly perishable (Kumar and others 1986; Johnston and Tompkin 1992). The redistribution of the higher initial microbial load from the more exposed areas and greater availability of nutrients resulting from breakage of the cells favor the growth of microorganisms (Trindade and others 2006).

24.7 Improving the Shelf Life of Mechanically Deboned Meat

Improvements in the shelf life of products may have an important economic impact, reducing losses attributed to spoilage and allowing the products to reach distant and new markets (Lemay and others 2002). New techniques such as high pressure, nanotechnology, irradiation, ingredients with functional properties, and so on are increasingly used to maximize the shelf life of the product, while improving the nutritional properties of foods (Viuda-Martos and others 2008).

24.7.1 Irradiation

One promising tool is processing food by irradiation, especially when conventional methods cannot be applied (Mayer-Miebach and others 2005). The potential application of ionizing radiation in food processing is based mainly on the fact that ionizing radiation damages the DNA very effectively so that living cells become inactivated; therefore, microorganisms, insect gametes, and plant meristems are prevented from reproducing, resulting in various preservative effects as a function of the absorbed radiation dose (Farkas 2006). Irradiation, as a method of meat preservation, has excellent potential for the elimination of pathogenic and spoilage microorganisms from meat and meat products, and has been viewed by most food safety officials and scientists as an effective critical control point in a hazard analysis and critical control points (HACCAP) system established for meat processing (Badr 2004). Another advantage of irradiation is that treatment causes practically no temperature rise in the product. Irradiation can be applied through packaging materials including those that cannot withstand heat. This also means that radiation treatment can be performed after packaging, thus avoiding recontamination or reinfestation of the product (Farkas 2006). Numerous authors have studied the application of irradiation to MDM, thus de Azevedo Gomes and others (2003a) irradiated samples of MDCM in frozen form with doses of 0.0, 3.0, and 4.0 kGy. The frozen chicken was stored at 2 ± 1°C for up to 12 days and evaluated for its sensory characteristics, color, lipid oxidation (TBARS) and total psychrotrophic bacterial count. As regards the sensory analyses, color, psychrotrophic bacterial counts and TBARS analyses as a whole, the MDCM samples irradiated with doses of 0.0, 3.0, and 4.0 kGy were acceptable under refrigerated storage for 4, 10, and 6 days, respectively. Thayer and Boyd (1992) reported that 90% of *Staphyloccocus aureus* in MDCM was killed by a dose of 0.36 kGy. D_{10} values in the range of 0.40–0.46 kGy for *S.*

aureus in different meat systems irradiated at 5°C have also been reported (Thayer and others 1995). de Azevedo Gomes and others (2003b) evaluated the 2-thiobarbituric acid (TBA) method for measuring lipid oxidation in samples of MDCM, both gamma-irradiated with doses of 4.0 and 5.0 kGy and nonirradiated, finding similar TBA test values for all the samples. Szczawińska and others (1991) analyzed MDCM irradiated at 0, 1.25, and 2.50 kGy, before storing at 5°C and 10°C. Irradiation at 1.25 and 2.50 kGy caused an average reduction in bacterial levels of 2.23 and 3.44 logs, respectively.

24.7.2 High Pressure

High hydrostatic pressure (HHP) in the range 100–800 MPa may help alleviate the growth of pathogenic microorganisms in food products, including meat. The nonthermal character of this technology provides a unique opportunity to the food industry for developing novel foods of superior nutritional and sensory quality (Tuboly and others 2003). HHP represents an attractive nonthermal process for meat products to avoid postprocessing contamination. When combined with antimicrobials like bacteriocins, the microbial death rate may be increased because of sublethal injuries to living cells. HHP is a powerful tool to control risks associated with *Salmonella* spp. and *Listeria monocytogenes* in raw or marinated meats (Hugas and others 2002).

Yuste and others (1999) analyzed mechanically recovered poultry meat (MRPM) inoculated with *Listeria innocua* 910 CECT at an approximate level of 108 CFU g^{-1}, treating vacuum-packaged samples with combinations of pressure (350, 400, 450, and 500 MPa). High pressure caused a marked bactericidal effect on *L. innocua*, and reductions higher than 7.5 log units were achieved in several cases. Tuboly and others (2003) studied MDTM treated by HHP (200 MPa; 20 min and 400 MPa; 20 min) and observed a significant reduction in the numbers of viable cells, an increase in thiobarbituric acid reactive substances (TBARs) and the formation of cholesterol oxidation products. Yuste and others (1998) evaluated the combined effect of high hydrostatic pressure processing, the addition of nisin and acidification on aerobic mesophilic and psychrotrophic bacterial populations in mechanically recovered poultry meat (MRPM) kept under refrigeration (2°C). In some samples a reduction of mesophile counts of between 3.44 and 5.38 log cfu g^{-1} was observed while psychrotrophs were reduced to undetectable levels.

24.7.3 Plant Extracts

Many sources of antioxidants and antibacterials of plant origin have been studied in recent years (Singh and others 2005; Viuda-Martos and others 2011). Among these, many aromatic plants and spices have been shown to be effective in retarding the processes of lipid peroxidation and microbial growth (Kulisic and others 2004; Viuda-Martos and others 2007, 2008). However, very little information is available concerning the effect of plant polyphenol extracts on the quality of MDM (Hassan and Fan 2005). Püssa and others (2008) studied the stability of mechanically deboned chicken and turkey meat, supplemented with 1%, 2%, or 4% of dried sea buckthorn (*Hippophae rhamnoides*) berry powder residues. These authors reported that the processing residue of sea buckthorn juice is a good functional supplement to MDM products that inhibits the oxidation of fatty acids and enriches the meat products with plant-derived health-beneficial polyphenols. Mielnik and others (2003) analyzed the effects of commercial rosemary antioxidants on oxidative stability of MDTM compared with Trolox C (vitamin E) and ascorbic acid (vitamin C). The investigation showed that the oxidative stability of MDTM stored in packages with free oxygen access was improved by adding antioxidants in the following order Trolox C > Ascorbic acid > rosemary extracts especially during long-term frozen storage. Raghavan and Richards (2006) studied the ability of cranberry press cake to inhibit lipid oxidation in mechanically separated turkey meat. The ability of cranberry extracts to inhibit lipid oxidation depends on the type and amount of specific constituent phenolic compounds, like quercetin, rather than the total amount of phenolic components. Increasing the amount of quercetin in the press cake extracts could increase the ability of the extracts to inhibit lipid oxidation in MST. In a similar study, Lee and others (2006) assessed the ability of the components of cranberry powder to inhibit lipid oxidation processes in mechanically separated turkey (MST) and cooked ground pork. They reported that quercetin, a nonglycosylated flavonol present in cranberry powder, inhibited lipid oxidation in MDTM. Kathirvel and others (2009) reported that an extract prepared from cran-

berry juice powder was particularly effective in inhibiting lipid oxidation in mechanically separated turkey (MST), providing more than 3 weeks of additional stability during −4°C storage at a usage level of 0.1% due to the presence of flavonoids such as quercetin and quercetin-3-*O*-(6″-benzoyl)-β-galactoside. Mielnik and others (2006) studied the efficiency of four concentrations of grape seed extract (0.0, 0.4, 0.8, and 1.6 g/kg) for retarding the oxidative rancidity and was tested with cooked mechanically deboned turkey breast meat. They conclude that grape seed extract could be very effective in inhibiting lipid oxidation of cooked turkey meat during chill-storage and acted in a concentration-dependent manner.

Hassan and Fan (2005) tested the antioxidant potential of cocoa leaf extracts at different concentrations (100, 200, 400, and 800 mg/kg) in a model meat system, based on MDCM, compared with a 1:1 butylated hydroxyl anisole (BHA)/butylated hydroxyl toluene (BHT) mixture (200 mg/kg) and green tea extract (200 mg/kg). These authors mentioned that the best antioxidant characteristic was shown by the BHA/BHT mix, although the performance was closely matched by the natural polyphenols from green tea and 800 mg/kg cocoa leaves. At lower concentrations of 200 and 400 mg/kg, the antioxidant potential of cocoa leaf extracts was about 50–80% of the BHA/BHT. Another component present in aromatic plants and spices, which may act as a natural antioxidant and antimicrobial, is the corresponding essential oil (Viuda-Martos and others 2008, 2010). These authors reported that the essential oils of some spices (sage, rosemary, thyme, oregano, and clove) were capable of chelating iron (II) and did so in a concentration-dependent manner. This property could be very useful since it would limit the presence of free iron in the MDM, reducing the reactions in which this metal participates. Spice essential oils can be considered good sources of natural compounds with a significant degree of antioxidant activity that could be used to retard lipid oxidation and antimicrobial growth in MDM.

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25

Breading

Teresa Sanz and Ana Salvador

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25.1 Generalities about Breading

The term breading essentially refers to the coating, traditionally formed by bread crumbs, which is applied in dry form to the outer surface of a food. A more general definition considered breading as thermally processed cereal-based foods (Dyson 1983).

The breading can be applied directly to the food substrate or, most frequently, to the surface of a previously battered food. In the latest case, the breaded food has a more complex structure as it also contains a layer of batter, which acts as an adhesive glue to fix the breading.

Most frequently, bread crumbs are used for breading, but breading can be simply composed of cereal flour, usually wheat, or they might contain also other ingredients such as gums, seasoning, colorants, and so on. The selection of the breading composition will depend on the required functionality (crispness, flavor, oil absorption, etc.).

In the production of bread crumbs, the breads that are used may come from regular bread production (whether fresh or returned stales) or may be baked according to special recipes, which may contain spice blends, increased shortening levels, special flavors, and other functional additives. Also the breads may be processed to differ in texture, either more compact or more expanded, so as to cover specific requirements and to achieve the highest variability (Jackel 1993). Some bread crumbs are manufactured through an extrusion process, without using loaves of baked bread. The crumbs produced by extrusion differ in appearance, flavor, mouth feel, and consistency from those processed from loaves of bread. The bread crumbs contain larger and more porous particles that are crunchier in texture than the denser and more finely ground particles produced by extrusion.

The breading must have sufficient strength and integrity to adhere to the food. In general, the higher density and more compact-textured bread crumbs, the less the oil and moisture absorption.

The selection of breading particle size also depends on the substrate dimensions. Golden medium-to-coarse breadcrumbs are preferable to achieve golden color and uniform coating in fish/chicken fillets or burgers. Fine-to-medium breadcrumbs are preferable for chicken nuggets, fish sticks, oysters, shrimp, and scallops (Zhang 2001).

25.2 Types of Breading

According to the ingredients and mainly, to the production method employed, breading is classified into different types. The most common types are as follows:

25.2.1 Flour Breaders

It consists of wheat flour, although it might contain other flours, starches, gums, colorants, and seasonings. This type of breading is the simplest and creates a home-style appearance (Kuntz 1997a).

25.2.2 Cracker Meal Type

This type of breading is formed using a cracker-type formulation. The ingredients are mixed with water to form a dough that is passed through a series of paired rollers forming a sheet ~1 in. thick. The dough sheet is baked out completely or baked to higher moisture, then requiring a secondary drying after grinding. The final moisture content of the breading is ~8%. This moisture level ensures a long time stability of the breading and contributes to the absorptive capacity. The crumbs obtained may be sieved for size.

Different from cracker baking, the manufacture of cracker meal-type breading does not require long fermentation times (Dyson 1983).

Cracker meal-type breading provides a hard and crunchy texture. However, texture modifications can be obtained by changes in the formulation.

This type of breading is widely used on fish products.

25.2.3 American Bread Crumbs

This type refers to bread crumbs obtained from baked loaves of yeast-raised bread. In this method, the dough mixing operation is followed by a fermentation time. The dough is divided into portions that are proofed and baked as in the production of sandwich bread. The loaves are dried to the required moisture, then ground and sieved into fine, medium, and coarse sizes.

This type of breading is more porous and provides a less tough, crispier texture than cracker meal-type breading. American bread crumbs also tend to darken more quickly.

25.2.4 Crispbreads, Extrusion Cooking

This type of breading is manufactured through an extrusion process, without using loaves of baked bread. A cooker–extruder system is employed. The flour is mixed under highly turbulent conditions in the presence of steam to a moisture content of ~18%. The steamed flour is then directly fed into the extruder. The particles obtained by this system are denser and more finely ground than the ones of American bread crumbs.

25.2.5 Japanese or Oriental-Style Bread Crumbs

This type is made by cooking traditional yeast bread dough using microwaves or electrical resistance, instead of oven-baking. The bread is cooked in less than one-half the time taken for conventional oven baking. The result is a crust-free, low-density loaf.

The breading obtained is of low density, very porous material.

25.3 Quality Properties of Breaded Food

25.3.1 Pick-up and Adhesiveness

Pick-up and adhesiveness are one of the most used methods to evaluate objectively the quality of breaded food (Cunningham and Tiede 1981, Scott 1987). The value of pick-up is regulated for breaded food, which cannot exceed 30% (Johnson and Hutchinson 1983).

Batter pick-up is an important factor for batter-coated products and for industrial manufacturers because the weight of the breaded layer in relation to the total weight of the breaded-coated food determines the process yield which affects the final cost of the product.

In battered products, pick-up is defined as the percentage of raw batter that adheres to an uncoated piece of food (Salvador and others 2002):

$$\text{Batter pick-up}\,(\%) = \frac{B - I}{B} \times 100$$

where B = mass of battered raw piece of food, and I = initial mass of raw unbattered piece of food.

In breaded products, there is a natural tendency for the bread crumbs or particles to fall off the coated product. Hsia and others (1992) defined pick-up in terms of the following formula:

$$\text{Cooked yield}\,(\%) = \frac{S}{I} \times 100$$

where S = mass of the cooked breaded piece of food after shaking, and I = initial mass of raw unbreaded piece of food. In this case, the pick-up takes into account possible losses in the adhered breading layer due to postcook handling.

Good fried coating adhesion to the substrate in the end product is closely related to the adhesion of the raw batter at the moment when it is applied (Fiszman and Salvador 2010).

On the other hand, adhesion can be defined as the chemical and physical bonding of a food coating with the food structure (Suderman and Cunningham 1983). Some of the factors that affect batter adhesion to food products are batter ingredients and predusting material. The bread crumb particle size is also important. Maskat and Kerr (2004) studied the effect of different particle size and they found that breadcrumbs with a particle size of <250 nm resulted in higher values of coating adhesion.

With respect to batter ingredients, adhesion is a critical characteristic of batter-coated food which has been associated with gums. The effectiveness of gums to improve adhesiveness is due to the gel-forming properties of some gums such as cellulose and its derivatives. Therefore, the use of gellan gum, pectin, carrageenan, and alginates in the presence of ionic salts has also been associated with improved adhesion (Kuntz 1997b). Hsia and others (1992) studied the breading adhesion of different hydrocolloids and found a high correlation between apparent viscosity and adhesiveness. However, xanthan gum was the only hydrocolloid that improved adhesion. Starches with high amylose content increase the adhesiveness of breading to the products (Harper 1981, Meyers 1990). Acioli-Moura and Yoon Kil Chang (2006) optimized an extruded breading with a content of 15% vital wheat gluten, 85 high amylose starch, and 30% corn meal. This breading presented good values of pick-up and adhesiveness (29.87% and 100% respectively). They found greatest values of adhesiveness with 9–24% of vital wheat gluten and 9–26% of high amylose starch, and pick-up values around 30% with 15–17% vital wheat gluten and 18–23% high amylose content.

Predust is a fine, dry material that is dusted onto the moist surface of the food substrate before further coating is applied. Predust material also can improve batter adhesion because it absorbs part of the water on the surface of the substrate and creates a rougher structure (Yang and Chen 1979). Many works have focused on improving batter adhesion through different predusts (Baker and others 1972, Mukprasirt and others 2000, Albert and others 2009a). Baker and others (1972), in a pioneering work, evaluated the predust material capacities of a series of ingredients from three large groups: starchy materials, proteins, and gums. They found that proteins and dried egg albumin, in particular, improve adhesiveness. Since then, little research has been conducted in this area. Mukprasirt and others (2000) studied adhesion of

rice flour-based batter combined with egg-albumin powder predusting to chicken drumsticks by laser scanning confocal microscopy and they found that all samples showed good adhesion between batter and substrate. Albert and others (2009a) studied three different hydrocolloids as predusting materials for battered fish nuggets and their performances in three different cooking procedures were evaluated. Oxidized starch, xanthan gum, and hydroxypropylmethylcellulose (HPMC) were compared with wheat flour (control), using deep frying, conventional oven, and microwave oven. Image analysis, an innovative method in this area, was used to quantify adhesion. Oxidized starch gave the best adhesion results for the three cooking methods and HPMC and xanthan gum showed better adhesion properties than wheat flour when a microwave oven was used.

However, adhesiveness is also heavily dependent on the nature of the surfaces. If the batter is applied to a surface that is too moist it can slip, leaving some areas uncovered or covered with a diluted material that will then form too thin a layer. On the contrary, if the predust is applied to very dry product surfaces it does not adhere to the product surface. If the product is frozen, a layer of ice results in pour adhesion. Predusting also helps reduce any voids that may be caused by entrapment of air pockets between the substrate and the batter during batter application, preventing "blow-off" and "pillowing," and also tends to increase batter pick-up (Fiszman 2008).

25.3.2 Texture

The most highly appreciated texture of a fried product is crispness. In breaded or battered products, one of the main texture characteristics is crispness/crunchiness. Crispness indicates freshness and high quality (Szczesniak 1988). The ideal coating should exhibit a structure that offers some resistance to the initial bite but then quickly melts away in the mouth (Loewe 1990). The crispness that the external layer acquires during frying depends on its initial composition, on the frying temperature and on time. During the frying process, it is dehydrated until it provides a crisp texture in the outer part while protecting the tenderness of the inner part (Altunakar and others 2004).

From a structural point of view, battered foods are very complex systems as they contain a wide range of different kinds of components, with both the coating and the food substrate undergoing substantial changes as a result of frying.

The use of various types of dextrins has been associated with an improvement in the crispness of final fried battered foods. Acid-converted dextrins of high amylose corn flour used in batter coatings proved effective in obtaining a crispy texture in battered food prefried and frozen for subsequent microwave cooking (Lenchin and Bell 1985). Potato strips dipped in an aqueous solution containing a hydrolyzed starch product including dextrins and maltodextrins remained crisp for an extended period of time after final frying (Calder and others 1994). Battered squid rings prepared with a dextrin-containing batter remained crisper for a longer period of time after frying (Baixauli and others 2003). Albert and others (2009b) evaluate the instrumental and sensorial crunchiness and general performance of two different dextrins (of high and medium solubility) in batter formulations finally cooked by three different procedures: deep frying, microwaving, and microwaving with susceptor packaging. They found that when the dextrin-containing nuggets were cooked by microwave and susceptor, they were perceived as crunchier as and oilier than the other samples.

Wheat flour gives flavor and texture to the breaded product, and its main components (starch and protein) play a structural role in the technological properties (Loewe 1990). Corn meal develops a good texture for the final product (Duxbury 1988) and vital wheat gluten increases viscosity of breading, leading to essential characteristics of texture and crispness of the final product because of the formation of a matrix during the process (Kalin 1979, Loewe 1990). Acioli-Moura and Yoon Kil Chang (2006) studied the technological properties of wheat-based extruded breading. They found that greater texture was achieved at a lower temperature and addition of high amylase starch or vital wheat gluten into the formulation increased texture values. They concluded that a desirable texture in the breading product could be reached with 13–23% of vital wheat gluten and 5–26% of high amylase starch. In fact, sensorial and technological properties of an extruded breading are strongly affected by the composition of formulation and operational parameters. The temperature of extrusion and moisture of formulation play important roles in the expansion of the extrudate, which affects the texture of the final breaded product (Attenburrow and others 1993, Ryu and Walker 1995).

The final heating method is decisive for retaining crispness as well as differences in composition and process. Varela and others (2008) developed a new method to assess the crispness of crusted products with a high-moisture core (chicken nuggets) after final cooking by four different processes (deep-frying, conventional oven, microwave oven, and microwave oven plus susceptor) by means of an integrated approach, measuring the force/displacement curves during cutting and the sound emitted simultaneously. They found that deep-fried samples and those cooked in a conventional oven presented jagged force curves and acoustic signals with many peaks, both characteristics of crispy products. The curve profiles of microwaved samples were drastically different and typical of tough, gummy products but the use of a susceptor package in microwave heating improved the crispness of the samples.

In breaded and battered poultry products, crispness is among the textural characteristics that are highly valued by consumers (Antonova and others 2003). Later, they found significant differences in the sensory crispness of chicken nuggets cooked by different methods. Fried samples were significantly crispier than chicken nuggets cooked in either a convection oven or a microwave oven (Antonova and others 2004).

The importance of crispness in the acceptability of crusted foods has created the need to define and measure this perception. Salvador and others (2002) proposed to evaluate the complete penetration curve profile of a fried coating to observe various aspects of texture. Some simplified methods have measured the fried crust alone (separated from the substrate) by puncturing it with a cylinder or a conical probe (Fan and others 1997, Mohamed and others 1998, Matsunaga and others 2003). However, measuring only the external crust has the limitation of not reflecting reality, as deformation of the outer layer while eating is not only a result of the mechanical behavior of the crust but also depends on the mechanics of the non-crispy core, where shear and compression forces are mixed (Varela and others 2008). The mechanical, morphological, and composition differences between the layers of this sandwich-like structure make it difficult to determine the contribution of each part (Luyten and others 2004), but the mechanics of the layers stacked together are essential for understanding the texture of the food pieces as whole.

25.3.3 Oil Absorption

Similar to other fried food, one problem associated to the pleasure of battered and breaded food consumption is the important amount of oil absorbed during the frying operation, which may arise in some cases to one-third of the total food product by weight (Mellema 2003). This high level of fat ensures a high level of satiety but constitutes a health risk, as saturated animal fats have become associated with obesity and coronary heart diseases. For this reason, extensive research has been carried out in the last decades in ways to reduce the oil absorption.

25.3.3.1 Oil Reduction by Physical or Chemical Treatment

The application of physical or chemical pretreatments to the breaded food or to the breading prior to frying has been applied as a way to reduce the oil absorption.

Breading particle size and porosity influence moisture loss and fat absorption during frying. A particle with a porous or open structure will absorb frying oil and will release moisture more quickly than a similarly sized dense granule. Porosity, together with mesh size, determines the rate of absorption but also largely determines the texture of the coating.

The absorption of oil and the effective rate of heat transfer in porous granules were higher than with dense granules (Dyson 1983).

The employ of an innovative technology for breaded chicken food production consisting in incorporation of a steam oven cooking before battering (predust-cooking in steam oven-battering-breading-flash frying) reduced in 9% the fat content in comparison with products obtained by the conventional technology (battering-breading-flash frying-cooking in conventional oven) (Bianchi and others 2009).

Microwave precooking was found to reduce fat absorption during frying. An interaction effect of microwave precooking and frying temperature and time on mass transfer was found. Precooking and frying temperature influenced oil uptake for precooked chicken nuggets breading part. Precooking at 6.7 W/g produced chicken nuggets with the least fat uptake at all the frying temperatures (Adedeji and others 2009).

25.3.3.2 Oil Reduction by Incorporating Food Ingredients and Additives

A very important approach to reduce oil absorption during frying consists in the incorporation in the breaded food of different food ingredients or/and additives. Among these, polysaccaharidic hydrocolloids and proteins have been the most widely investigated.

25.3.3.2.1 Employ of Cellulose Derivatives

The role of cellulose derivatives as fat reducers during frying has been extensively studied. In particular, cellulose ethers methylcellulose (MC) and HPMC have been reported as effective fat and moisture barriers by many authors (Meyers 1990, Balasubramaniam and others 1997, Mallikarjunan and others 1997, Sanz and others 2004, Salvador and others 2008, Primo-Martín and others 2010). This barrier effect has been mainly associated to their thermal gelation ability. In solution, these polymers are completely hydrated and there is little polymer-to-polymer interaction other than simple entanglement. As the temperature is increased, these cellulose polymers gradually lose their hydration water and the viscosity decreases. When the gel point is reached, sufficient dehydration of the polymer occurs to cause polymer-to-polymer interaction to predominate and the solution starts to gel. When the solution is cooled, the process begins to reverse. Thus, thermal gelation is reversible and can be repeated as desired.

In contact with hot-oil thermal gelation of MC or HPMC present in a breaded food forms a gel or film that together with their water retention capacity protects against moisture loss and the entry of oil during the frying process.

The gelation temperature and the stiffness of the resulting gel depend on the level of methoxyl and hydroxypropyl substitution, and on the molecular weight of the cellulose ether. Gelation of MC and HPMC is mainly caused by hydrophobic interaction between molecules containing methoxyl groups. However, the presence of hydroxypropyl substitution significantly alters gelation properties: for the same degree of methoxyl substitution, an increase in hydroxypropyl substitution increases the gelation temperature and diminishes the stiffness of the resulting gel, so for HPMC the gelation temperature is higher and the stiffness of the resulting gel is lower than for MC. As for the effect of molecular weight, gel stiffness increases with the molecular weight of the cellulose used. Molecular weight does not affect the gelation temperature (Sarkar 1979).

The influence of the differences in the gelation properties among MC and HPMC in the oil and moisture barrier properties was recently studied in deep-fried battered snacks. The highest oil reduction was found for the cellulose with the lowest level of methoxyl substitution, even though its moisture content was lower. This combination of lower oil and moisture content conferred this cellulose type the best crispness characteristics (Primo-Martín and others 2010).

Concentration has also been related to oil reduction efficiency. In fried battered squid rings, the increase in MC concentration in the batter from 1% to 2% decreased oil absorption and increased moisture retention, both after the prefrying step (30 s of frying) and after final frying subsequent to freezing (Sanz and others 2004).

As with other hydrocolloids, the functionality of MC and HPMC in a battered or breaded food should request that they are correctly hydrated. Good dispersion can be achieved either by dry blending or in hot water. In batter and/or breading, the dry blending technique consists in the mixture of the hydrocolloid with the other dried ingredients of the batter or breading. Once the molecules are dispersed, hydration occurs when mixing with water during dough formation. The temperature of the water is important to achieve a correct hydratation and viscosity development. The required temperature decreases as the number of methoxyl substitutes rises. For HPMC, water temperature should be lower than 25°C while for MC should be lower than 13°C. In the hot-water dispersion, the hydrocolloid is mixed with a small amount of water (from 1/5 to 1/3 of the required water) at 80–90°C. Subsequently, the rest of the water is added as very cold water or ice (hot–cold technique), allowing hydratation.

The dispersion/hydratation technique employed has been found to affect the oil and moisture barrier effectiveness. HPMC was found to be more efficient as a barrier when incorporated previously hydrated (hot–cold technique) than when it was hydrated together with the other batter ingredients (dry-blending technique). The reason was associated with a better hydrocolloid hydratation when the hot–cold technique was used (Meyers and Conklin 1990). In battered food, another factor that has been related to the

barrier efficiency is the batter temperature during the batter operation previous to frying, due to the effect of temperature in hydratation and functionality. In batters containing MC, a reduction in the barrier efficiency was observed when batter temperature was increased from 5°C to 25°C. The reduction in effectivity was associated with a decrease in viscosity and in water retention capacity, which reveals a decrease in the hydrocolloid functionality (Sanz and others 2004).

Apart from incorporating MC or/and HPMC in a batter, they can also be applied as an edible film around the pieces of food by dipping them in a solution of MC or HPMC prepared by the hot–cold technique. The influence in the oil reduction capacity of applying HPMC and MC as a film before breading or adding them to the breading formulation was studied in marinated chicken strips (Holownia and others 2000). The most efficient method was to add the dry hydrocolloid to the breading formulation. Application as a film before breading inhibited the migration of moisture from the substrate to the crust but did not reduce the amount of oil absorbed by the crust. The application of the film after the breading was not possible due to a lack of adhesion.

Another cellulose-derived hydrocolloid that has been used to reduce oil absorption is sodium carboxymethylcellulose, although its efficiency against fat absorption is much lower than MC and HPMC.

The incorporation of carboxymethylcellulose (CMC) in a batter-based legume snack reduced the amount of oil absorbed during frying. The greatest barrier efficiency was obtained at a concentration of 2% (Priya and others 1996).

Incorporation of either 1% CMC or 1% HPMC in fish nuggets significantly decreased oil absorption in comparison with the control and other batters (Chen and others 2009).

In contrast to MC and HPMC, incorporation of CMC in a deep-fried battered snack was not found effective to reduce the oil absorption during frying (Primo-Martín and others 2010).

25.3.3.2.2 Employ of other Hydrocolloids

Apart from cellulose derivatives, other hydrocolloids that proved to be effective barriers against fat absorption in breaded food were gellan gum and pectin.

Gellan gum is a heteropolysaccharide produced by *Pseudomonas elodea*. The application of gellam gum can be made as a hot solution: the food is dipped into the solution and the film forms as it cools. Another possibility is to dip the food into a cold gellan solution and gellation occurs after ions such as Na^+, Ca^{2+}, Mg^{2+}, and K^+ are added (Duxbury 1993). Gellan gum has also been added with CaCl to the dry ingredient mixture of batters for chicken, fish, cheese, and vegetables, resulting in low oil absorption and appropriate crispness characteristics. In breaded food, gellan solutions have been used to coat the crumbs used as breading; the final product had lower oil absorption and was crispy (Chalupa and Sanderson 1994).

As for pectin, in combination with Ca^{2+}, it was proved effective to reduce the fat absorption in a batter-coated and breaded product. The Ca^{2+} was added to the breading (This was accomplished by dry blending, spray drying, agglomerating, baking the calcium source into the bread crumb or any combination of the above.) and the batter-coated and breaded food was treated with a solution of calcium-reactive pectin. The hydrophilic film that is formed during reaction between the Ca^{2+} and the pectin reduced the oil absorption during frying. The level of Ca^{2+} added to the breading was an important factor to consider as sufficient available calcium for efficient reaction with the pectin should be assured (Gerrish and others 1997).

25.3.3.2.3 Employ of Proteins

The use of proteins to reduce the fat absorption during frying mainly concerns to whey protein. Whey protein is composed of various proteins, the main protein of which is the β-lactoglobulin, a globular protein with an isoelectric point of 5.1. Other important globular proteins of whey are α-lactalbumin and bovine serum albumin. All of these proteins can form thermally induced gels that may alter the porosity of the product, thus lowering moisture loss due to evaporation and subsequent oil absorption into the fried food when used as a coating.

In comparison with soy protein isolate and egg albumin (EA), whey protein isolate (WPI) was found to be more effective on improving the quality parameters of deep-fat-fried chicken nuggets. WPI addition in the batter at 3% significantly reduced the oil content of the fried nuggets and provided the hardest

and crunchiest product. EA also reduced the oil content significantly but yielded softer products (Dogan and others 2005).

The effect of incorporating MC or WPI either in the predust or in the batter in the quality properties of breaded chicken nuggets after pressure frying was also studied. No significant effect on fat content was found in any case. However, chicken nuggets in which MC was incorporated into the predust and fried using nitrogen gas-enhanced product crispness (Ballard and Mallikarjunan 2006).

The employ of WPI solution as a postbreading dip was found a promising alternative in reducing fat content during frying. Fat reduction and the most appropriate WPI concentration were dependent on the type of breading employed. In crackermeal patties, the highest lipid reduction (31.2%) was found for patties treated with 5% WPI solution, while for Japanese breadcrumb patties was 37.5% with 10% WPI solution. The oil barrier properties were also dependent on pH, the highest oil reduction was observed at low pH (2–3) (Mah and others 2008).

Application of a solution of WPI in previously predust-battered and breaded chicken breast strips reduced the oil absorption during frying. However, no fat reduction was observed if the WPI coating was applied directly in chicken strips or in wheat flour-predusted chicken strips (Dragich and Krochta 2010).

25.4 Innovation in Breaded Food

Due to the increased health concern of consumers, oil absorption reduction continues to be an important future issue in breaded food. In this regard, an important necessity is to reduce the fat absorption during frying without scarifying crispness. Oil absorption mainly occurs due to moisture replacement, and so oil reduction is typically associated with a reduction of crispness sensation. The number of studies that investigate the relationship among oil reduction and crispness is scarce so further research will be required in this field.

Research in ways of creating new textures which provide different crispness sensations as well as new colors and flavors will always be necessary as the market is always demanding food products with innovative properties. New crumb sizes and geometries, enriched colors, combination of flavors and seasonings, new sophisticated ingredients are some of the innovation possibilities.

Another future research need is the quality improvement of breaded food finally cooked by microwave. In comparison with frying, microwave cooking confers a nonpleasant soft and soggy texture, because in this type of heating water is conducted from the inside to the outside. New research on ingredients or mixtures of ingredients that avoid water migration from the coated food to the outer breaded crust will help in improving the quality of microwave-breaded food. Also more knowledge about ingredients that may confer crispness on microwave heating may be convenient.

Related to the health issue also stands the increase in the fiber content of the breading. The employ of whole-grain ingredients and the incorporation of fibers from a variety of sources will increase the nutritional properties of the breading. The study of the effect of fibers in fat absorption is another future concern. The incorporation of a retrogradated resistant starch (type 3 resistant starch) in a batter formula increased the fiber content and reduced fat absorption during frying (Sanz and others 2008, 2010).

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26

Marination: Ingredient Technology

Brian S. Smith

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26.1 Introduction

Adding functional ingredients to a marinade can influence product yield, cook loss, oxidative and microbial stability, and operational efficiency. Functional ingredient classes include salts, sweeteners, phosphates, starches, gums and other hydrocolloids, nonmeat proteins, and spices and flavoring systems. Many ingredients perform multiple functions when properly selected and incorporated through marinating. For example, aside from their contribution to flavor, ingredients such as sodium chloride, sugars, and sodium or potassium lactate chemically bind water and effectively reduce water activity, which may extend shelf life. The combination of sodium chloride with phosphate enhances water-holding capacity of the meat system by aiding muscle protein extraction, positively impacting yield and product texture, and minimizing package purge and cooking losses. Phosphates also have chelating properties that effectively reduce oxidation and warmed over flavor. Certain sweeteners participate in Maillard browning to enhance product flavor and appearance. Starches, gums, and other hydrocolloids effectively manage moisture to improve yield, manipulate texture, and minimize purge. Nonmeat proteins also control moisture but often are formulated to replace meat for economic purposes. Certain nonmeat proteins may also contribute to marinade viscosity which can influence absorption and retention. Freeze–thaw stability can be manipulated by the addition of certain sugars and modified starches. Water, while typically the

transport medium for other ingredients in a marinade, can impact the efficacy of certain other functional ingredients. Chilling and softening source water for marinades can provide significant benefits in minimizing the oxidation and improving the protein extraction.

Considerations that should be factored into the development of marinade formulations include ionic strength, marinade pH, marinade viscosity, and marinade temperature. Postmortem events such as salting, pH adjustments, temperature, surface area, and cooking all have a dramatic impact on the ability of the muscle to absorb and retain water. Water-holding capacity, or the ability of raw muscle myofibrils to swell and maintain cellular and added water, is lowest at its isoelectric point. The isoelectric point of muscle proteins is the pH at which there exist an equal number of positive and negative charges. As the muscle pH moves further away from the isoelectric point, water-holding capacity increases (Wilding and others 1986). The pH and buffering capacity of marinade solutions will impact the ability of the meat substrate to retain the solution. Buffering capacity should be viewed as the ability of the solution to resist changes in pH when acids or bases are added. This is critical when processing with high acid solutions. Alkaline phosphates are the best tool to properly manage marinade pH for maximum water-holding capacity. Ionic strength of the solution relates to the chemical binding of water in the meat protein lattice structure (Offer and Trinick 1983). Meat proteins have a negative charge in postmortem muscle. Interstitial space is increased when additional anions are added, typically from the PO_4^- contribution of phosphates and the Cl^- contribution of salt. The increase in negative charges helps repel the myofibrils from each other during the mechanical phase of the marinade incorporation. Wilding and others (1986) determined that myofibrils swelled two to three times their normal diameter in the presence of 0.6 M potassium chloride (KCl). This concentration would equate to a solution of about 0.44%. When cations are added to the meat system, the chemical attraction of the positive charges to the negatively charged ends of the myofibrils promotes chemical binding of water to the proteins. Viscosity of the marinade solution is also a variable that must be measured for optimal performance. From an equipment point of view, multineedle injectors will require a fairly narrow range of solution viscosity for maximum marinade distribution and retention. Needle diameter, pump pressure, and solution temperature will also influence the results of solution viscosity, where smaller needles will equate to higher injector pressures. The primary ingredient contributors to marinade viscosity are proteins, since they hydrate in cold water. Other ingredients that promote viscosity are pregel (instant) starches and hydrocolloid gums (carrageenan, xanthan, and guar are typical examples). The benefits of increased marinade viscosity are improved protein extraction due to the additional friction of the viscous solution and improved retention since the more viscous solutions are less likely to weep out of the marinated product. Marinade temperature is also critical: 2–4°C provides food safety measures as well as optimizes protein extraction which equates to maximum binding sites as proteins are solubilized. Also, cold solutions will not warm the meat substrate, and solutions should be maintained so that they are as cold or are colder than the meat that is being marinated.

26.2 Ingredient Classes

26.2.1 Water

For most meat formulations, water is normally second only to the meat raw materials with regard to inclusion level. Furthermore, water may be the most overlooked ingredient with regard to the amount of the attention it receives. Many factors including hardness, pH, dissolved solids, and temperature can impact the functionality and final quality of the finished meat product. The measurement of hardness, measured as calcium carbonate (Table 26.1), is likely to be the most important factor to monitor in source water for food processing. Increased levels of calcium, magnesium, iron, and other minerals contribute to water hardness and have a dramatic impact on the ability of many ingredients to solubilize and may even reduce functionality. Specifically, sodium or potassium phosphates used in brine and marinade systems are powerful antioxidants and water conditioners. When incorporated into solutions of hard or very hard water, the phosphates will chelate the minerals in the water substrate and the net result will be a reduced functional level in the meat system. Water pH is another variable that should be controlled. Many food-manufacturing plants using a municipal water supply may see wide fluctuations in water analyses,

TABLE 26.1

Water Hardness Levels

Hardness Classification	$CaCO_3$ Dissolved (ppm)
Soft	0–60
Moderately hard	61–120
Hard	121–180
Very hard	>180

especially pH, as the municipality will manage water characteristics for broad-based criteria, not necessarily for the most effective pH and hardness for food processing. Temperature management of ingredient water is critical for optimal success in food processing. In meat processing operations, product quality is highly variable when the water that is used in the marinade or brine solution is not cold. Cold water is important for many reasons, including improved protein extraction efficiency, maintaining cold meat temperature for microbial stability, managing functional ingredient performance by delaying hydration and hydrolysis of various proteins, hydrocolloids, and phosphates. Water contains organic matter, dissolved solids, and minerals that form scale in distribution pipes, fixtures, equipment, injector needles, and on surfaces. Films form on surfaces in contact with water and reduce the efficiency of cleaning and sanitizing agents. Scale, films, residues, and other deposits reduce efficiency, performance, and longevity of systems and equipment and may ultimately lead to harborage issues with microorganisms since the films and scale protect them from sanitizers. Therefore, cold, softened water with stable pH values is critical for processing efficiency, ingredient performance, and microbial control for both meat and facilities.

26.2.2 Salt (Sodium Chloride)

Salt has been a part of food preservation and human history since ancient times and is likely the first ingredient that was used to preserve food. The historical use of salt as a preservative in muscle foods has been well documented and is due to its antimicrobial properties (Rhee 1999). Salt has played a major role in world history and culture. The Latin word *salarium argentum* means "salt money" and was so important that it served as currency that Roman soldiers were partially paid with. The English word "salary" is derived from this early term. Salt still plays a very important role in food processing as well as many industrial applications.

Salt has an empirical formula of 60.66% chloride and 39.34% sodium, with a molecular weight of 58.4 g/mol. The salt crystals are isometric and cube shaped, but can be acquired in a variety of source and physical form options. The primary manufacturing processes for salt include solar drying, pan evaporation, and mining from salt deposits or salt domes. The manufacturing process also impacts the crystal size, shape, and surface area. Proper selection of salt is important to maximize the performance in many meat-manufacturing processes. Many of the flaked and crystal-modified products have very large surface areas with considerable cavitation on the particles, which contributes to improved solubility in brine-mixing systems. Proper selection of salts with large surface area, crystal size, and shape can also improve the seasoning blends by minimizing settling of the components. Flaked and crystal-modified salts can also improve the salty taste perception and allow processors to decrease the usage over smooth particle salts and achieve the same degree of saltiness.

Salt enhances flavor, tenderizes meat due to increased ionic strength, increases water-holding capacity through salt-soluble protein extraction, and increases juiciness (Huffman and others 1981). The primary functions of salt in muscle food processing and marinating include

- Lowering water activity
- Providing flavor
- Antimicrobial
- Improving salt-soluble protein extraction (bind, yield, and texture)

- Increasing ionic strength of meat systems
- Increasing water-holding capacity
- Improving fat binding and emulsification properties

Utilization of salt is necessary for the extraction and solubilization of myofibrillar proteins, specifically actin and myosin, in meat formulations. This property is critical in the binding of water and fat, creation of the meat gel matrix, and is highly related to the concentration or brine strength and the ability to impact the lean meat components. The chloride ion is the part of salt that improves water-holding capacity by increasing ionic strength. The shift in negative ions from the chloride helps the meat proteins shift away from the isoelectric point (higher ratio of negative to positive charges), thus improving the ability of the proteins to bind water.

While important efforts are underway to reduce the sodium and salt in food formulations, it is safe to say that salt will never be removed from the processed meat formulations. Salt is an important contributor to food safety and shelf-life concerns and functions by lowering the water activity in meat products and making conditions less desirable for microbial growth. Higher salt levels generally provide a greater bacteriostatic effect. Salt provides a large contribution to the characteristic flavor associated with marinated meat, poultry, and seafood and processed meat products, although tempering with sweeteners is often necessary when higher concentrations of salt are used. The sodium ion is primarily responsible for flavor contribution. Substituting other salts, such as potassium chloride, may have similar functional benefits, but without technical intervention, bitter flavor notes will result due to the potassium ions.

One notable downside to salt is the prooxidant properties that can be found at typical inclusion levels, particularly in frozen products such as pork sausage and beef patties. Several theories abound, implicating the Na^+ (Ellis and others 1968) and the Cl^- (Osinchak and others 1992), but neither theory seems to be repeatable. One of the most plausible explanations is the impact of salt on the structural integrity of muscle and adipose cells. Perhaps this event brings catalysts, such as iron, into more frequent contact with unsaturated lipids (Rhee 1999).

26.2.3 Curing Agents

Sodium nitrate and sodium nitrite are used to produce cured meat products. Earlier on, with the use of salt as a preservative and drying agent, it is likely that people noticed that certain salts preserved meat better than others. In the late 1800s, sodium nitrate was identified as the compound that is responsible for the pink color in cured meats. Later, it was determined that the nitrates were reduced to nitrites by bacteria. Sodium nitrates are generally used in long, slow-curing operations because they must be reduced to sodium nitrite to be functional. Potassium nitrite and potassium nitrate are also approved for similar curing results. Cure color chemistry is predicated on the reaction of nitrite with myoglobin and heat resulting in a stable cured meat color. The reaction is defined as

$$\text{Myoglobin} + \text{Nitrite} \rightarrow \text{Nitric oxide myoglobin} + \text{Heat} \rightarrow \text{Nitrosohemochromogen}$$

Nitrites have many benefits including antioxidant properties, stable cured color, and characteristic cured meat flavor, but the most important benefit of nitrites is the prevention of the growth of *Clostridium botulinum* and *Clostridium perfringens*. This food safety measure alone provides ample rationale for the use of nitrites in meat products. Legal limits for the use of nitrites and nitrates are dictated on the meat weight in a formula, whether pumped, tumbled, or dry cured. The maximum allowable amount of nitrate or nitrite that can be used is product, process, and curing agent specific. The limits are shown in Table 26.2.

26.2.4 Curing Accelerators

Cure accelerators are critical ingredients in many cured meat products since they contribute to rapid and complete cure reactions. The improved cure reaction speed can be provided by ingredients that are considered reducing agents or acidulants. The cure reaction takes place faster at lower pH and chemically reduced conditions. Usage levels for curing accelerators are based on the ingoing level calculated on the

TABLE 26.2

Nitrite Use Levels in Meat Products

	Immersion Cured	Massaged or Pumped	Comminuted	Dry Cured	Bacon
Sodium/potassium nitrite	200	200	156	625	120
Sodium/potassium nitrate	700	700	1718	2187	n/a

green weight of the meat and/or poultry and/or meat/poultry by-products in the formula. Cure accelerators include reductants, such as sodium erythorbate and sodium ascorbate, and acidulants, including citric acid, ascorbic acid, sodium acid pyrophosphate, and glucono-delta-lactone. Usage limitations for sodium erythorbate and sodium ascorbate are 7/8 ounce per 100 pounds of meat (546.875 ppm), whereas erythorbic acid and ascorbic acid are regulated at 469 ppm of the meat component and citric acid and sodium citrate may be used to replace up to half of the previously discussed cure accelerators.

26.2.5 Phosphates

Sodium and potassium salts of phosphoric acid are the foundation for any nonmeat ingredient formulation. While most ingredients directly manage added water in the formulation, phosphates manage the meat proteins and thus the water already inherent in the meat raw materials plus additional formula water. Phosphates perform many functions and are possibly the most cost-effective ingredient available today. As shown in Table 26.3, proper inclusion of phosphates can have many positive effects on the meat system and final product.

Phosphates are typically manufactured using three primary drying methodologies that include drum drying, spray drying, and agglomeration; the solubility similarly moves from least soluble to most soluble, respectively. Benefits of improved solubility include faster marinade and pickle makeup, less sensitivity to solubility in cold water, increased salt tolerance, and improved yields and performance in hard-water systems.

Food grade phosphates are manufactured through the reaction of raw phosphoric acid with either sodium or potassium hydroxide yielding a salt of phosphoric acid. The primary sources for phosphorus rock are in Israel in the Negev Desert, Morocco, and parts of the United States, including Florida and North Carolina. Molecular weight characterization of phosphate products is found in the $P_2O_5^{2-}$ component, and this ratio to the entire phosphate molecular weight explains a great deal about the efficiency of that particular phosphate chain length. A single phosphate chain length can rarely be used in the marinade

TABLE 26.3

Properties Affected by Phosphates in Meat Systems

Property	Mechanism
Antioxidant	Chelating metal ions prevents their participation in oxidation reactions
Emulsification	Stabilizes emulsions by increasing protein extraction efficiency and relaxation of the actomyosin protein matrix to more efficiently encapsulate fat droplets
Texture	More efficient protein extraction
Protein extraction	Improves salt-soluble protein extraction by increasing ionic strength and adding like charges to relax and open up meat protein structures
pH	Alkaline phosphate products increase pH and thus improve water-holding capacity. Many of these phosphates have pH ranges from 7.5 to 12.0 in 1% solutions and increase pH values of the meat system
Buffering capacity	Phosphates (especially monophosphates) add buffering capacity to meat systems and therefore help the system resist changes when acids (or bases) are added to the system

TABLE 26.4

Properties of Phosphates Containing Various Chain Lengths

Chain Length Designation	Chain Length	Primary Properties
Mono (ortho)-phosphate	1	Buffering capacity
Di (pyro)-phosphate	2	Dissociation of actin and myosin; chelates magnesium
Tripolyphosphate	3	Chelates calcium, improves water-holding capacity; predominant component of most phosphate blends
Hexametaphosphate	6+	Improves solubility; chelates calcium

provide optimal functionality in any meat system. Each phosphate chain length has specific functional properties, and a blend of these chain lengths with an alkaline pH often provides optimal meat product performance in a tumble or injection marination systems. The kinetics of water uptake by meat during marination is phosphate-type dependent. The amount of water uptake is as follows: pyrophosphate ≥ tripolyphosphate > hexametaphosphate > orthophosphate (Xiong 1999). Table 26.4 shows the specific properties exhibited by specific chain lengths.

The efficiency of various phosphates is based on chain length and has been studied for many years. Early studies by European researchers (Bendall 1954) indicated significant benefits to meat functionality with added phosphates. In particular, this research documented the benefits of specific phosphate chain lengths on the ability to swell myofibrils. Pyro- or diphosphate was the most functional, giving myofibril swelling levels at over 50% of the original myofibril volume in the presence of 1% sodium chloride. In contrast, polyphosphates were only able to achieve myofibril volume increases of 30% over the initial volume. These data have been supported by many researchers in more recent experiments (Xiong 1999; Trout and Schmidt 1986).

26.2.6 Protein-Based Functional Ingredients

Protein-based functional ingredients typically bind water, provide emulsification properties, modify texture, and often bind fat due to their amphiphilic nature. Generally, protein ingredients are cost-effective on a hydrated basis and can replace meat, improve cooking yield, and provide stability for processing stresses. The major protein categories in processed meat products include those from soy, dairy, egg, and meat or animal by-products.

26.2.6.1 Soybeans

Soybeans have been reportedly cultivated for over 5000 years and originated in China. George Washington Carver can be credited with realizing the potential for the oil and protein-rich legume. Carver had been making soy products since 1903, when he began his peanut work, concluding that "the soy bean, with its numerous varieties, is almost, if not quite, as versatile as the peanut with its 300 products" (Carver 1936) (p. 716). Soybean meal and oil are the primary components in raw soybeans, with soybean oil being the most widely used edible oil worldwide. After harvest, soybeans are taken to a processing facility where the beans are cleaned, cracked, dehulled, and the oil is extracted. The food grade defatted soy flakes are then processed into various soy protein products. After the food grade raw material is collected (known commercially as defatted flakes or white flakes), it can then be converted into texturized soy flour, or further processed through extraction and pH manipulation into products with higher protein content, and thus improved functionality. Soy proteins are classified for labeling purposes based on protein content (Table 26.5).

For utilization in marinade systems, only products suitable for suspension in brines will be discussed further. Functional soy protein concentrates and isolated soy proteins are the most useful soy protein products for marinades. The more functional protein concentrates and isolated soy protein products provide increased water-holding capacity, cleaner (less beany) flavors, whiter colors, improved fat binding

TABLE 26.5

Typical Proximate Values (%) for Soy Protein Products (As Used Basis)

Soy Product	Protein Content	Soluble Carbohydrate	Insoluble Carbohydrate (Fiber)	Moisture, Ash, Other
Soy flour	52	16	16	16
Soy protein concentrate	65	—	22	13
Isolated soy protein	86	—	—	14

and emulsification, marinade viscosity, and gelling properties. Isolated soy proteins have water absorption values that range from 150% to 400% (Singh and others 2008), which is a primary reason that isolated soy protein addition to meat formulations has the ability to decrease drip loss (Porcella and others 2001). When studied in low-fat meat batter systems, isolated soy protein has been shown to improve the emulsion stability and water-holding capacity (Lin and Mei 2000). Soy protein products may also be vitamin and mineral fortified for child nutrition applications.

Injected or enhanced products of all types can benefit from soy protein inclusion. A wide variety of poultry products including boneless meat, bone-in parts, and whole birds are routinely injected or tumbled for marinade uptake. These products can be fresh and frozen retail and food service, fully cooked, par cooked, and breaded. Soy protein concentrates do not gel in the classical sense, yet make significant contributions to product moisture retention, juiciness, and tenderness. Due to the low gel strength, functional soy protein concentrates are ideal for fully cooked bone-in poultry, especially dark meat. The highly dispersible soy protein concentrate will evenly distribute throughout the meat tissue and limit gel pockets. When similar applications are attempted with isolated soy proteins, or many gum products, the accumulation of gel pockets between muscle bundles, where muscles and bone join together, and under the skin can lead to undesirable product appearance. Isolated soy protein is an excellent application for boneless marinated meat and poultry, especially when the gel matrix can develop and provide structure and firmness. This property translates directly to yield quality benefits in portion control products. Fully cooked strips, slices, and diced product with isolated soy protein will have improved texture, more uniform particles, and improved slicing yield. Fully cooked whole muscle products and deli meats are also applications that benefit from soy proteins. Soy protein ingredients are also effective at slowing oxidative processes. Fully cooked meat balls evaluated during frozen storage were shown to have suppressed lipid oxidation with isolated soy protein, which was slightly more effective than whey protein concentrate at inhibiting lipid oxidation (Ulu 2004). On the other hand, Porcella and others (2001) reported no differences in rancidity in chorizo with control and added isolated soy protein, but measured Thiobarbituric acid reactive substances at <1 MDA mg/sample kg.

26.2.6.2 Dairy-Based Proteins

Dairy-based proteins are essentially produced from cows' milk. Benefits of milk proteins include a clean flavor profile, high water-binding properties, and the ability to improve texture through gelation. These properties are ideal for the justification to include milk protein products in processed meat formulations. The basic composition of raw milk is shown in Table 26.6.

The protein content (3.4%) in fluid milk is comprised of caseins (2.8%) and whey (0.6%). The conversion of fluid milk to food grade dry ingredients is accomplished by various precipitation, coagulation, and acid processes. The most abundant protein, casein, is relatively expensive and may be limited by cost to higher-end meat products. Whey proteins, however, are relatively inexpensive and contribute measurable functionality improvements. Typical protein contents of various milk protein products are shown in Table 26.7.

Cost considerations often limit the use of milk proteins in meat formulations, especially when the sometimes volatile nature of commodity milk pricing is factored in. Several studies have evaluated the functional properties of the various milk protein products and from that information, a relative cost–benefit ratio can be determined. Whey proteins are globular in nature, and with proper modification, the proteins will unfold to improve surface area, binding sites, and gelling properties. One study demonstrated greater

TABLE 26.6

Composition of Raw Cows' Milk

Component	Percentage
Water	87.3
Milk sugar (lactose)	4.8
Minerals (ash)	0.8
Fat	3.7
Protein	3.4
Total	100.0

hardness values when textural studies were conducted for modified whey protein concentrate and caseinate (Barbut 2006). From these data, we can extrapolate that these milk protein products had stronger gel properties and this was subsequently confirmed with microstructural observations. Dairy-based proteins can also improve the sensory properties of enhanced fresh meat products. Hayes and others (2006) reported that whey protein concentrate provided significant improvements in juiciness, tenderness, and Warner–Bratzler shear force in injected pork loins when compared with noninjected control pork loins. Even though a minimal amount of whey protein was used (10% pump over green weight, 3.3% in brine, 0.3% in the injected product), consumers preferred the whey protein-enhanced product over the control product that contained similar concentrations of salt and phosphate in the injected product. Another potential benefit of dairy proteins in marinated poultry is the lightening effect. Lightening poultry dark meat is highly desirable for value-added products. While not always repeatable, some research does promote the lightening effect of dairy proteins in poultry dark meat (Prabhu and Husak 2007).

In summary, dairy protein products are quite useful in marinated meat and poultry products. Inclusion of sweet dairy whey can provide some water binding, clean flavor, and color improvements with minimal cost. More highly specialized ingredients such as whey protein concentrates and sodium and calcium caseinates can provide gel strength and significant functionality, although cost may be a consideration. Usage levels will be defined by the meat food product as dairy proteins will be considered a binder or extender.

26.2.6.3 Meat-Based Proteins

Spray-dried stocks and broths are finding increased use in marinated products. Besides the natural labeling opportunities, meat-based stocks and broths provide protein and thus, viscosity, improved water-binding capacity, emulsification properties, and significant flavor contribution. Additionally, no allergen issues are found with meat protein-based stocks and broths.

26.2.7 Carbohydrate-Based Ingredients

Many types of carbohydrate-based ingredients are used in meat products to improve the functionality of extended and processed items. Modified food starches, hydrocolloids, and gums predominate in this category. Sweeteners are also important in marinated meat and poultry formulations and are predominantly used for binding water, counteracting the harshness of salt, providing desirable color and imparting flavor.

TABLE 26.7

Protein Content of Milk Protein Products

Product	Typical Protein Content
Whey	16
Whey protein concentrate	34
Nonfat dry milk	33
Sodium caseinate	88
Calcium caseinate	88

26.2.7.1 Modified Food Starches

Many different plant sources are used in the manufacture of modified food starches (Table 26.8). These sources normally include corn, potato, rice, tapioca, and wheat. Starches for food applications are further classified based on the percentage of amylose and amylopectin, two glucose polymers that make up starches. Amylopectin units are highly branched chains of glucose, whereas, amylose units are straight chains of glucose molecules. These chains can range from 2000 to 200,000 glucose units. Plants store energy in this format and release glucose when energy is needed by hydrolyzing the starch stored in organelles called amyloplasts. In corn starch nomenclature, waxy maize starches are derived from waxy corn containing nearly 100% amylopectin, and dent starches are derived from dent corn plants and contain approximately 70% amylopectin and 30% amylose. Dent, or field corn, can be recognized by the small indention, or dent, on the top of each kernel. This property impacts the texture, pasting temperature, and viscosity of the hydrated starch in food products.

Regardless of plant source, starches are available in several forms, with the primary distinction being native or modified. Native starches are minimally processed, but require significant heat to paste the starch granules, and the lack of stabilization leads to a high rate of retrogradation, or realignment of the starch granules. The net effect of retrogradation is purge and loss of water. Modified starches are more highly processed to impart a wide variety of properties that are beneficial to food manufacturing, including marination of meat. Modification may be through the processes of substitution and/or cross-linking. Substitution is the process of chemical insertion of large polymerized molecules on the starch chain. These molecules effectively take up space and minimize the retrogradation effect, thus stabilizing the starch chains against purge and further improving the freeze/thaw properties. Cross-linking involves tying together the starch chains to improve the resistance to processing stress. These stresses may include excessive heat such as retort conditions, high acid, and high shear. Again, the effect of cross-linking on the starch products promotes stabilized ingredients that have the ability to maintain the hydration water in the processing systems and has the added benefit of lowered gelatinization temperatures.

Starches are available in cook-up and pregelatinized forms. Cook-up starches are selected to match the application based on desired hydration temperature and the expected processing stresses placed on the finished food product. For instance, a fully cooked chicken product designed to be held at high temperature on a restaurant steam table will require a starch with different characteristics than a starch selected for a marinated beef product that will be cooked to a lower internal temperature and served immediately. Pregelatinized starches are precooked and redried to allow for cold swelling in water solutions. While most of the applications for pregelatinized starches are in cold process dressing and sauce manufacturing, the use in marinades does have an advantage. Incorporating a cold swelling starch into a marinade

TABLE 26.8

Characterization of Starch Granules

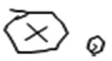
Source	Morphology	Average Diameter (μm)	Shape
Corn	Discs	15	
Potato	Ellipses	70	
Rice	Granules	1–2	
Tapioca	Bell shapes, discs	20	
Wheat	Granules (two sizes)	5, 30	

TABLE 26.9

Carrageenan Type and the Characteristics They Impart in Processed Meat Products

Carrageenan Form	Gel Characteristics	Solubility
Iota	Elastic, clear, freeze/thaw stability	Hot water
Kappa	Strong, rigid gels, slightly opaque	Hot water
Lambda	No/weak gels	Hot or cold water

will increase viscosity and improve retention in the raw meat, poultry, or seafood product. Generally, cook-up starches are used in marinated products, but no functional gains are realized until sufficient heat is applied to allow the starch to paste and bind water.

Moisture management is a primary characteristic of starches. When heated to the proper temperature, starch granules swell as they take up water that provides viscosity moisture retention in the food products. Modified food starch binds water in the muscle food substrate and added water in the marinade improves juiciness and moisture in the product and reduces purge from cook out juices during packaging, storage, and distribution. Furthermore, many modified starches are excellent at improving freeze/thaw stability by stabilizing the water and ice crystal formation process. Generally, inclusion of starches in meat and poultry products improves the firmness and texture through gel formation. Addition of starches to reduced fat products is a proven method to improve texture when fat is replaced with water (Hughes and others 1998). Improved slicing yields in deli meats are typically a benefit from starch inclusion.

Proper starch selection is critical for success. Technologists must pair the environment of thermal processing, pH, shear, and storage conditions with the degree of modification of the starch to insure the proper hydration and pasting temperature and stability under stress and storage.

26.2.7.2 Gums and Hydrocolloids

Extremely high water-holding capacity is a trait mostly associated with hydrocolloids (Lin and Mei 2000). Carrageenan, guar, locust bean, and xanthan are typical for inclusion in marinades and solutions. Most are available with a variety of characteristics. Carrageenan is manufactured by an extraction process from red seaweed. The extract is found in three forms, all of which have different properties. The properties of each form are described in Table 26.9.

When used in the presence of cations, such as salt, carrageenan has improved dispersion properties and increased gel temperatures. These gels are thermally reversible which promotes their use in deli meats. The strong gels formed by inclusion of carrageenans provide a matrix that improves slicing properties, especially with extremely thin-sliced logs and loaves. Carrageenans are highly synergistic and work quite well in combination with modified food starches, soy proteins, and other gums, such as locust bean. One caution with carrageenan, however, is that low pH conditions generally reduce the gel strength.

26.2.7.3 Sweeteners

Sweeteners and carbohydrates provide a variety of functions in food formulations. They provide flavor, lower water activity, increase solid content, lower costs, provide Maillard browning, and promote desirable color characteristics. Corn-based carbohydrates are generally described based on a measure of dextrose equivalence or DE. DE is a quantitative measure of the degree of starch polymer hydrolysis and the higher the DE, the greater the extent of starch hydrolysis. Furthermore, DE is a measure of reducing power compared to a dextrose standard of 100. Cane- or beet-sourced sucrose, or sugar, can also be found in many marinade formulations. Sweetness and solubility are generally inversely related to the properties of hydroscopicity and bulk density. Ranked from least sweet to most sweet, corn-based carbohydrates include

$$\text{Maltodextrin} < \text{Corn syrup(solids)} < \text{Dextrose} < \text{Fructose}$$

Sucrose, or sugar, is manufactured commercially from sugarcane and sugar beets and is available in several forms including granulated white sugar, brown sugar (containing the natural molasses), and in

natural, minimally processed forms. Other sweeteners may include honey, maple syrup, and a variety of nonnutritive sweeteners. Besides adding a variety of characteristic flavors, sweeteners can provide body to marinade solutions. Many brines and marinades are high in salt and salty flavored compounds and sweeteners also help soften the harshness of the salty flavors. Various sweeteners can also be used to promote a particular product characteristic, such as color and flavor that sets the product apart in the market place.

26.2.8 Antioxidants

Raw-marinated products that are predominantly red meat are susceptible to oxidation due to polyunsaturated fatty acids and phospholipids. In addition, myoglobin is susceptible to oxidation which is very important in the display of refrigerated, case-ready products. In these muscle food systems, the use of antioxidants is important for flavor preservation and prevention of lipid and protein oxidation. There are two predominant ways to incorporate antioxidants into products. Synthetic oxidants can be used and include butylated hydroxyanisole, butylated hydroxytoluene, and tert-butylhydroquinone. Natural antioxidants can also be used, especially if a natural label is desired. Natural antioxidants include rosemary, green tea, grape seed extract, and oregano. However, caution should be exercised when using these antioxidants because some natural antioxidants can promote bitter flavors (Sant'Angelo 2010). Vitamins C, E, and A, green tea, and several extracts from fruits including pomegranate, acai, and rooibos are all listed in the top 10 antioxidant food ingredients from 2007 to 2009 (Sant'Angelo 2010).

There are three primary functions of antioxidants. Hydroxyl groups function as hydrogen donators that attach to peroxides and slow down the oxidation reaction. In addition, they function as chelators of cations that induce oxidation and hydrolysis of fatty acids and phospholipids. Antioxidants also prevent the oxidation of the iron in the heme ring of myoglobin and preserve the red color of products when stored in the presence of oxygen under lights in refrigerated meat cases. Carbon side chains are also important components on the antioxidants in cooked products because they provide carry over and allow the product to be heated without losing antioxidant effectiveness. Use of antioxidants can often provide 2–5 days of shelf-life extension for refrigerated products.

26.2.9 Antimicrobials

As noted in CFR 21 (Food and Drug Administration 2007), many new antimicrobials are approved in meat and poultry products. Many of these are designed for use on the surface of carcasses and primal cuts. For the purpose of this discussion, only those common ingredients found in marinade systems will be covered. Antimicrobials may be defined as chemical compounds added to muscle foods products that suppress, inhibit, or destroy pathogenic or spoilage microorganisms. The efficacy of the variety of antimicrobials varies widely. Organic acids are commonly used in meat and poultry products. Citric, lactic, and acetic acid are the principal organic acids that see widespread use as antimicrobials in meat and poultry (Hirshfield and others 2003). Acidic blends that may include propionic acid, octanoic (caprylic) acid, and certain peroxyacids are also used in marinated meat products. The limitations of organic acids are predicated on their potential to negatively impact product color, flavor, and odor. Additionally, the potential to lower the meat pH can be detrimental on the water-holding capacity of the marinated meat system. Hence, the most effective use of organic acids may be through application as topical antimicrobial agents.

Ricke (2003) discussed the use of organic acids in marinated meat products with respect to concentration, type of acid, exposure time, temperature, physical and chemical nature of the meat substrate, and microorganism type. The mechanism of action against microbial growth is complex and not completely understood, but is generally considered to involve penetration of the undissociated acid through the cell wall and the subsequent dissociation in the cytoplasm. Proton release within the cytoplasm results in an initial pH decline while the anion results in metabolic disruption and osmotic effects. Undissociated acids may also partition into the bacterial cell membrane at concentrations dependant on their partition coefficient (lipid solubility) and interfere with the activity of membrane proteins, leading to growth inhibition (Hirshfield and others 2003). The net result is generally a static response to injury or cell death.

The salts of organic acids are more useful when incorporated into brines and marinades. Through the manufacturing process, the salts of organic acids are pH buffered to a level more conducive to injecting

or tumbling into meat products. Shelef (1994) reviewed the antimicrobial effectiveness of lactic acid salts and specific studies have documented the benefits of lactates in roast beef (Papadopoulos and others 1991), fresh ground pork (O'Connor and others 1993), and pork sausage (Lamkey and others 1991). Besides effectiveness as a broad spectrum of antimicrobial, sodium and potassium lactates have been shown to be effective against several pathogens, including *Listeria monocytogenes* (Shelef and Yang 1991; Legan and others 2004), *Escherichia coli* O157:H7 (Miller and Acuff 1994), and the germination and growth of *Clostridium* spp. in vacuum-packaged roast beef (Sabah and others 2003). In addition to the antimicrobial and flavor impact, lactates function to increase color stability of enhanced pork (Jensen and others 2003) and fresh pork sausage (Bradley and others 2009; Seyfert and others 2006), and enhanced modified atmosphere packaging (MAP) beef (Mancini and others 2004). Sodium and potassium lactate can be used as antimicrobial agents in various meat and poultry products at levels of up to 4.8% by weight of total formulation.

The salts derived from acetic acid are also effective as antimicrobials. The sodium form of acetic acid is sodium acetate and the compound sodium diacetate (an equimolar composition of sodium acetate and acetic acid) are well documented to inhibit the microbial growth. Sodium diacetate is effective against *L. monocytogenes*, especially when combined with sodium or potassium lactate (Seman and others 2002; Legan and others 2004). Sodium acetate, sodium diacetate, and potassium diacetate are approved for use in various meat and poultry products at levels of up to 0.25% of the product formula. Finally, sodium citrate (sodium salt of citric acid) is reported to manage the germination and outgrowth of *C. perfringens* in beef and pork systems (Thippereddi and others 2003). When used at up to 1.3% of the product formulation, sodium citrate buffered to a pH of 5.6 with citric acid can be used in marinated whole muscle meat and poultry products, both cured and uncured.

26.2.10 Smoke, Spices, and Herbs

Imparting specific flavor characteristics to meat, poultry, and seafood have been accomplished with smoking, spices, and herbs since man began eating the meat. The spice trade has influenced the course of history. After Marco Polo's trip to China in the 1200s, European efforts to seek a more direct route to the spice-rich Orient was the reason for Columbus' initial voyage in 1492. While infinite combinations of spices, herbs, and smokes can be used in a wide variety of food products, this section focuses on the internal use of these ingredients when marinades and brines are used.

Spices are derived from the bark (cinnamon), root (garlic and onion), buds (cloves), seeds (mustard), berries (black pepper), or fruit (paprika) of plants and trees, whereas herbs are leaves (parsley, thyme, oregano, and sage) of slow-growing shrubs (Hertzler 2001). Spices and herbs deliver not only their characteristic flavors and aromas, but also visual appeal through color and particulates. While tumble marinating may use ground, crushed, or chopped spices and herbs, needle injection is not an option for particulates of any significant size. A small portion of the ingredient blend might contain ground spices, but anything larger than 60 mesh would be problematic due to the clogging of needles since the spices and herbs are not soluble. A much more consistent approach is through the use of spice and herb extractives. Solvent extraction of the volatile oils of spices and herbs can concentrate the desirable flavor and color components. Not only do these extracts plate easily on dry ingredients such as salt, maltodextrin, and sweeteners, but they are also more consistent in flavor, price volatility, and microbiological loads.

The use of smoke can be accomplished in a variety of methods. Natural wood smoking is performed by burning wood chips, sawdust, or logs and directing the smoke vapors over the product. Liquid smoke is derived from the pyrolysis of wood in controlled conditions to capture the key smoke components of carbonyls, phenolics, and acids. Furthermore, liquid smoke is manufactured to reduce the tar and ash components that are insoluble in water and create the majority of cleanup residue in cooking houses. Each class of compounds performs a specific purpose. Acids provide tartness and flavor and contribute to skin formation on smoked items, while phenols supply the primary flavor components and antimicrobial and antioxidant properties (Estrada-Muńoz and others 1998). Carbonyls are the source for smoke color and minor flavor components. Liquid smoke can be applied through atomization, drenching or dipping, spraying, inclusion into casings and nets, and through internal addition as an ingredient.

Utilizing liquid smoke as an ingredient requires specific selection based on the characteristics of the marinade or pickle solution. Liquid smoke can be added directly to a comminuted meat product at the mixer or chopper. The use of liquid smoke in a brine or marinade requires further criteria. If the product is cured (contains nitrite), a neutralized smoke must be used in the brine. Using a liquid smoke that has not been neutralized could lead to the acid promoting the breakdown of sodium nitrite to nitrous oxide, a potentially lethal gas. This is not a critical issue in solutions that do not contain nitrite. However, to be able to disperse the liquid smoke in the marinade, an emulsifier, such as polysorbate, should be a component of the liquid smoke product.

Advantages of direct addition of liquid smoke include uniform flavor throughout the product, minimal equipment needs, and incorporation of antioxidant properties throughout the entire meat product. Furthermore, smoke flavor can be added to products cooked in impermeable bags or casings. Disadvantages of internal smoke addition include the lack of external color development and the requirement to label the product as "Smoke Flavoring Added" as a qualifier.

26.3 Reduced Sodium Content

Sodium content is at the forefront of consumer, health, and regulatory priority lists with significant momentum to consider government intervention to regulate and reduce the sodium content of food products (Neuman 2010; Terwilliger 2008). However, simply reducing salt levels is not an acceptable option as yield, bind, shelf life, purge, and flavor properties all suffer. Furthermore, a review of all other sources of sodium in the meat and poultry product formulation should be reviewed. Placing a priority on new product development efforts with reduced sodium claims is warranted, with reduced sodium food product introductions topping the list of new food and beverage brand introductions (Food Business News 2008). It should be noted that many factors including genetic predisposition, exercise, and especially the diet as a whole impact the effect that sodium has on the health of individual people (Satin 2007).

While some functional level of salt is necessary for most marinated muscle foods formulations to be effective, four strategies can be used to reduce sodium content in marinated products (Desmond 2006): (1) reducing the inclusion level of salt, (2) replacing sodium salts with potassium salts, (3) using low-sodium-containing flavor enhancers, and (4) evaluating the physical form of the salt crystal. Reducing the salt level is most effective when reducing the salt of products containing a higher, rather than lower salt inclusion level. Ruusunen and others (2001) reported that sensory evaluation of hams with salt levels that were reduced to 1.7% were rated as salty as hams with either 2.0% or 2.3% salt, but still rated as more salty than hams with 1.1% and 1.4 % salt. Regarding functionality, Barbut and others (1988) tested frankfurters with various salt levels and phosphate type. When phosphate was not included as a treatment variable, the researchers determined that no differences existed in emulsion stability or smokehouse yield at 3.0%, 2.5%, or 2.0% sodium chloride, but when the levels were reduced to 1.5% and 1.0%, smokehouse yields were significantly reduced and emulsion stability suffered. Several options exist for replacing sodium salts with potassium salts in marinades. The two primary negatives for this strategy are increased cost and likelihood of bitter flavor notes. Potassium chloride, potassium phosphate, and potassium lactate are all commercially available products that can effectively replace their sodium counterparts. Adding or increasing sweeteners in the formulation or incorporating one of several commercially available natural flavors that act as bitter blockers may increase the chance of effective sodium reduction without flavor problems. Incidentally, potassium salts are generally highly soluble. As mentioned in the previous salt section, selecting a salt product with increased surface area can also allow for salt reduction with minimal flavor impact.

ACKNOWLEDGMENTS

The author would like to acknowledge the contributions of Stephen G. Campano, Hawkins, Inc., for his contribution to the content of the Antimicrobials section and Chris M. Salamone, John R. White Co. Inc., for his contribution to the content of Section 26.2.10 of this chapter, "Smoke, Spices, and Herbs."

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27

Marination: Processing Technology

J. Byron Williams

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27.1 Introduction

What is marination and why is it important in today's muscle foods industry? Marination has many different meanings depending on whether it is for home and restaurant use or industry application. In the context of this chapter and in muscle foods processing, it generally means the addition of liquids including functional ingredients, spices, and flavorings to food products prior to cooking. Other definitions describe marination similarly, stating that it is a technique used to improve tenderness, juiciness, and enrich flavor of meat (Brooks 2007). The marination time before cooking and the type of marinade, whether within the processing plant, restaurant, or at home, can vary widely depending on the product and its intended use. For muscle foods processors, chefs, and entrepreneurs, marination generally means the addition of several functional ingredients that can add flavor and improve tenderness while improving moisture retention, juiciness, and certainly yields. This type of marination application generally refers to enhancement of the product and is in direct relationship with product yield. To consumers and some food service operators, marination is simply the process of soaking or adding ingredients to food products for some time prior to cooking in order to enhance the product by adding flavor, moisture, and tenderness. This is often a slow process that involves passive transport of the marinade mixture into the meat products by absorption or osmosis (Brooks 2007).

The physical marination process can involve several steps and can either be a very simple process or a highly complex and expensive process, depending on the volume, time, type of product, and desired outcome. The basic practices of marination are accomplished by a single process or a combination of processes: (1) static soaking, (2) massaging, (3) injecting, (4) tumbling, and (5) mixing/blending to incorporate solutions and ingredients for many species, and is routinely used for chicken breast meat products (Smith and Young 2007). The mixing/blending process is mainly for ground or chunked products, while the other methods are used more in whole muscle type products, such as boneless hams, boneless chicken breasts, catfish fillets, or other intact muscle products. The discussion in this chapter will focus on the first four methods of marination listed, since they are more commonly practiced in marination systems. Equipment

and ingredients must work collectively to optimize the marinating process, resulting in value-added, high-quality products with increased profitability and consumer satisfaction.

27.2 Marination Mechanics

27.2.1 Definitions

To facilitate a more thorough understanding of the various marination practices mentioned above, it is beneficial to explore the simple definitions of these terms. According to Guralnik (1974, p. 1350), soaking is the "act of submerging or keeping in a liquid as for thorough wetting, softening, for hydrotherapy." Guralnik (1974) defines massaging as "rubbing, kneading, etc. of part of the body, usually with the hands, as to stimulate circulation and make muscles and joints pliable or flexible, while 'tumbling' is typically described as an act of tossing about or rolling around to put into disorder by tossing here and there to disarrange" (p. 872). This act of tossing about is usually accomplished by using a revolving box or drum into which the materials are placed and tumbled about for mixing, polishing, and similar tasks. Injection is defined by Guralnik (1974) as "the act of forcing or driving a fluid into some passage, cavity, or chamber; especially to introduce or force a liquid into some part of the body by means of a syringe, hypodermic needle or similar device" (p. 725). The method of mixing/blending, primarily used in sausage manufacturing, is more mechanical with more action of paddles and/or blades inside an open vessel (usually) than the massage technique. Mixing and blending will not be further discussed since this chapter is dealing primarily with whole muscle or larger muscle chunks, further processing techniques and functional ingredients. Mixing/blending can be used for smaller pieces (usually less than the size of a fist) in the process of making chunked and formed products. These chunked and formed products can also be processed/marinated using tumblers.

27.2.2 Devices and Equipment

In muscle foods processing plants and larger-scale production atmospheres, the terms used to describe marination processes mean something slightly different, although the principles are very similar and identical in some cases. The soaking technique is utilized more for in home use or very small establishments such as individual restaurants that conduct marination activities prior to cooking various muscle foods. This method is the least efficient and most time consuming of the four listed to accomplish uniform flavor throughout the product and to increase yields for value-added products. Soaking simply involves placing the raw product and marinade solution into a container, usually with a flat bottom and open top, and allowing the raw product to sit in the solution under refrigeration for a period of time prior to final preparation and cooking. This time period can vary from a few hours up to a few days (1–4) depending on the type of product, marinade solution, and flavor intensity desired. This technique is not consistent or efficient enough to justify the space and time required to be profitable for larger scale operations.

Massaging of muscle food products relies mainly on abrasion, through meat against meat contact and through meat contacting the walls of the container or vessel (Romans and others 2001). Typical massagers rely on multiple smooth paddles (usually rounded) aligned horizontally on a vertical shaft that agitates slowly, massaging the muscle, while minimizing tearing and particle size reduction. A design of a typical paddle massager is available in several publications, but a very typical original massager design is shown in *Processed Meats*, third edition by Pearson and Gillet (1999) on page 153. One issue with a square-shaped massaging device is that the product in the corners is less likely to receive mechanical action, and therefore be less functional and inconsistent with other products in the same container.

Tumbling of muscle food products relies on gravitational impact of product pieces dropping from the top to the bottom of the drum as it rotates and abrasion of product pieces rubbing against each other to extract the myofibrillar proteins that are necessary for binding pieces of meat together (Romans and others 2001). Most tumblers are made using a very similar design to a cement mixer, a drum with a

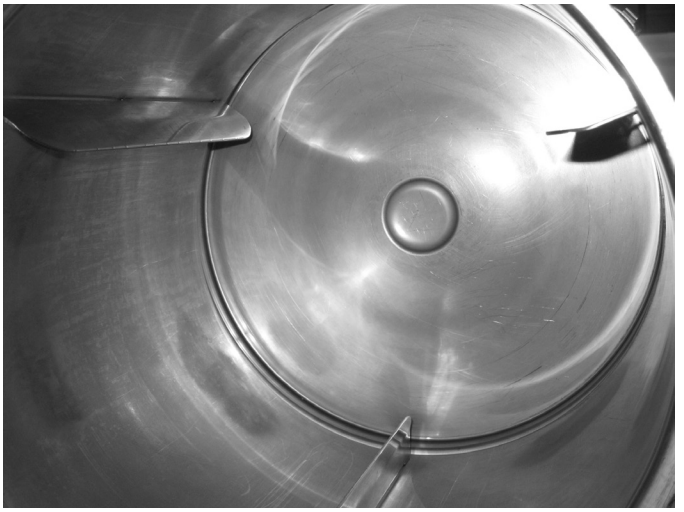

FIGURE 27.1 Photograph of small tumbler with baffles.

helix-shaped baffle or baffles inside (Figure 27.1), with or without added paddles or other devices inside the drum (Toldrá 2007). These devices range from very small (table top 10 kg) to very large (over 9000 kg capacity) and very simple (drum with 2–3 rounded baffles) to quite complex with many features such as: automatic loading and unloading, tilt adjustments, jacketed walls for chilling capacity, adjustable rotation speeds, timers, product temperature monitor log, and other similar features. All tumblers should be equipped with the ability to pull and continuously maintain strong vacuum, ≥20 mmHg to minimize air pockets in finished products, and to aid in protein functionality in water/pickle/marinade absorption.

Injection equipment, like the initial and simple definition listed earlier, is usually a stand-alone piece of equipment that has a walking carrier bed with a head containing multiple injection needles through which the marinade/brine/cure solution is passed through into the product as the muscles pass through the injection equipment. The head containing the needles moves with up and down strokes in a timed sequence with the walking bed of the injector. Both bone-in and boneless products can be injected, however, for bone-in products, the injector head must be equipped so that the needles (usually spring loaded) can float upward into the head when they contact a hard surface while allowing the other needles to continue penetrating the muscle. As the needles make contact with the product and resistance is encountered, the fluid solution is released into the needle and then forced under pressure into the muscle as the needle penetrates through the muscle mass. Different manufacturers' equipment models vary with some being used for both bone-in and boneless product while others are used specifically for one type of product. Equipment type and usage will depend on individual operations, volumes, and plant layouts. Most injectors are capable of variable pumping speed, head pressure, and conveyor bed speed to regulate ingoing solution percentage depending on the type and volume of product (Toldrá 2007). The injection principle can be very simple (hypodermic needle or syringe used in home kitchens and restaurants) or be accomplished through the use of multineedle high-speed complex machines that are capable of injecting several thousand kilograms of product per hour. Recently, advancements have been announced for a spray injection system that forces brine or curing solutions into meat pieces utilizing high-velocity micro droplets (Meatingplace 2010). The injection process is most often used in conjunction with tumbling or massaging equipment to complete the processing steps required for most boneless muscle food products since injection action alone does little to extract proteins that are imperative for the binding of water and the binding of meat pieces together. Some whole, single muscle or intact bone-in products may not need any further protein extraction for additional functionality and hence only need the injection process prior to final processing. Boneless muscles are often utilized in further processed products that are made with multiple pieces or muscles that were formed into one larger shaped/formed finished product such as a boneless buffet ham, boneless roast beef log for slicing, or a boneless poultry log and others. However,

there are some single muscle items that are processed with coatings/seasonings that require some protein extraction to create the adhesion properties, "glue," to hold the spices on the external surfaces of the individual portions, that is, seasoned chicken breast, seasoned brisket, peppered roasts, seasoned/flavored tenderloin.

Whether massaging and/or tumbling, both are mechanical processes applied to a product over time with the goal of bringing salt-soluble proteins to the surface of the product without damaging the tissue. The salt-soluble proteins, mainly actin and myosin, help bind water and liquids throughout the product and facilitate in seasoning absorption, retention, and adherence to the surface especially with very coarse ground seasonings. Additionally, there are many other processing benefits involved with massaging and/or tumbling such as: tenderization due to the mechanical actions; pliability improvement; uniformity in appearance and shape; increased moisture, flavor, and yield; and decreased production cost per unit volume while adding value to the end product (Schmidt 1987). The main difference between massagers and tumblers is that massaging is a gentler, much slower mechanical action than tumbling. Massaging relies more on piece to piece abrasion with some abrasion of the meat pieces against the paddles and container walls. Tumbling is usually a more forceful action with the meat pieces being picked up by baffles or other structures inside the tumbler drum and allowing the pieces to fall onto other pieces in addition to more aggressive contact with the metal structures within the tumbler device. Depending on the product, tumbling procedures are completed in considerably shorter time than massaging procedures. Ultimately, processors benefit from savings with tumbling through less product inventory on hand, increased product throughput and turnover, reduced total product cost due to increased yield, reduced product shrinkage, added value and versatility of the product, and overall quality improvement of the product. Customers will benefit from noticeable improvements in juiciness and tenderness of the products, enhanced flavor through less purge and cooking loss, overall improved taste, improved appearance with more uniform shapes and color, and increased uniformity of salt/marinade flavor throughout the product.

The injection system is the most commonly used system to apply brine and/or marinade solutions to meat products. As stated previously, many injected products are subsequently either massaged or tumbled to accomplish protein extraction for certain products used in stuffing operations, external seasoning applications, and for those products that processors desire protein extraction from mechanical action. The injection process, if done correctly, will give the most uniform distribution of ingredients throughout muscle pieces in the least amount of time. Injection will also improve tenderness through the mechanical action of the numerous needles penetrating the muscle tissues. For many processors and products, the most advantageous process is the combination of injection to get the marinade and curing solutions uniformly into muscle tissues followed by tumbling. This combination provides the ultimate overall product enhancement and maximum finished product throughput in the least amount of time.

27.2.3 Advantages and Disadvantages

Soaking

- Minimal equipment investment.
- Simple one-step process.
- Allows value addition to very delicate products with minimal damage from mechanical action.
- Requires a long amount of time for absorption.
- Requires a large amount of refrigerated space for containers.
- Requires large volumes of raw product inventory to be in the plant.
- Inconsistencies in product color, flavor uniformity, and moisture retention.
- Lower cooking yields.

Massaging

- Greater enhancement of delicate products when compared to soaking.
- Lower equipment investment than injector plus tumbler costs.

- Allows for reduced action of muscles where minimal protein extraction may be desired in specific products.
- It is useful for products that need long-term effects of slow action and absorption.
- Increases the time of production due to longer processes.
- Requires more refrigerated space for holding uncooked product.
- Results in more inventory of unfinished product in the plant.
- Depending on the massaging equipment used (rectangular), some inconsistencies of protein extraction, lack of uniformity in color, and flavor could be issues.
- Lower finished yields for some products.

Tumbling

- Pliability improvement of raw product which will improve stuffing uniformity and consistency.
- Uniform appearance of raw and finished product.
- Allows for maximum protein extraction and binding capacity of muscles.
- Can be utilized to marinade smaller pieces not conducive or efficient for the injection process.
- Provides optimum environment for maximum absorption of added ingredients.
- Allows for precise amounts of added ingredients to be added and controlled.
- Yield improvements and subsequent cost reduction.
- Facilitates mechanical addition of external spices/seasonings, especially nondissolvable and whole spices for a desired external appearance.
- Increased initial costs in equipment.
- Not conducive to very delicate muscles/cuts due to more aggressive movement—may result in torn pieces with less than desirable size or usability.

Injection

- High-cost equipment.
- Not feasible or optimal for solution addition to small pieces or very thin strips.
- Faster cure/ingredient absorption time.
- More uniform and even distribution of ingredients into the muscle tissues.
- More uniformity of internal color development from ingredients (especially cure).
- Allows multiple ingredient addition in a single process (dissolvable ingredients).
- Most consistent yield improvement.
- Multiple product usage on same injector even with solution change over.
- Most prevalent method used today for solution/cure/marinade addition to muscle products.

Injection/tumbling combination

- Increased cost of equipment investment but with yield improvement will give a very good return on investment.
- Increased labor for product handling and transfer.
- Allows advantages of both systems as listed above to be combined into the final product.
- Minimizes solution loss while maximizing yields and weight gain.
- Reduces total processing time prior to cooking/smoking/final processing steps.
- Sensory attribute enhancement but can increase bacterial numbers (Guner and others 2008).

27.2.4 Technical Points

All of the marination processes listed above involve many of the basics of meat chemistry discussed in other chapters within this text and functional ingredients which are discussed in the chapter on marination ingredients. The main functional ingredients of water, salt, phosphate, and cure for cured products are

vital in most of the products that have added solutions, especially those that are made from multiple pieces that are put back together to make larger products to look like one uniform muscle. Each ingredient has an important specific function in the enhancement process: salt for solubilization of the myofibular proteins, phosphate for enhancement of water-binding abilities of the proteins, and certainly water as the media of carrying these ingredients into the muscle tissues and for the added weight for yield improvement. This process can be explored in other sections of this text and additional references provide the essentials of the meat chemistry, which are critical, but will not be discussed in this section. Ultimate results of the processes used are judged on (a) yield before cooking and after cooling of cooked product, (b) adhesion of meat pieces to one another to give an intact whole muscle product appearance or sliceability, and (c) internal structure of the meat after final processing (Ranken 2000).

There are some critical factors that will affect product outcome and ultimate quality. Pale, soft, and exudative (PSE) muscles have lost most of their water-holding capacity (WHC) and therefore do not perform efficiently in marinated products. However, they can be mixed with other high-quality protein functional muscles at up to about 15–20%. This combination allows processors to utilize some of these lower quality products while relying upon the majority volume of the higher quality products for functionality. Under these conditions, there are usually enough high-quality proteins to bind the added solution and bind the lower quality muscles with the higher quality muscles to make acceptable end products. A disadvantage to this could be some color variation within the products due to the extreme paleness of some PSE meat. In extreme cases, these muscles should be separated and not used for processed whole or multimuscle products. Another comparable issue is raw materials with high collagen content used in sectioned and formed type meat products. This will cause similar conditions as when used in comminuted or emulsified products in that there may be jelly pockets and voids within the finished cooked formed product and poor binding of muscle pieces.

There are different opinions and reported results about tumbling and massaging under vacuum. Smith and Young (2007) reported that type of pressure had no effect on broiler breast and fillets during tumble marination, except on marinade uptake, which disappeared with a 1 h holding time prior to cooking. Others have reported that there are no effects of vacuum on the uptake or retention with fish muscle and only small degrees of brine distribution differences in pre- and postrigor pork muscle (Martin 2001; Zayes and others 1976; Solomon and others 1980). For most products and processing, it has been shown that protein extraction and muscle binding is enhanced if the mechanical actions are applied under vacuum (Hedrick and others 1994). While there may be some disagreement on specific effects of vacuum in the uptake and retention of solution, it is imperative to remove as much air as possible from solution-enhanced muscles that are going to be stuffed into larger casings, molds, or devices to make products with multiple muscle pieces put together to have a one piece final product. If not, there will be air pockets and cavities within the finished product due to trapped air that can cause casing splits in the cooking cycle. Furthermore, trapped air can affect to some extent, single muscle products if they are macerated or have creases and crevices on the exterior surfaces that can fold over to form cavities with trapped air. The majority of tumblers are equipped with vacuum capability and its use is encouraged for most solution enhanced/marinated products.

Another technical issue and concern is the use of acid marinades. Using very acidic marinades or marinade ingredients that are acidic can cause denaturation of the extracted proteins in muscle tissue. Therefore, very acidic marinades can cause poor binding of muscle pieces, loss of moisture retention and internal cavities, all yielding finished products with poor overall appearance and sliceability (Figure 27.2). These conditions all end in dissatisfied customers, poor yields, and loss of profitability.

Raw material size variation should be minimized within any marination/solution enhancement operation regardless of the method used. Extreme size variation of muscles or pieces will cause unevenness in solution uptake, color differences, inefficient time usage of equipment, and generally inconsistent finished products. Optimal performance is dependent on the uniformity in piece weight and dimension. As an example, it is not recommended to mix whole or half breasts with strips or whole boneless hams with individual smaller ham muscles and expect to optimize the product uniformity and yields while maximizing the efficient use of the equipment (Figure 27.3).

Timing of marination postmortem with injection, tumbling, massaging, soaking, or a combination has been reported to have varying effects on end product tenderness, moisture, and flavor, especially in

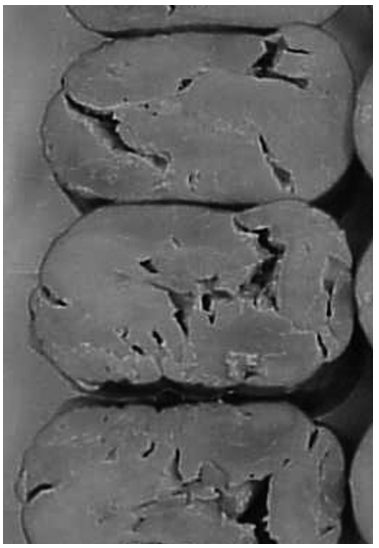

FIGURE 27.2 Photograph of processed ham with acidic marinade, low tumbler vacuum, and overcooking.

broiler breast fillets (Saha and others 2009; Alvarado and Sams 2004). These authors reported enhancement of broiler breast meat tenderness, juiciness, and texture by marination methods which can improve tenderness in prerigor-boned breast meat more than postrigor-deboned meat. However, their research confirmed that postrigor-boned and marinated breast meat received the highest consumer acceptability scores. This pattern is certainly true for red meat species but is not as clearly documented for fish muscle. As concluded by Xiong and Kupski (1999), in a study monitoring marinade penetration with phosphate and varying salt levels in breast fillets, the proper selection and combination of marinade ingredients and the processing schedule is of utmost importance. One must also note that these factors may vary from product to product and species to species, so the same marinade and process schedule will most likely not work for multiple products.

FIGURE 27.3 Photograph of slicing logs made with multiple ham muscles under optimum tumbling and vacuum conditions.

27.2.5 Processing Tips and Recommendations

After this abbreviated discussion, which method do you use to marinate and/or enhance your products? If ground, cracked or whole spices are desired to be added to the product, then you will need to marinate the dissolved liquid portions of the solution into the product first and then coat the exterior with the other ingredients since they will not dissolve or pass through injector needles. Clogged injector needles are a processing operational nightmare and will usually result in a new set of needles with a costly price tag. Other additives such as fiber ingredients, whole grains, starches, gums, and carrageenans can be very beneficial to certain products but require careful and timed addition to the process. Some can be dissolved and injected, but it is imperative for continual agitation in the solution tank and a filter screen device attached to the injector to filter excess solution prior to it reentering the injector to preclude needles being clogged with muscle connective tissue and any other particulates of the marinade solution. Most processes with external spices or rubs will use an injection process for the liquid portion absorption, followed by tumbling to get maximum absorption and create protein extraction on the product surface, then add other dry ingredients and tumble until the product is uniformly coated prior to stuffing, cooking, and other processing measures. For very small volume operations with delicate product and/or lack of equipment or capital to purchase injectors and/or tumblers; soaking is the preferred method of marination. For those products that require no surface protein extraction, soaking or injection only would most likely work best. Tumbling would improve yields and some sensory characteristics, but would require close attention and very little mechanical action in an effort to preclude extraction of an undesirable amount of protein for these specific products.

Experience and practice indicate that the optimum marination process involves the injection of the marinade/curing solution very close to the desired input level within the product followed by immediate vacuum tumbling. The solution should be adjusted to the exact desired amount by weight into the tumbler. If the injection percentage is targeted to be 0.5–1% below the targeted ingoing amount, then adjustment with free solution into the tumbler at the precise amount of added ingredients can be easily accomplished. A simple formula for percent gain is: marinated or injected weight *minus* the green nonmarinated weight *divided* by the nonmarinated or green weight *multiplied* by 100: {%gain = (marinated weight – green weight)/(green weight) × 100}. This combination process reduces time, increases volume, maximizes yields, improves texture and other sensory characteristics, and allows strict control of the product which results in uniformity and consistency of the finished product.

A few other processing tips regardless of which process is used are: (1) make sure the proper phosphate is used for the application and that it is dissolved completely in the water before any other ingredients are added; (2) keep solution cold and agitated while it is being added and/or injected; (3) inject close to the desired percent gain, just slightly below (it is much easier to add rather than take out solution after the product has been injected and in the tumbler); (4) add free solution to the tumbler/massager to bring up to the desired level (maximum amount to add as free solution is about 3%); (5) keep everything cold including the room where injecting, tumbling, or soaking is occurring (2–4 °C is an optimum range); and (6) intermittent tumbling cycle with continuous vacuum (7–10 rpm, 22 + mmHg vacuum, rotate for 8–15 min then rest for at least the same amount of time, repeating this cycle until the desired extraction and absorption is accomplished). These are general starting guidelines that may need to be adjusted depending on the type of product and the desired end product outcome. If injecting, use only side port (opening) needles of the smallest feasible size according to the product being injected. Smaller needle holes made into the product usually results in better retention of the liquid solution and less loss through damaged tissue at the injection sites. Following massaging, tumbling, injection, or a combination of methods, it is desirable to allow products to rest or sit in a cold container and environment for several hours prior to heating or cooking. This allows further solution migration and uniformity throughout the muscle tissues and enhances uniform color and flavor development. If products sit for an extended period of time and become very stiff and not very pliable, it is a good practice to place the product into a clean tumbler, pull a good vacuum and tumble for a few minutes to loosen the product prior to immediate stuffing and cooking. This makes the product more pliable, provides stuffing uniformity, and prevents air cavities and voids.

27.2.6 Conclusions and Suggestions

Whichever method of marination is chosen, it is critical to begin with quality raw materials. It is important to be very cautious with temperatures and avoid any abuse since these methods, especially injection and tumbling, tend to spread the microflora that is present while increasing the surface area. This type of abuse can result in increased microbial counts if not controlled carefully. This can be particularly problematic in solutions with very low salt concentration and higher concentrations of sweeteners. Additionally, it is advised to be cautious when using ingredients that alter the pH drastically, especially acidic ingredients.

Another point mentioned earlier in this chapter is size and uniformity of product pieces. This is very critical for any of the methods discussed as it can result in inconsistent finished products. Products produced in state or federally inspected establishments must follow labeling restrictions which are applicable to most meat and poultry products according to the amount of marinade/solution added and/or analytical analysis. Remember, value addition is the main objective while the effectiveness of most liquid marination systems depends ultimately on the water-holding capacity of the raw material. Last but certainly not least, the finished product must look very appealing to the customer and taste even better to the consumer.

ACKNOWLEDGMENTS

My sincere thanks and appreciation are expressed to my graduate student, Courtney A. Crist, for her diligence, patience, and thoroughness in assisting me with the preparation of this manuscript. Her expertise in editing and technical skills with references is graciously appreciated. Also, gratitude and appreciation is expressed to my colleague, Dr. M. Wes Schilling, the section editor, for giving me the opportunity to contribute a portion to this text.

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28

Drying: Principles and Applications

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28.1 Introduction

Meat drying may be fundamentally defined as the removal of most of the water present in meat by evaporation or sublimation. Consequently, the water activity (a_w) is lowered thereby hindering microbial growth and biochemical reactions. The dried meat product will thus have a longer shelf life. Drying using simple natural conditions has been practiced for centuries with the primary purpose of preserving meat. Such drying is still practiced today in many developing countries where other means of meat preservation (e.g., frozen storage) may be too costly or inexistent. In contrast, in developed countries, drying is often only one of the various processing steps (salting, fermentation, smoking) used during the manufacture of specific meat products.

There exists a broad range of traditional dried or intermediate moisture meats originating from different developing states, such as the Brazilian *carne do sol* and *charque* and the Sahelian *kilishi*. The same is true for industrialized countries where traditional dried meats, such as bacon, cured ham and *Grisons* meat, enjoy a good popularity among consumers. Yet, there is a scarcity of statistical data on the world production of dried meat products. This could partially be explained by the fact that a large number of traditional products, especially from developing countries, are generally meant for the domestic market.

This chapter first presents the current conventional techniques used for meat drying and their underlying principles. Applications of these conventional techniques to produce various dried meat products are then illustrated. The quality of dried meat products during processing and storage is then discussed.

28.2 Meat Drying: Conventional Techniques and Principles

28.2.1 Conventional Techniques for Meat Drying

The conventional techniques for meat drying may be used at small, semi-industrial or industrial scales. A major example among the conventional techniques is the convective drying, based on heating using solar energy, microwave, or hot air stream. Drying may be used as the only technique for production of highly dehydrated and shelf-stable end-products, as is often the case in hot areas and developing countries, where chilling and refrigeration infrastructures are lacking. Drying may also be combined with other techniques such as smoking, salting, seasoning, curing and ripening, leading to intermediate moisture products like ham and sausages. The choice of such combined treatment mainly aims at enhancing the organoleptic characteristics (flavor, texture) and improving the palatability of the end-product, to suit consumer requirements.

Sun-drying is among the oldest techniques used for meat preservation, especially in the Southern countries, as it is adapted to home-scale constraints. The meat, either raw or seasoned, is cut into strips or flat pieces. These pieces are exposed to the sun, directly laid on the ground or raised on locally made trays, or sometimes hung on hooks. The advantage of this simple and cheap technique is the use of the natural and renewable sun energy and of local materials. The main disadvantage is the poor quality of the end-products, because of the lack of hygiene and the meat contamination by blueflies, insects, rodents, and dust. Cabinet solar dryers have been developed, where warm air is conducted through a hermetically closed chamber, thereby avoiding any undesirable contamination from outside. Different types of low-cost solar dryers have been implemented in many African countries (FAO 1993). However, sun-drying is highly dependent on the weather conditions. It is proven that during rainy seasons, sun-drying is not sufficient to ensure complete preservation of the meat, because of high relative humidity and frequent day/night fluctuations of temperature.

To overcome this problem, the convective hot air-drying is a successful alternative. Various types of dryers based on the transport of convective hot air are available, functioning on both batch and continuous modes. These equipments generally consist of a ventilated drying chamber where the air conditions (i.e., temperature, relative humidity, and velocity) are well controlled. The meat pieces are hung in the drying chamber and submitted to the hot air stream, for a period of time necessary to achieve the required characteristics of the end-product, in terms of sanitary quality and sensory features. The process is operated at moderate temperatures (under 35°C), in the case of refrigerated storage of the end-product, or at higher temperatures (50–60°C) when drying is used as a unique treatment for raw material preservation. For cured and fermented meats, drying is associated with the product ripening and the drying–ripening temperatures range from 10°C to 15°C.

Fluidized-bed dryers are successfully used for meat drying in temperate climates, mainly for use in canned soups. Meat is cut into slices, cooked, minced, or cut into small cubes, and is then dried at 70°C.

Alternative drying technologies, consisting of treatment by superheated steam followed by either heat pump drying or hot air-drying at 55°C, were tested at laboratory scale for chicken drying with the purpose of use in ready-to-eat noodles. These alternative technologies showed interesting results such as decreased drying time, less shrinkage and better rehydration capacity of the end-product. However, they are not yet widely used at industrial scale. Recently, a new drying-ripening process: Quick-Dry-Slice has been proposed for sliced products. Sausages are fermented to the desired pH and are then frozen, sliced, and dried in a continuous system that combines convective and vacuum drying. This new process reduced the traditional drying–ripening time to 30 min without any defect in the sausage quality and safety (Arnau and others 2007). Sebastian and others (2005) investigated the combination of radiative and convective heat transfers for the drying step in the traditional processing of *boucané* in La Réunion

Island (France). Radiant metal plates were heated by the combustion smoke, which improved the quality of the end-product by cutting down the benzo(a)pyrene production.

28.2.2 Principles of Meat Drying

As for major categories of foodstuffs, the convective drying of meat is ruled by the classical principles of this process. Air-drying, in particular, is an ancient process used to preserve foods, wherein the solid to be dried is exposed to a continuously flowing hot stream of air causing water to evaporate (Ratti 2001). The main purposes of meat drying are product preservation through a decrease in its water content and a_w, as well as the development of required sensory characteristics. Drying decreases the water content of raw meat (70–80%, wet basis) to a safe value, in the range of 12–15% (wet basis) when drying is the sole preservation process, and in the range of 28–50% when drying is combined with other techniques such as salting.

Drying also reduces the meat a_w until the safe value of 0.6 (0.7–0.75 for intermediate moisture meat products) is reached. The a_w is a measure of the amount of available water remaining after drying which may contribute to the degradation of the meat quality, through microbial or biochemical mechanisms. The measurement of a_w describes the moisture equilibrium between the meat and the surrounding air. The moisture sorption isotherm is a sigmoidal curve describing the equilibrium relationship between the water content and the a_w of a product. It represents the amount of water adsorbed in the meat as a function of the relative humidity of the air surrounding the material. Figure 28.1 shows an example of a moisture sorption isotherm. The moisture sorption isotherm can help optimize the meat-drying process to obtain defined end-product characteristics.

Heating is the driving force of convective meat drying. The hot air is characterized by temperature, relative humidity, and velocity. These parameters should be well chosen with respect to the type of meat and end-product. They should also be controlled throughout the process, using adequate sensors in the drying chamber. In addition, the characteristics of the product, that is, its size, shape, structure, and proximate composition, are key factors that determine the drying time needed for the end-product to reach safe water content and a_w.

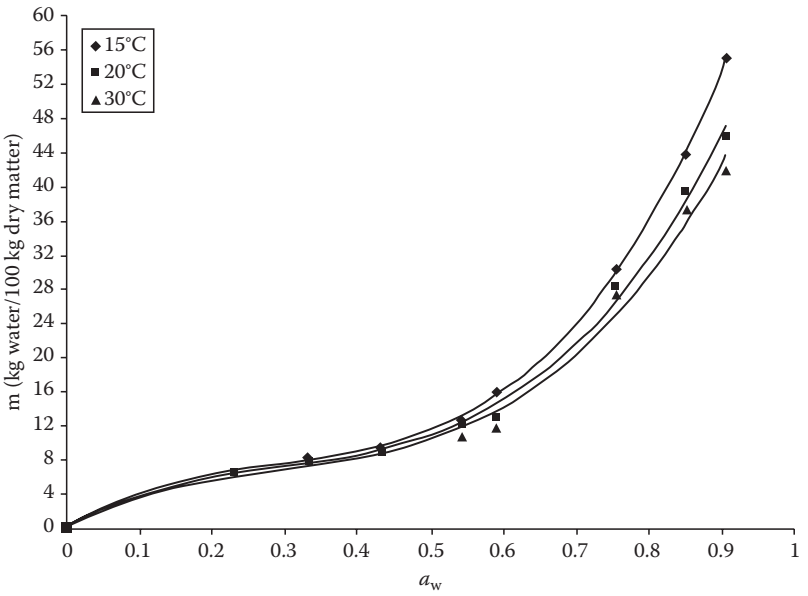

FIGURE 28.1 Moisture adsorption isotherms of dehydrated slices of *pastirma* at 15°C, 20°C, and 30°C. (Reprinted from *Meat Science*, 71, Aktas N, Gürses A. Moisture adsorption properties and adsorption isosteric heat of dehydrated slices of Pastirma (Turkish dry meat product), pp. 571–576, Copyright (2005). With permission from Elsevier.)

Convective meat drying involves two phenomena: (1) heat transfer from the hot air to the product, and (2) mass or water transfer in the reverse direction. Water transfer involves the evaporation of water from the product surface, simultaneously accompanied by the migration of water inside the product toward the surface. This latter migration is essentially a diffusion-based mechanism.

Drying curves illustrate the transfer phenomena occurring during meat drying. They are called drying kinetics when the decrease in meat water content is plotted against drying time. Another form of drying curves consists of plotting the drying rate against the water content. Three zones can be distinguished on a typical drying curve, corresponding to the three stages occurring during food drying: (1) induction period (beginning of the drying with fast removal of water), (2) constant-rate drying period (corresponding to the constant evaporation of water at the product surface), and (3) falling-rate drying period (corresponding to the most difficult removal of water because of its slow diffusion to the product surface). Figure 28.2 illustrates the drying rate of kilishi under sun-drying and convective hot-air conditions.

Drying kinetics allow understanding of the mechanisms of water transport occurring under specific meat-drying conditions. The presence of fat in meat products tends to slow down the drying rate because of its barrier effect on water transfer. The higher the fat content, the lower the drying rate.

28.2.3 Meat Preparation Prior to Drying

Dried meats are generally manufactured from bovine meat and to less extent from sheep, mutton, goat, yak, or camel. Cured meats are commonly made from pork and in some cases from beef or turkey. Meat drying can be performed either on fresh raw material, a few hours after slaughtering, or on refrigerated and/or frozen meat. Frozen meat must be properly thawed prior to further processing, by tempering the meat at chilling temperatures for up to 48 h. The preparatory operations prior to drying are common to most meat-processing techniques. Meat preparation consists of cutting the carcass into primary or retail cuts, trimming to remove undesirable material (e.g., damaged tissues, extra fat, connective tissue, nerves, and bones). Meat is then cut into strips or flat pieces which are either directly air-dried or undergo treatments such as salting or spicing before air-drying. For some meat-based products, the meat must be chopped and then mixed to ingredients and seasonings, and in certain cases, inoculated with starter microbial cultures.

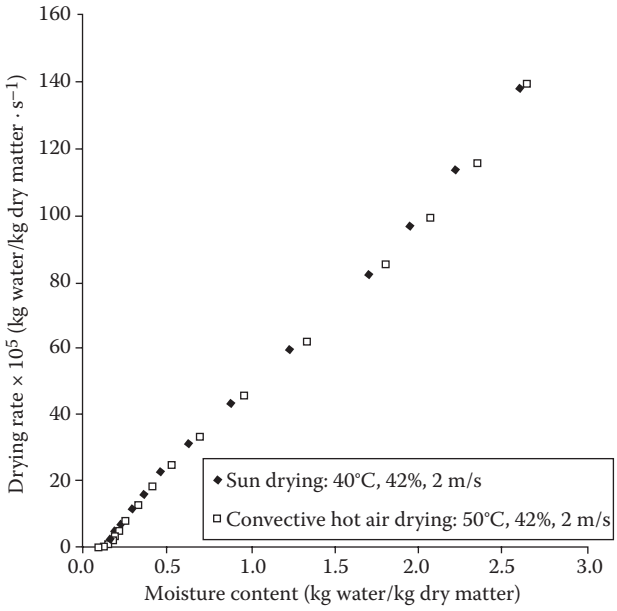

FIGURE 28.2 Drying rate of beef before coating (kilishi processing), under sun-drying and convective hot-air drying. (Reprinted from *Meat Science*, 50(1), Kalilou S, Collignan A, Zakhia N. Optimizing the traditional processing of beef into Kilishi, pp. 21–32, Copyright (1998). With permission from Elsevier.)

28.3 Meat Drying: Applications

In the following, a range of meat products produced solely or partly by drying is described. Examples of popular dried meat products and some of their characteristics are summarized in Table 28.1. As meat drying is often combined with other techniques such as dry or wet salting, smoking, and fermentation, the products were classified according to the unit operations involved in their processing.

TABLE 28.1

Physicochemical Characteristics of Dried Meat Products

Type	Meat Products	Origin	WC (% wb)	a_w	SC (% wb)	References
Dried	Kilishi	Sahel African Countries	7.5–20	0.65	—	Igene and others (1989); Kalilou and others (1998); Kalilou and Zakhia (1999)
	Sharmoot	Chad	8–13	—	—	Hugoo (1993)
Salted dried	Biltong	South Africa	8–44	0.60–0.84	3.5–7.7	Van der Riet (1976)
	Unam inung	Nigeria	31–56	—	—	Solomon and others (1994)
	Carne do sol	Brazil	64–70	0.94	5–6	Norman and Corte (1985)
	Charque (or charqui)	Brazil	44–45	0.87–0.91	12–15	Norman and Corte (1985)
	Tasajo	Cuba	33	0.75	24	Chenoll and others (2007)
	Sou gan	China	8–16	0.4–0.66	3.7–5.1	Rao (1997)
	Dendeng gilling	Indonesia	19–21	0.62–0.66	6.9–7.7	Rao (1997)
	Pastirma	East Mediterranean	39–52	0.85–0.90	2.7–9	Kilic (2009)
	Cecina	Mexico	61–65	0.89–0.90	8.1–10.0	Reyez-Cano and others (1994)
Salted dried smoked	Banda	Nigeria	8–16	—	—	Obanu (1988)
	Kundi	Nigeria	48.8–62.9	0.73–0.90	0.5	Alonge (1987)
	Boucané	Réunion	25	0.83	—	Poligné and others (2001)
Salted fermented dried	Uzice beef prshuta	Serbia	40	—	4.3	Tomic and others (2007)
	Salted dried duck fillets	France	—	0.93	5	Poma (1998)
	Dry-cured lacón	Spain	40	0.77	12	Lorenzo and others (2003)
	Parma ham	Italy	59–64	0.92	4–6	Baldini and Raczynski (1979)
	Ham	Argentina	48–53	0.77–0.81	9.4–15.5	Aguilera (1990)
	Serrano ham	Venezuela	47	0.88	8.9	Aguilera (1990)
	Serrano ham	Spain	52	0.9	6–7	Arnau and Monfort (1998)
	Cecina de León	Spain	50–60	0.87–0.93	5.7–7.5	Molinero and others (2008)
	Bresaola	Italy	61–63	0.88–0.95	—	Paleari and others (2000, 2002)
	Grisons meat	Switzerland	46–53	—	—	Office Fédéral de l'Agriculture (1999)
	Kaddid	Morocco	7.5–14.3	0.49–0.65	7–12	Bennani and others (1995)
	French fermented sausage	France	35–37	0.89	4.0–5.4	Durand (1999)
	Sucuk	Turkey	26–47	0.82–0.85	2.5–3.5	Kilic (2009)

Notes: WC, water content; SC, salt content; wb, wet basis.

28.3.1 Dried Meats

Simple meat drying is more common in Africa even if Rao (1997) mentioned about dried meats essentially from mutton produced in Pakistan (*ladi*, *khadit*, *prasanda*, or *patav*), India, and Nepal. Traditional dried meats are usually cut into fine slices, and sun dried. They can be eaten as snacks or rehydrated and incorporated into local meals and sauces before consumption.

Kilishi is a sun-dried, spiced, and roasted traditional African meat product of long shelf-life at ambient temperatures (up to 12 months (Obanu 1988)) and high consumer acceptability. It is widely consumed in Sahel African countries and is highly valued in both rural and urban areas of Niger and Nigeria (Igene and others 1989; Kalilou and Zakhia 1999). Traditionally, *kilishi* is made from rump or shoulder of beef, but goat, sheep or camel meat can also be used. Its process flow diagram is shown in Figure 28.3. The fat and tendons are removed from the meat and then it is cut with a knife into slices 3–4 mm thick and

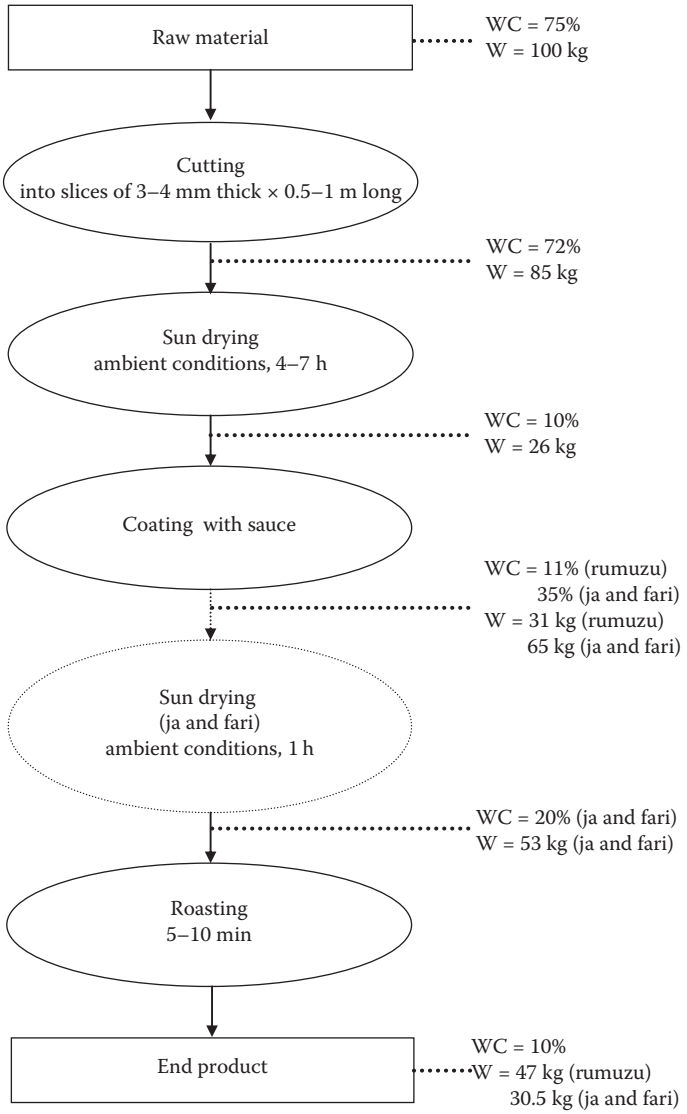

FIGURE 28.3 Kilishi process flow diagram (W, weight; WC, water content; dotted line represents intermediate products; dotted box represents optional step). (Adapted from Kalilou S, Zakhia N. 1999. Traditional methods for processing meat in Niger. *Tropical Science* 39:18–22.)

0.5–1.0 m long. The strips are dried in the sun on raised beds of millet straw on wooden tables for 4–7 h, depending on climatic conditions. The strips are turned periodically so that they dry evenly. The dried meat is coated with a sauce that can be of three different types. For *fari kilishi*, the sauce contains salt, different spices, and condiments, for *ja kilishi*, the sauce is prepared as for *fari* but is red colored with *Bixa orellana* and for *rumuzu kilishi*, the strips of dried meat are soaked in groundnut oil and then dusted with a similar mixture of spices. Finally, the coated dried strips are roasted over a wood fire for 5–10 min. Grilling makes the sauce stick to the meat and completes the drying process, giving the product its characteristic flavor. The brown to black color and the brittleness are the criteria used to determine whether the meat is sufficiently dry. Drying is a critical stage, especially in the wet season when the meat does not dry well and the risk of spoilage by microorganisms is considerable (Kalilou and Zakhia 1999).

The processing of *quitab* and *ndariko* (in Sahel African countries) (Laurent 1981; Obanu 1988) and *sharmoot* (typical in Chad and Sudan) (Hugoo 1993; Varnam and Sutherlands 1995) is similar to that of *kilishi*, in terms of cutting into strips and sun-drying for 3–7 days but they are not subsequently coated or grilled.

28.3.2 Salted Dried Meats

Either dry- or wet-salting can be performed prior to drying. In most cases, local spices are added to salt. The greatest variety of salted dried meat products can be found in Africa (*biltong* and *droëwors* in South Africa, *unam inung*), in Latin America, (*charque* (or *charqui*), *carne-do-sol*, and *tasajo*), and in Asia (*dendeng*, *sou gan* . . .). *Basturma* (or *pastirma*) is famous in the Mediterranean basin. A North American product is *beef jerky*, which was traditionally sliced dried beef. Nowadays, *jerky* products are characterized by a considerable diversity in technological procedures (comminution, curing, marination, cooking, smoking, drying, and packaging) with different kinds of raw materials (beef, pork, poultry, and venison), spices, and other functional additives such as antioxidants and stabilizers (Carr and others 1997; Choi and others 2008).

Biltong and *droëwors* are ready-to-eat dried meat products originating from beef or game meat (see Figure 28.4). They are regarded in South Africa as a delicacy and are gaining international popularity as a snack food with markets in the United Kingdom, Australia, Portugal, and the United States (Attwell 2003). Nearly all muscles in the carcass may be used for *biltong* but the largest hindquarter muscles are most suitable. Meat is cut into long strips (typically $4 \times 2.5 \times 20–30$ cm^3) and salted, more commonly dry-salted. Sodium chloride is the principal curing ingredient, although nitrates/nitrites are also used. Other ingredients such as brown sugar, vinegar, pepper, coriander, and other spices may be added. The addition of 0.1% potassium sorbate is now recommended and permitted in South Africa. Meat is left in the cure for some hours, plunged quickly into hot water with vinegar. Drying is traditionally accomplished by hanging strips in a well-ventilated area for 1–2 weeks and drying chambers are employed for large-scale production thus shortening drying time to a few days (Van der Riet 1982). Nowadays, it is produced commercially at a large scale and sold in sticks, slices, nibbles, and cubes, and in the ground or pulverized form. It may be stored for months without refrigeration. Van Der Riet (1976) showed there is a fair amount of variation in moisture content (8–44%) and a_w (0.6–0.84) of *biltong*. Nowadays, many consumers prefer moist biltong with moisture content higher than 40% (Nortjé and others 2005). The processing of *droëwors* is similar: small pieces of beef are ground, seasoned, stuffed into casings, and dried (Burnham and others 2008).

Unam inung is a cured pork product that is very popular in the Southeastern states of Nigeria. It is most commonly served with cassava chips or boiled yam and plantain. The product is traditionally prepared by heavily salting sliced pork belly which is then sun dried and packed in a dry clay pot. In some cases, the product may also be smoked. When required for consumption or for the market, the desired quantity is removed from the pot, washed, and boiled. The traditional production techniques have not been improved to cope with modern scientific requirements and with increasing demand (Solomon and others 1994).

Latin American and mainly Brazilian *carne-de-sol* and *charque* are produced using similar procedures. However, they are quite different in terms of shelf life as the first one is lightly salted and dried. It is thus processed in small scale and manufactured where it is consumed whereas *charque* is produced

FIGURE 28.4 Biltong and droëwors.

in regions having an abundant supply of raw materials and is transported over the country. They are traditionally manufactured from bovine muscle although sheep and goat meats are suitable. *Charque* processing (Figure 28.5) consists of cutting meat into large thick (2–4 cm) flat pieces (called mantas), followed by wet-salting or brining (immersion of the pieces in a salted agitated brine). The salt solution ($NaCl + NaNO_2$) may also be injected into the muscles. After removal from the brine, the meat pieces are stacked into piles separated from each other by 1 mm-thick layers of coarse salt (dry-salting). After 1 or 2 days, the meat is restacked and the uppermost meat pieces are repositioned to the bottom of the new piles. This maneuver is known as *tombo* and is repeated every 24 h (three to five times). After rinsing off excess salt from the meat surface, meat is dried directly in the sun (unless the weather is unfavorable) on wooden or stainless-steel rails for 4–5 days. At night, meat pieces are collected and piled on the concrete floor and are covered. Finally, *charque* is packed in sackcloth of jute or vacuum-packed in plastic bags and commercialized. Its salt content (12–15%) and a_w (0.87–0.91) allow storage at ambient temperature. Partially desalted *charque* is often used as an ingredient in a typical Brazilian food dish known as *feijoada* (with black beans). When the weather is cloudy, an alternative processing is winter pillage that consists of stacking meat pieces in piles up to 3 m in height immediately after the third tumbling and without washing. The piles are maintained in this state up to 4 months before being washed and dried in the sun according to procedures described earlier (Norman and Corte 1985; Pinto and others 2002; Shimokomaki and others 1998). *Carne-de-sol* processing procedures are very similar to those used for *charque*, however, there is only one step of dry-salting and the product is rarely exposed directly to the sun; instead, it is dehydrated in covered and well-ventilated areas. Being lightly salted (salt content 5–6%) and partially dehydrated (a_w 0.94), it should be stored at 10°C. Prior to cooking and consumption, *carne-do-sol* is desalted by soaking in cold water for 1–2 h. This makes it appealing as a fresh meat substitute (Norman and Corte 1985). *Tasajo* is a salted dried meat product made in Cuba as a version of *charque* (Andujar and others 2001; Chenoll and others 2007).

In Southeast Asia, salted dried meat products often have a sweet taste that differentiates them from other raw-cured meat in other parts of the world. Examples include *sou gan* in China, *dendeng* in Indonesia from beef, pork, or chicken and *bak kua* in Malaysia from beef or pork. They are made from thin sliced, cured (with sugar, salt, soy sauce, and spices), sun dried, and sometimes roasted or cooked meats. They have a high sugar content (>20%) (Chang and others 1996; Kuo and Ockerman 1985; Leistner 1985; Rao 1997). Some salted dried meats are also found in Myanmar, Pakistan, Philippines (*tapa*), Thailand, and India (Rao 1997).

Basturma (or *pastirma*) is a traditional intermediate moisture meat product highly esteemed, especially in the eastern Mediterranean countries (Egypt, Greece, and Turkey) and in Armenia. Whole semitendinous

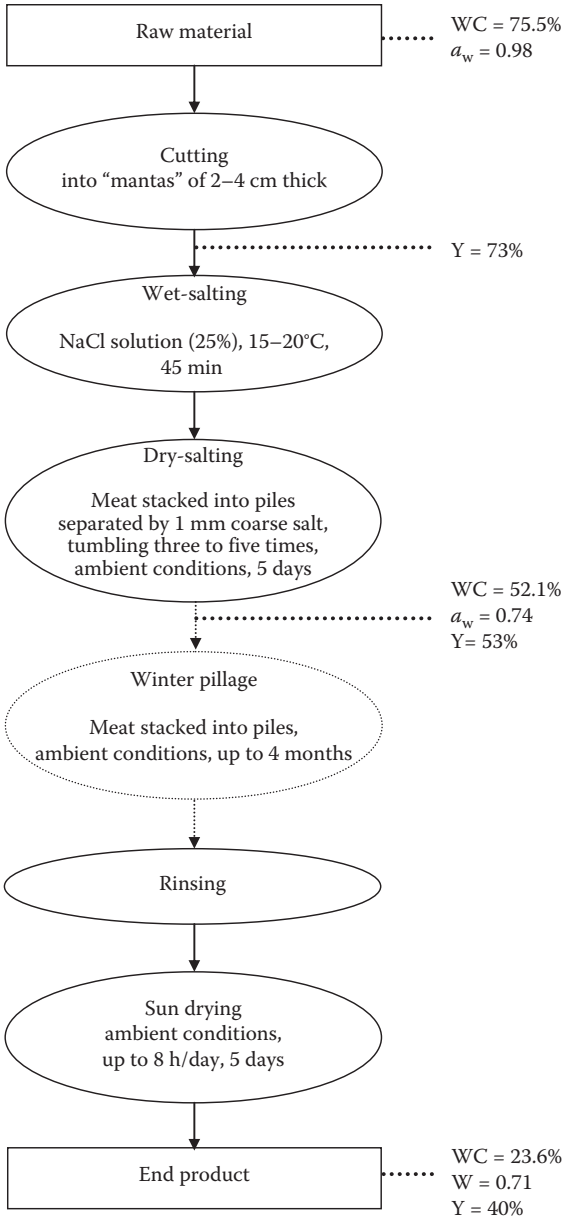

FIGURE 28.5 *Charque* making process (W, weight; WC, water content; Y, yield; the dotted line represents intermediate products; the dotted box represents optional step). (Adapted from Norman GA, Corte OO. 1985. Dried salted meats: Charque and carne-de-sol. FAO technical papers: 38pp; Torres EAFS, Shimokomaki M, Franco BDGM, Landgraf M. 1994. *Meat Science* 38:229–234.)

muscles of beef or buffalo are used and several incisions are made in each muscle at several points to facilitate salt penetration. Different procedures are described in the literature (El-Khateib 1997; Kaban 2009; Kotzekidou 1992). One example is described here. A mixture of 15 kg of coarse salt and 15 g potassium nitrate is used for salting 100 kg of meat. The salt mixture is inserted in the incisions and rubbed on the surface of the muscle. The salted meat is stacked in a stainless-steel vessel, where it remains for a few days at room temperature. Each piece of meat is then rinsed thoroughly with tap water to remove the excess salt and the pieces are pressed under a hydraulic press for 48 h. The pressed, salted meat can be cut

into smaller pieces that are again stacked and compressed for another 48 h. The individual pieces are then entirely covered with a thin layer of paste called *cemen*, consisting of freshly ground garlic, red paprika powder, and a mixture of wheat flour and *helba* (ground seeds of *Trigonella foenum graecum*). The pasted pieces are hung to dry at room temperature in a well-ventilated area for 4 days. The end-product is then packed with or without vacuum in transparent plastic bags (Kotzekidou 1992).

28.3.3 Smoked Dried Meats

Prior to smoke-drying, meat may be salted (either by dry or wet-salting) and sometimes cooked. The most known smoked meats in Africa are *kundi* and *banda* in Sahel African countries and *kitoza* in Madagascar. *Banda* is obtained by cutting meat from reject cattle into pieces boiled in water during 15–30 min and smoked during 18–30 h leading to further cooking and drying. The product is a very stable product with a shelf life of 6–12 months (Obanu 1988). *Kundi* is produced by smoking fresh beef, camel, or horse meat at high temperatures (165–180°C) in the Northern parts of Nigeria and is storable without refrigeration. Meat may also be salted before smoking (Alonge 1987). *Kitoza* processing consists of cutting beef or pork meat into strips, salting (either dry or wet), and hung over a wooden fire for 2–3 days (Laurent 1981).

Bacon is a famous cured and smoked meat product originating from the Wiltshire country (UK). The traditional Wiltshire process involves curing an entire side of a trimmed half pork carcass. The meat is first injected then immersed in a curing brine ($NaCl + NaNO_3 + NaNO_2$) for 3–5 days at 5°C. Maturation involves drainage and drying of the bacon and equalization of the concentration of curing ingredients through the meat. Conditions in the maturation room are important; the temperature should be controlled at 4–5°C and the relative humidity (RH) at 82.5–85%. Maturation for 6–7 days is considered ideal, leading to "green" bacon. The majority of Wiltshire bacon is sold without further processing but a proportion is smoked. The first, "cold smoking" is performed at 32–38°C for 15–18 h, and the second "hot smoking" uses a temperature around 60°C for 2–4 h. Small quantities of Wiltshire bacon are dried without smoking. Drying involves holding bacon in a chamber at 30–33°C for 18 h. Bacon is finally vacuum packed and, where appropriate, sliced and transported to retail outlets (Varnam and Sutherlands 1995).

Boucané is a salted/dried/smoked pork product well known in La Réunion (French Island in Indian Ocean), traditionally produced in the mountainous and isolated parts of the island. Pork belly is cut manually into strips that are then salted, either by dry-salting or brining for 24 h. Some spices might be added during salting. The salted strips are hung over a fire in a chamber measuring 36 m^3 called "boucan" or in smoking metal drums. *Boucané* presents a water content of around 25% and a of a_w of 0.83. Prior to consumption, *boucané* is desalted and added to traditional stews (Poligné and others 2001).

28.3.4 Fermented/Ripened Meats

Fermentation is an additional step in the processing of certain types of salted/dried meats. There are still some debates about the definition of fermented meats. Some authors distinguish fermented meats (essentially comminuted meats like sausages) and ripened meats (essentially whole muscles like raw hams). In their mind, fermentation refers to the production of lactic acid by lactic acid bacteria and the term ripening refers to all chemical processes resulting in development of flavor and texture due to microorganisms or endogenous meat enzymes. Other authors think that both dry sausages and raw ham should be considered fermented meats because both show changes during production and storage as a result of microbial activity. Thus, it is common to call fermented meat products those in which enzymes have contributed to their final characteristics, be they truly fermented or not (Campbell-Platt and Cook 1995; Toldra 2007).

The most famous examples in this category of meat products are the dry hams and dry-fermented sausages. Fermented products are found in most part of the world although Europe is the major producer and consumer. They can be divided in two groups, based on the form of the meat:

1. Products made by using large, whole pieces of meat: dry-cured hams, made from pork, form a large part of this group but also products like *cecina*, *bresaola*, *grisons meat*, or *bindenfleisch*, *uzice beef prshta* (traditional Serbian dry-cured beef (Tomic and others 2007)), *dried duck*

fillets (Poma 1998). In some processes, the meat is cut into smaller strips but fermented as whole pieces. These products are found in hotter regions of the world where it is necessary to cut the meat into thin strips to achieve rapid drying before putrefaction can set in, for example, *gueddid* or *khaddid* and *jirge* (sun-dried fermented beef, mutton or goat in Africa (Obanu 1988)).

2. The second group is made from meat that has been chopped into small pieces. These are the *fermented sausages.*

Although the procedures for the manufacture of *dry*-cured hams are highly variable depending on traditions and the types of raw material, there are common stages to all of them. The salting is done at low temperature normally 5–10°C for 10–15 days. Further salt is usually added and this continues for another 20–50 days. Then, during the drying-maturation stage, the temperature is increased in stages over several weeks or months. Typically, they are increased from 13–18°C to around 30–35°C, or ambient temperatures in the later stages of drying. Smoking at 30–40°C can be done over a period of days or weeks with some of the hams. At the prevailing high-salt concentrations in the meat, coagulase-negative micrococci and staphylococci tend to dominate the microflora (Campbell-Platt and Cook 1995). Hams are produced in Europe (S*errano* and *Iberian* in Spain, *Parma* in Italy, *Bayonne* in France, *Fenalaar* in Norway, *Black forest* in Germany) and North America (*country hams*) with some production in South America and Asia (chinese *Jinhua ham*) (Toldra 2007).

Cecina de León is a salted dried and smoked beef manufactured almost exclusively in the province of León (Northwest of Spain). Biceps femoris or semimenbranosus beef muscles are covered with a mixture of salt and nitrite during 3 days at 3–4°C and a relative humidity of 85–90%. Then, they are washed to remove excess salt and held under the same conditions for another 30 days (postsalting stage). The meat is then placed in a smokehouse set at 12–15°C and is smoked 20 days. Then, it is dried for 40 days at 10–12°C and 75–80% RH. Finally, meat is aged for several weeks (a minimum of 60 days) (Garcia and others 1995). *Cecina de León* making process is summarized in Figure 28.6. In Mexico, *cecina* is a salted dried but nonsmoked beef product consumed to a large extent (Reyez-Cano and others 1994).

Bresaola (Italy) is produced using similar procedures except that it is not smoked. Drying is done in three steps (7 days at 20–22°C, 60–65% RH; 15 days at 15–16°C, 70–80% RH; 20 days more to reach 30% weight loss). Paleari and others (2000, 2002) prepared similar products with buffalo, deer, horse, wild boar, and goat meats.

A similar product is the *Grisons meat*, also called *bindenfleisch* or *buendnerfleisch*, well known in France and Switzerland. Fat and nerves are totally removed from the beef cuts, which are then let to rest for 1 week at refrigeration temperatures. This resting period allows meat maturation and improves its tenderness. Each piece is then carefully rubbed with a mixture of coarse salt, nitrate, and spices. The salted meat pieces are tightly laid in well-ventilated containers at 6–8°C for 2–4 weeks. Coarse salt is spread between the meat layers and renewed every 5 days. At the end of this ripening stage, the meat pieces are well rinsed and slowly dried for 1–4 months or more at 10–15°C and 75–80% RH (Frentz 1974).

Gueddid (Tunisia) or *kaddid* (Morocco) is produced by cutting bovine or sheep meat in little slices, then adding ingredients (salt, garlic, olive oil) followed by drying of the sample in the sun for 7–10 days (Bennani and others 1995; Essid and others 2007). It is considered as a fermented meat product (Ben Belgacem and others 2008; Essid and others 2007, 2009) even if the ripening time is shorter than thus observed for all the products described above.

In the same way, the results of Pinto and others (2002) on *charqui* (modification of the microflora during the processing, coagulase negative staphylococcus predominant in the finished product) suggest that *charqui* is a fermented meat product.

Manufacturing of fermented dry sausages consists of comminuting meat in a grinder and adding curing salts (NaCl and nitrates), carbohydrates, and seasonings to the ground meat. To direct fermentation, back-slopping, in which some meats from a previous successful fermentation is added to encourage establishment of the desired microflora, was originally used. Nowadays, many manufacturers use starter cultures for industrial products, while traditional ones are not inoculated, the fermentation relying on the indigenous microflora from the meat, brine ingredients, or the environment. The mixture is then stuffed into natural, synthetic, or collagen casings. The filled casings are left to ferment at controlled temperature (varying from 10°C to 24°C) and relative humidity (around 90%) for a period of 1–7 days. Semidry

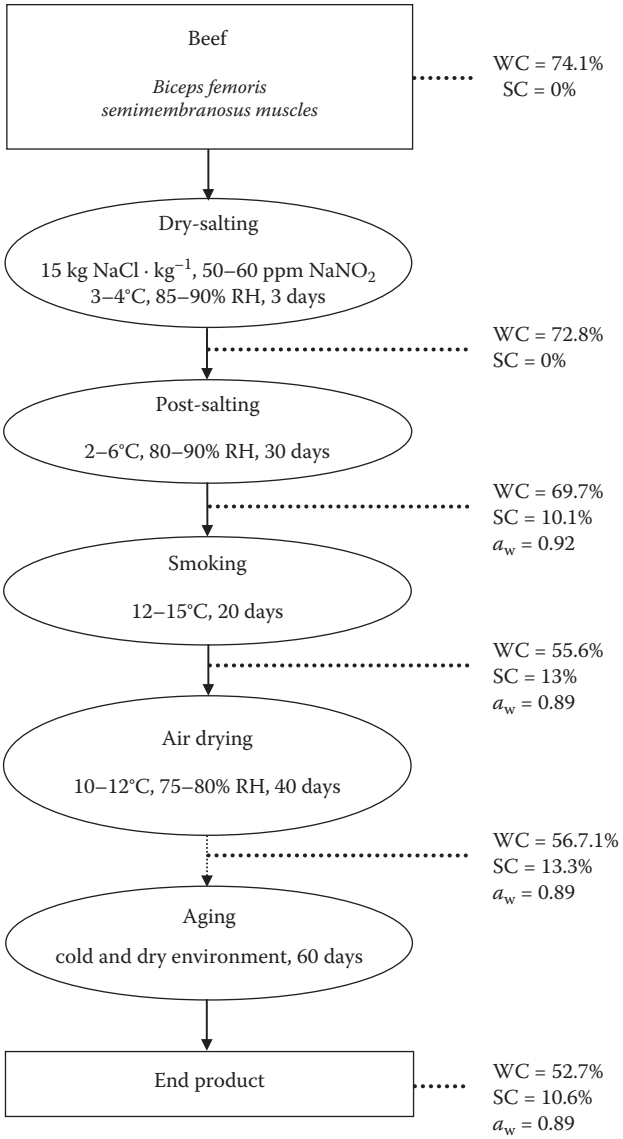

FIGURE 28.6 *Cecina de Leon* making process (SC, salt content; Y, yield; W, weight; WC, water content; the dotted line represents intermediate products; the dotted box represents optional step). (Adapted from Garcia I, Zumalacarregui JM, Diez V. 1995. *Food Microbiology* 12:309–315.)

sausages are fermented at higher temperature (30–40°C) for a few days. Then fermented sausages are dried. Conditions of drying vary in terms of temperature and time. The temperature ranges between 10°C and 20°C with progressively decreasing relative humidity (starting at 80%) (Lebert and others 2007; Ricke and others 2001). In the case of French *saucisson*, the drying lasts 4 weeks at 13°C and 77% RH (Durand 1999). Some sausages are smoked or cooked before being dried. This diversity of procedures leads to variable water contents. The water content of fully dried sausages is usually not more than 35%, which corresponds to a a_w of 0.85–0.90. Semidry sausages have water contents between 45% and 50% and a_w about 0.9–0.95 (Ricke and others 2001).

Fermented sausages from pork and beef have their longest tradition and are widespread in southern and central Europe. The most representative examples of dry sausages are *chorizo*, *pepperoni*, and *salamis*. In Italy, Spain (also called *salsichon*), and France (*saucisson*), *salamis* are made from pork while in

Germany they usually contain pork and beef. Turkish *soudjuk* or *sucuk* is made from beef meat and sheep fat and is traditionally manufactured without starter cultures by spontaneous fermentation (Kilic 2009). Many fermented sausages can be found in North America (semidry sausages *lebanon bologna* made from beef) and in Asia (*lap cheong*, *nham*) (Toldra and others 2007).

Fermented sausages are produced from many other meats: poultry (Santchurn and Collignan 2007), duck, mutton, lamb, camel, goat, ostrich and horse, buffalo, and game meats (Vural and Ozvural 2007).

28.4 Changes in Meat Quality during Drying and Storage

28.4.1 Changes in Meat Quality during Drying

Changes that occur in meat during drying are closely linked to the meat chemical nature and operations carried out prior to drying along with the processing conditions. Water loss leads to a decrease in a_w which in turn affects biochemical and enzymatic reactions occurring in the meat. High-temperature drying may be associated with a cooking effect while moderate-temperature drying would rather favor fermentative reactions.

28.4.1.1 Microbial Changes

Drying aims primarily at preserving meat and making it unfavorable to spoilage and pathogenic microorganisms. However, drying has a low lethal effect on microorganisms. Many of the microorganisms present in the original meat will therefore remain, albeit in a dormant state, in the dried product. The initial flora of fresh meat will change following (1) the pretreatments the meat is subjected to (size reduction, precooking, addition of salt, nitrites, spices), (2) the fall in a_w resulting from a decrease in water content, and (3) temperature of drying (hot drying or cold drying/ripening). For instance, any size reduction process like grinding or chopping will tend to increase and spread contamination to the bulk of meat. As drying proceeds, microbial proliferation slows down and eventually stops when the minimum a_w for growth is reached. Many dried meat products undergo a fermentation stage despite the fact that no starter culture is purposely added. On the other hand, starter cultures may be deliberately added to promote specific organoleptic properties. Microbial enzymes play an important role in the development of the acidic pH (fermentation of sugars into lactic acid), of the color and flavor (conversion of nitrate into nitrite) of cured meat products. Microorganism-mediated proteolysis gives rise to a range of compounds contributing to the flavor profile of fermented products (Molinero and others 2008).

28.4.1.2 Physicochemical Changes

As a result of water loss during drying, meat loses weight and shrinks. The dripping away of molten fat during hot-air drying may further reduce mass yield. Volume shrinkage of meat is accompanied by a wrinkled appearance and a hardened texture. Surface hardening or crusting is one of the main problems that may arise during drying of meat products. The crusted surface layer is harder and less permeable than the inner part of the product (Gou and others 2005).

Water loss automatically results in a concentration of dry solids. Fat, protein, and carbohydrate contents therefore increase. Other changes may occur in the meat pH, free amino acids, and free fatty acid contents (Rahman and others 2005).

28.4.1.3 Biochemical Changes

During hot-air drying, sarcoplasmic and myofibrillar proteins of meat denature, which is associated with a decrease in the water-holding capacity and rehydration capacity. The lower the air temperature during drying, the lesser will be the loss in water-holding capacity and the greater the degree of reconstitutability. At around 60–65°C, meat collagen starts its transformation into gelatin. This conversion does not occur appreciably below 100°C. However, if the meat has undergone precooking prior to drying, then the collagen will be transformed into gelatin, which on drying will form granules.

Aroma compounds including aliphatic hydrocarbons, aldehydes, and ketones are also produced as a result of lipid oxidation. Furans, sulfur, and nitrogen compounds are produced by Maillard-like reactions. Variations in drying methods and conditions may account for significant flavor differences between similar products (Zhang and others 2006).

In general, during drying, lean meat color changes from red to brown. The drying method may also affect the intensity of color change (Guizani and others 2008). In fact, meat color begins to change at 40°C, from raw meat color to an off-white gray to a brownish tint depending on the temperature, type of muscle and the sarcoplasmic hemoprotein behavior. Hemoproteins, which are key factors in color development, start to coagulate at around 65°C, precipitating with other muscle proteins. In the temperature range 45–63°C, the lightness of beef tends to increase. At higher temperatures, 67–79°C, beef internally turns dark brownish gray due to biochemical modifications in myoglobin and hemoglobin. Heat damage during drying is characterized by toughness, grittiness, and burnt flavor.

In contrast to hot air-drying, during low-temperature drying as is usually practiced for meat curing, in the range of 3–7°C, other biochemical reactions intervene to develop the characteristic organoleptic properties of the dry-cured product. The final product characteristics are in fact the net result of the combined effect of all processing steps including that of drying. Of major significance is the salting step. Salt interacts with meat proteins to form complexes that have higher water-binding capacity. Salt also solubilizes myofibrillar proteins that eventually form a gel matrix accounting for the texture of the product. In addition, salt has a darkening effect on meat color. Color and flavor may in fact be deliberately modified through addition of nitrites. The attractive red color and characteristic flavor of dry-cured meat result from the reaction between myoglobin and nitrites.

In normal postmortem circumstances, the meat enzymes, cathepsins, and calpains would be inactivated at the ultimate pH of meat. However, meat conditioning may lead to a rise in meat pH accounting for residual activity of these enzymes. Under near-ambient conditions, the first stage of air-drying may be accompanied by rapid biochemical and enzymatic-ripening processes. On the other hand, enzymatic activity remains negligible during artificial hot-air drying. In the case of drying of cured products, both meat and microbial enzymes break down meat components, essentially proteins and fats, to form a number of compounds that contribute to the flavor profile of the product.

28.4.2 Changes in Quality during Storage of Dried Meat

Biochemical, physicochemical, and microbiological changes may occur during storage of dried meat, the extent of which depends on a_w, pH, type of packaging, and storage air conditions (temperature and relative humidity). These alterations are mainly due to the activity of microorganisms surviving at low a_w, along with some enzymatic and chemical reactions like browning or oxidation.

Although most dried meats are shelf-stable with regard to food safety (due to lower a_w, pH), a proper packaging system is important for chemical and microbial shelf stability. Chemical deterioration in dried meats most often refers to lipid oxidation. Oxidation is usually started by the action of a catalyst: heat, light, or oxygen. Many different compounds form during oxidation, imparting a rancid flavor and odor in meat. However, microbial deterioration is usually due to mold growth on the surface.

Different packaging may be used to improve storage conditions. Generally, the products are packaged under vacuum or modified atmosphere. Vacuum packaging of dried meats removes oxygen, thus reducing rancidity. Packaging also prevents potential mold growth (that can lead to a rise in the pH, which may then allow growth of pathogens). Hermetic sealing in opaque, gas-impervious films (polyethylene, polypropylene, polyester) and/or storage in the dark prevent color, aroma, and other oxidative deteriorations. Various types of edible coatings, lipid based, polysaccharide based, or protein based, may also be used.

28.5 Conclusion

Drying remains an important step in the meat-processing sector, both in developing and developed countries, leading to a wide range of stable meat products. Meat drying is most often conducted on a small scale and is closely linked to the customs and traditions of specific regions or countries, more so

in the developing countries. Dried meat products form an important component in the diet of many populations. Some of them are even considered as specialty items in the gastronomy of both Western countries (e.g., Parma Hams, Grisons Meat, fermented sausage) and South countries (e.g., Brazilian charqui, South African biltong). In recent years, there has been a growing interest among the scientific community for traditional products including dried meat products. It is expected that this interest will translate, in the near future, not only into better understanding of the scientific principles underlying the processing of these products, but also into an enhancement of their overall quality, safety, and popularization.

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29

Thermal Technology

Isabel Guerrero-Legarreta and Raquel García-Barrientos

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29.1 Introduction

Heating is probably the cheapest and most efficient preservation method; its main objective is to ensure the destruction of most pathogens and spoilage microorganisms and to inactivate enzyme, in such a way as to prevent spoilage reactions and proliferation of undesirable microflora. Depending on the expected shelf life, a specific food is subjected to a set of conditions, of varying intensity. If a food is cooked, only partial elimination of spoilage microorganisms and enzymes takes place, but additional preservation methods are necessary; conversely, canning allows the destruction of virtually all microorganisms and their spores, able to proliferate and/or produce toxins, specially *Clostridium botulinum*, as well as spoilage microorganisms. The result of some thermal-processing methods, such as canning, is the production of self-stable foods, with a considerably long shelf-life, without the need of applying and other special storage conditions. However, thermal processing usually alters sensory and physical characteristics; in some cases, changes in physical structure are highly desirable, and are even part of the process itself, as is the case of luncheon meat where an emulsion system changes to a gel as a result of thermal processing.

Therefore, a compromise must be reached between food safety and the desirable, or minimum, sensory and physical properties alteration. When a thermal process is applied for the first time, it is calculated taking as basis the destruction of most harmful or the most abundant microorganism present, or the enzyme or enzymes that require to be inactivated to prevent spoilage for a reasonable long time. Usually, *C. botulinum* destruction is taken as a calculation basis, although other bacteria can be considered for meat processing, such as *Staphylococcus aureus* or *Streptococcus thermophilus*, depending if an uncooked or processed meat is processed.

29.2 Microorganisms in Meat

Microbial populations in foods are not static; as these are heterogeneous substrate, a given microbial population is established depending on nutrient availability. This is even more noticeable when a food material is processed and its chemical and physical characteristics change. In cured meats, considerable microbial reduction is obtained due to the action of nitrites and salt on a_w and pH. However, these products require further processing or refrigeration to extend their shelf life. Microbial contamination can occur during meat fabrication; the populations present can be *Enterobacteria*, *Clostridium* spp., some psychorphiles such as *Lactobacillus viridescens* (Jay 2000) and *Leuconostoc* (Makela and others 1992).

There is a specific association in refrigerated meats of nonfermentative Gram-negatives belonging to genera *Pseudomonas, Alcaligens, Flavobacterium, Shewella*, and *Moraxella*. The addition of salt, nitrates, and nitrites and phosphates during curing completely changes the microbial association to Gram positives, such as *Micrococcus, Lactobacillus*, *Carnobacterium*, and *Brochothrix* (Zamudio 2006).

Although the striated muscle of a healthy animal is considered as sterile, meat microbial contamination starts from a number of sources: water, skin, viscera, and workers' clothing and handling. It is mainly composed by yeasts, bacilli, and bacteria such as *Moraxella*, *Acinetobacter*, flavobacteria, Enterobacteriaceae, *E. coli*, *Salmonella* spp., *Shewanella putrefaciens*, and *Listeria* spp. (Zamudio 2006). After prolonged refrigerated storage, psychrophiles are the main responsible of meat spoilage, mainly by *Brochothrix thermosphacta*, *Carnobacterium* spp., *Lactobacillus* spp., *Leuconostoc* spp., *Weissella* spp., *Pseudomonas* spp., *Acinetobacter*, and *Psychrobacter inmobilis* (Borch and others 1996; Zamudio 2006). In poorly refrigerated meats, *Clostridium perfringens* and Enterobacteriaceae are present (Ingram and Dainty 1971).

In general, no signs of deterioration become evident in meats contaminated with pathogens. There are few exceptions, such as contamination with proteoytic strains of *C. botulinum*, causing off-odors although not intense enough as to be taken as a pathogen indicator (Doods and Austin 1997). Some of the most important microorganisms causing food poisoning, involving meat and meat products are *Salmonella* spp., *Escherichia coli* O157:H7, *Yersinia enterocolítica*, *Campylobacter jejuni*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Clostridium perfringes*, *C. botulinum*, *Bacillus cereus,* and *Staphylococcus aureus* (Zamudio 2006).

29.3 Thermal Destruction of Microorganisms

It is calculated from the point of view of sanitation as well as extending food shelf life. Vegetative cells are destroyed at temperatures slightly higher than their maximum temperature to grow, whereas spores can survive much higher temperatures. Two principles must be taken into consideration:

1. All microbial cells and spores, able to grow and/or to produce toxins must be eliminated.
2. Spoilage microorganisms must be reduced to a safe lowest limit.

From the commercial point of view, a food is commercially sterile if it is free from *Bacillus stearothermophilus* or *C. perfringens.* Sporulated thermophiles are important only if a food will be stored at high temperatures. Thermal processes that eliminate *C. botulinum* and *C. sporogenes*

produce a thermostable food of long shelf life, without the need of other preservation method (Lawson and others 1994).

29.4 Heat Transfer Mechanisms

Process calculations consider vegetative cell destruction following the ratio:

$$\frac{dc}{dt} = kc$$

that is, cell concentration (dc) decreases with time (dt) in a direct proportion to viable cell concentration (c). This is a logarithmic destruction, decreasing one long cycle (for instance, 10^3–10^2) as time linearly increases. This calculation has the advantage of allowing death rate comparison among microbial populations, as all microorganisms follow a log destruction pattern (Guerrero-Legarreta 2006).

In all heat transfer operation, it is necessary to know the amount of heat to be transferred. This allows calculating the transport parameters in the system. As this is a dynamic process, heat flow is proportional to the driving force and inverse to the resistance to flow. The heat transfer mechanisms are conduction, convection, and radiation.

29.4.1 Conduction

Heat is transmitted within a body due to adjacent molecules. This is the transfer mechanisms occurring in solids; in solid pieces in brine in canned foods; or in pastes that become solid during heating, such as luncheon meats. Conduction follows the Fourier's law:

$$q = k\ (A\Delta T / L)$$

where:

A—area

ΔT—temperature difference

L—material thickness

k—thermal conductivity coefficient: in meats: $k = 1.464$ kg cal/h m^2 °C (Stiebing 1992); in stainless steel: $k = 45.872$ kg cal/h m^2 °C (Green and Maloney 1997).

29.4.2 Convection

It takes place in fluids, is due to different densities moving during heating or cooling. It is the mechanisms occurring in syrups, sausages, and brines. Heat propagation is faster if an external force is applied, such as stirring, reducing temperature difference to a minimum. It is based on Newton's law:

$$q = h\, A\Delta T$$

where:

A—area

ΔT—temperature difference

h—depends on flow properties, surface and flow rate of the heating medium, for example (Himmelblau 1997): boiling water: $h = 1464$ to 19,520 kg cal/h m^2 °C; air: $h = 2.44$ to 24.4 kg cal/h m^2 °C.

In food canning, heating by this mechanisms occurs from a heating medium (water or steam) through a barrier (the can) to a cold fluid within the can. Heat penetration in cans depends on the transfer mechanisms within the food. Very viscous or solid foods are heated by conduction, a slower mechanism; this mechanism occurs in intact tissues such as large meat pieces. Low viscous foods or containing finely chopped meat particles, such as instant soups, have higher heat penetration coefficients due that the leading mechanisms is convection; the mechanisms can be accelerated if the can is rotated or stirred, as is the case of continuous retorts.

29.4.3 Radiation

Heat is transferred by electromagnetic waves from a body and absorbed to another body. Infrared heating ($\lambda = 0.8$ a 400 μm) is used sometimes in foods, waves absorb by the food and transform into heat. Microwaves ($\lambda = 1$ mm to 1 m) are also an application of radiation to food heating. It is based on the alignment of water molecules when exposed to an electric field that changes its direction at around 5×10^9 times per second, the moving molecules cause friction and produce heat. Microwaves can inactivate enzymes and denature proteins; therefore, microbial destruction can be achieved by this means (Guerrero-Legarreta 2006). However, radiation is not common in food processing.

29.5 Thermal Technologies

Heat transfer is a phenomenon directed by energy interchange; as a material is heated, molecules move faster; the kinetic energy increases the absorbed caloric energy. Heat capacity (C_p, p means that it occurs at constant pressure) defines the amount of caloric energy necessary to increase the temperature to a given mass unit. As mentioned before, application of a given thermal treatment intensity depends on the required shelf life. It can be as mild as cooking where only few vegetative cells and enzymes are inactivated, to sterilization where practically all the microorganisms are destroyed. Canning, a treatment at intermediate severity, can range from semipreserves to tropical preserves with shelf life from 6 months at <5°C up to 1 year at 40°C, respectively (Guerrero-Legarreta 2001). Mild heat treatments, however, need an extra preservation method, such as refrigeration for cooked meats to prevent proliferation of spoilage microorganisms and pathogens as well as toxin production, in some cases. Eventhough, heat transfer principles are the same for all thermal technologies, although each one has different aim. Depending on the treatment severity, they can be divided into scalding, cooking, pasteurization, and sterilization (Hanson 1990).

29.5.1 Scalding

It is mainly applied at temperatures around 65°C to tissues before freezing, drying, or canning. If applied before freezing or drying, the main objective is to inactivate enzymes in order to prevent changes in color, flavor, and nutritional value due to enzyme activity. Meat scalding before removes gas trapped in the muscle fibers and favors vacuum formation during canning or vacuum packaging.

29.5.2 Cooking

The primary objective is to improve the sensory quality. It can be considered as a preservation method as cooked meats have a longer shelf life than raw meats. During this process, two main preservation mechanisms occur: destruction of microorganisms and enzyme inactivation, although other changes also take place altering color, texture, and flavor, as well as an improvement in meat digestibility. The term "cooking" includes five operations, depending on the method heat is applied: oven cooking, roasting, grilling, frying, boiling, and steaming. In oven cooking, grilling and roasting temperatures around 100°C are applied as dry heat, whereas boiling and steaming uses boiling water; frying implies using boiling oil at >200°C.

29.5.3 Pasteurization

Although it is process commonly applied to fluids in the food industry, solids can be also subjected to this process. It destroys pathogenic vegetative cells, but spores and heat resistant microorganisms can survive, therefore, this method is applied to meat and meat products where another preservation method will be used, in such a way that the combine methods, or hurdles, that minimize surviving microbial cell loads. The methods used together with pasteurization are refrigeration, chemical additive addition such as nitrates, vacuum packaging, and fermentation.

Time-temperature ratio depends on the food heat resistance and its sensitivity to heating. Sausage pasteurization mainly consists in heating the product up to 60°C at the geometric center. It is used to reduce microbial populations, in order to stop the lactic fermentation in fermented sausages once lactic acid reaches a given concentration; in these sausages, pasteurization time depends on sausage diameter, the smaller the diameter, the less the necessary pasteurization time.

29.5.4 Sterilization

In a sterile product, no viable microorganism is present. However, the term "sterile" in not suitable for food processing, as the sterility criterion for food is the growth inhibition of vegetative cells and spores in normal storage conditions. Therefore, in foods, the term used is "commercial sterility," "bacteriologically inactive," or "partially sterile." These foods have a considerably extended shelf life without any other preservation method. Usually *C. botulinum* and *Clostridium sporogens* are used as indicators for meats, and process calculations are based on the destruction of these bacteria.

29.6 Process Calculations

In order to establish the thermal process severity, calculations require two types of basic information: (1) heat resistance of a given microorganism taken as process indicator, usually *C. botulinum* or *C. perfringens*; and (2) previous handling of the products: for meats, postmortem handling, sanitation, etc. It is also necessary to define the expected shelf life of the product and thermal sensitivity of the product (possible alterations in color, flavor, odor, texture, etc.). Several inactivation parameters are usually calculated:

D-value: It is the time interval, at a given temperature, necessary to destroy 90% of a given microbial population. This value varies among microorganisms, and allows comparing thermal destruction among populations. For instance:

At 110°C, 90% *C. sporogenes* population (10^5–10^4) is reduced if heating is kept for 10 min ($D_{110°C} = 10$ min)

At 115°C, the necessary time for the same reduction is 3 min ($D_{115°C} = 3$ min)

At 120°C, only 1 min is necessary ($D_{120°C} = 1$ min)

On the other hand, at different heating times, the destroyed population increases with time of exposure.

Z-values: It is the temperature necessary for reducing *D* values in 1/10. For example: if $Z = 10$°C; *D*-value at 100°C is 50 min; at 110°C is 5 min; at 120°C is 0.5 min.

F-values: It is a calculation of the overall process efficiency. Total *F*-value of a given process is the sum of lethality efficiency of every part of the process (heating, holding, cooling). *F*-values are used to compare severity of different thermal treatments, according to microbial resistance to process conditions. *F* and *D* values are related by the following equation:

$$F = D(\log a - \log b)$$

where

a—initial cell load

b—final cell load

Process lethality is calculated by the equation: $\log (t/F) = (250 - T)/Z$. In fact, total process lethality is the sum of the lethality calculated for every minute of the entire process, resulting in a process curve, the area under the curve represent total lethality. Low acidity foods, such as meats, are heated at temperature that ensure reduction of *C. botulinum* spores in 12 log cycles, or 12D, with a final contamination of one *C. botulinum* spore per g, an extremely low probability of finding a spore, or $1/10^{12}$. In order to reach this reduction, heating must be 12 times more severe than $D_{121} = 0.2$ min, therefore, the meat must be processes for 2.52 min at 120°C. The process lethality is $F = 2.5$, known as botulinum cook.

29.7 Heat Penetration in Meats

Heat penetration in any food depends on factors such as structure, composition, and physicochemical characteristics; storage conditions following a thermal process; heat transfer properties of the package or container; and the heating medium. However, thermal properties of the food itself such as thermal conductivity and specific heat, and physical properties such as density and geometry, lead heat distribution within the product. Heat conductivity is the thermal flow rate throughout the product and depends on food composition. Average thermal conductivity of meats is 1.89 kJ/h m °K (Stiebing 1992).

In meat products, as in most foods, heat and mass transfer take place as the same time. This double operation have effect on the biological and biochemical characteristics of the products, such as microbial and enzyme inactivation due to protein unfolding and, therefore, microbial cells becoming unable to reproduce and proteins losing their activity, in the case of enzymes. When a meat product is cooked in an oven, the following mechanisms take place: heat is transferred from the heating medium to the food; heat is transferred within the food, as a transient state; mass is transferred within the food; water. (Mass is transferred from the food to the heating medium.)

High heat transfer coefficient means high heat transfer rate by convection mechanisms from the heating medium to the meat surface. Two simultaneous phenomena occur during cooking: (1) heat convection from the medium to the meat surface, and (2) conduction from the surface toward the inner part of the product (Thumel 1995). If the heat transfer coefficient to the product surface is small, such as in gas free convection, the limiting factor during cooking is the convection from the heating medium to the product surface; but the coefficient is high, such is condensing steam, the limiting factor is conduction within the product. In the first case, the product can be undercooked in the inside and the microbial and enzyme inactivation is deficient. In the second case, the product can be overcooked in the surface, but undercooked in the inside due to extreme dryness in the surface, preventing homogenous drying. In both cases, adequate sanitation is not achieved.

In other products, due to their spatial changes and hydrodynamic interactions between meat components and additives, if any, structure also changes. This is the case of luncheon meats or pâtés, emulsion-like products. In these products, the continuous phase is made of muscle fibers, connective tissue fractions, carbohydrates, and other additives; the disperse phase is made of fat droplets of more than 100 μm in diameter; the high-fat content allows the spreadability of the product (Guerrero-Legarreta 2010). The products change their aggregation state during thermal processing from semifluid to solid, turing an emulsion to a gel by protein unfolding and interlinking during heating. As convection occurs in fluids whereas conduction is a heat mechanism for solids, convection changes to conduction (Xiong and Blanshard 1994). In a meat emulsion, thermal conductivity increases with process temperature and moisture content; thermal properties change as phase melts, proteins denature, and water evaporates. As the transfer mechanisms changes from convection to conduction during gelling of a meat emulsion, heating rate also varies (Valiente 1997).

29.8 Thermal Technologies in the Meat Industry

Most meat are processed by forced convection in batch or continuous ovens oven; the most widely used equipment for this type of process is the smokehouse, where cooking and smoking are applied at the same time. In batch operations, meat (sausages, hams, ribs, bacons, etc.) is loaded into the smokehouse; the smallest smokehouses can processes 180 kg at a time, the largest up to 25,000 kg. Continuous smokehouses are fed with automatic conveyors, taking the meat through various heating and cooling zones (Hanson 1990). These equipments process 2200–5500 kg/h sausages or up to 10,000 kg/h hams, bacons, eviscerated turkey, and so on. Oven processing cooks meats by media such as free or forced convection of hot air and water, or steam, depending on the necessary heat transfer coefficient (2.5 kcal/h m^2 °K for free convecting gases to 15,000 kcal/h m^2 °K for condensing steam) (Green and Maloney 1997).

29.9 Factors Protecting Microflora in Meats during Thermal Processing

There is a close relationship between the type of microorganisms present in a food and its physicochemical characteristics that can increase the resistance to heat processing and, therefore, processes intensity must be increased in order to achieve the desired microbial destruction. Process efficiency depends on pH, water activity, food composition (fat, protein, and carbohydrate composition), and structure, among others.

29.9.1 pH

Low acid foods (pH > 4.5), such as meats, need more intense heat treatment due to the possible pathogen presence; in fact, pH 4.5 is for *C. botulinum* growth and toxin production.

29.9.2 Water Activity

Generally, dry products do not require heat treatment. Aw limiting value for *C. botulinum* grows is 0.97 for psychrotroph species and 0.95 for mesophile species.

29.9.3 Redox Potential

Some pathogens, such as *Staphylococcus* spp. can grow in low oxygen concentrations. Raw meat has –50 mV redox potential, reduced when meat is processed through heating. Vacuum packaging and reducing agents, such as ascorbates, decrease redox potential even further.

29.9.4 Food Composition

Carbohydrates, fats, and proteins have low heat capacity, therefore, are poor thermal conductors and project microorganisms against thermal processing. Muscle fiber direction also affect conductivity: in a perpendicular caloric flow applied to 78.5% moisture content beef at 0°C, thermal conductivity is 1.351 kg cal/h m^2 °C, whereas at the same condition with parallel flow, thermal conductivity is 1.385 kg cal/h m^2 °C (Pérez and Calvelo 1984).

29.9.5 Physical Structure

Although muscles from healthy animals are considered as sterile in the inner part, microbial contamination starts in the surface due to handling, contact with utensils, workers clothes, water, etc. Microorganisms colonize the inner muscle layers by following the perimysial layers. Therefore, physical structures are a barrier and a means for microbial colonization.

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30

Meat-Curing Technology

J. M. Martin

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30.1 Introduction

The origin of meat curing can be traced, back to the third century BC, when Cato recorded careful instructions for the dry curing of hams. From this historical perspective, meat curing may be defined as the addition of salt to meats for the purpose of preservation. This allowed for carryover of meat from time of plenty to times of scarcity. As the centuries passed, production of salted meats flourished. It is

probable that during this time humans discovered, quite by accident, the effects on meat color of saltpeter (nitrate) as a result of its presence as an impurity in salt. Therefore, a more modem definition of meat curing could be the addition of salt and nitrate/nitrite to meat that produces the color and flavor we associate with cured meats.

The first dry cured meat products were probably inferior by today's standards. Scientific principles of curing meats were not applied until the later part of the nineteenth century when the growing meat-packaging industry began to search for ways to improve the quality. With the successful advent of refrigeration, curing of meats evolved from being a means of preserving meat to a fantastically complex and diverse segment of the meat industry, which should continue to provide a unique and diversified variety in the food supply.

30.2 Principles

30.2.1 Meat-Curing Ingredients

30.2.1.1 Salt

Salt is the most basic ingredient in all curing brines and dry mixes. Without the inclusion of salt, the curing process would be impossible. This ingredient provides one of the basic flavors and is essential in solubilizing muscle proteins.

Granulated or grain salt was commonly called "corn" (from the old Norse korn meaning "grain"), thus the term corned beef (Townsend and Olson 1987). Due to its undesirably harsh flavor, salt is often used in conjunction with various sweeteners to provide a milder flavor. Additionally, salt improves yields and influences textural characteristics (Shackelford 1989). Food grade salt that is of the highest purity should be used in meat-curing practices. Impurities such as metals (copper, iron, and chromium) accelerate the development of lipid oxidation and concomitant rancidity in cured meats. Although salt may be of very high purity, it nonetheless contributes to meat lipid oxidation (Andersen and Skibsted 1991). Nitrite and phosphates, which will be discussed later, help retard this effect.

The amount of salt used in brines and dry mixtures can vary considerably. Usually, the level is self-limiting. Extreme levels of salt makes the product too salty, and too little salt can result in inadequate protein extraction. Most curing brines range from 35° to 85° salometer. Brine strength is checked by use of the salometer, a hydrometer that is graduated to show the extent of saturation of brines (Townsend and Olson 1987).

30.2.1.2 Sweeteners

There are several types of sugars that may be used in formulating curing brines and dry mixtures. Some examples of commonly used sugars are sucrose, dextrose, and corn syrup. These constitute the most commonly used sweeteners by the meat industry today. Sweeteners function to counteract the harshness of salt, and provide roundness and enrichment of flavor. Sucrose also functions as a preservative, but the levels required to provide this effect would probably render most cured meats too sweet.

The types and usage levels usually depend on the product. Dextrose is about 70% as intense as sucrose, whereas most corn syrups are about 42% as sweet as sugar. Corn syrup was formerly restricted to 2% solids on a dry weight basis of the product. However, its usage is now unrestricted and, as with other sweeteners, its usage is typically self-limiting. Generally, economics play some role in determining which type of sweetener to use. The degree of browning imparted to the finished product should also be a consideration. The Maillard browning reaction may become too pronounced with some sugars and excessive carmalization and burned flavors are the result.

It is a common industry practice to purchase liquid dextrose and corn syrup in bulk quantities. This allows the respective sweetener to be pumped directly to brine formulation stations or instantly into mixers. This eliminates the need for valuable warehouse space to store bulk quantities of bagged dry product.

30.2.1.3 Nitrate and Nitrite

Through attempts to preserve and extend the available meat supply, early humans used salt as a means of preservation. Nitrate impurities in salt used for this purpose led to the observation of reddish pink colors in meat (Binkerd and Kolari 1975). This observation lead to the direct addition of nitrate to preserved meat in order to maintain a uniform color. During the last century, research demonstrated that nitrate that is added to meats is reduced to nitrite through bacterial degradation. Hence, it was deduced that nitrite was responsible for the fixation of cured meat color through its reaction with the water-soluble proteins myoglobin and hemoglobin (Sofos 1981). The Bureau of Animal Industry of the United States Department of Agriculture permitted the official use of nitrate in meat curing in 1908. Additional research resulted in the USDA allowing the direct addition of nitrite to meat products as part of the curing recipe (Kerr et al. 1926). The properties nitrite contributes to cured meat are indeed unique. The original function of nitrite in meat curing was the production of the cured meat color. In addition to color, nitrite works as an antibacterial agent, it retards the progression of oxidative rancidity, and it has a profound influence on the flavor of cured meats. No other known chemical additive can accomplish these things to preserve cured meat products. There are no current usable substitutes for nitrite as a preservative (Bailey and Swain 1973).

30.2.2 Chemistry of Nitrite

Sodium nitrite is the salt of a relatively weak acid and a strong base. It is a pale yellow crystalline substance that is readily water soluble. Aqueous solutions are highly ionized and slightly alkaline. The nitrite ion is considered to be a highly reactive ion and can serve as both a reducing and an oxidizing agent (Price and Schweigert 1987).

The word nitrite is used generically to signify both the anion NO_2^-, and the neutral nitrous acid HNO_2, but it is the latter which forms nitrosating compounds (Sebranek and Fox 1985). Very low nitrous acid concentrations can generate extremely reactive nitrosating species (Price and Schweigert 1987). The initial step in the reaction sequence beginning with nitrous acid is the generation of a nitrosating species of the neutral radical nitrous oxide (N_2O). The strongest nitrosating species are a form of a positively charged (electrophillic) nitrogen oxide, either in its simplest form as the nitrosonium ion (NO^+) or as part of a larger molecule. In a strong acid, two species are formed, the nitrous acidium ($H_2NO_2^+$) and NO^+ ions.

$$HNO_2 + H^+ \rightarrow H_2NO_2^+ \rightarrow H_2O + NO^+$$

In biological systems, the Van Slyke reaction is a classic example in which gaseous nitrogen can be liberated from the α-hydroxyl acid (Sebranek and Fox 1985):

$$RCHNH_2COOH + HONO \rightarrow RCHOHCOOH + N_2 + H_2O$$

This reaction is used in the estimation of the amount of free α-amino groups in biological systems. In meat curing, some of the added nitrite may disappear as a result of this reaction. When cured meats are placed in highly acidic solutions, nitrite disappears rapidly. The Van Slyke reaction appears to be the major pathway for its depletion (Price and Schweigert 1987).

Another important form of nitrite is nitric oxide (NO). The nitrogen is reduced by one electron from nitrite–nitrogen and may be formed from dismutation or reducing reactions. NO is an electron-pair donor and forms very stable complexes with the transition metals. The coordinate-covalent complex of NO with the heme pigments of meat (nitrosylmyoglobin (MbNO), nitrosylhemoglobin, and dinitrosylhemochrome) form the pink and red color of cured meats (Fox 1966).

NO in the presence of air is rapidly oxidized. This accounts, at least partially, for the instability of cured meat color when exposed to air:

$$2NO + O_2 \rightarrow 2NO_2$$

The rapid speed of this reaction may also explain the antioxidant properties of nitrite because it is very fast. Furthermore, NO is a radical chain terminator (De la Mare and Vaughn 1957), which would explain

why it is so effective in retarding the development of lipid oxidation. Additionally, research has proposed three cooperative mechanisms for the antioxidant properties that nitrite imparts to cured meats: (1) the formation of MbNO, which has antioxidant properties; (2) on heating, MbNO forms a stable complex, nitrosylhemochrome, that blocks the catalytic activity of heme iron and also prevents the release of heme iron as nonheme iron, which is a very effective catalyst; and (3) nitrite appears to chelate nonheme iron, and possibly copper and cobalt, forming a stable complex, thus inhibiting catalytic activity (Morrissey and Tichivangana 1985). These findings are in agreement with Kanner et al. (1991), who reported that NO, liganded to ferrous complexes, acts to prevent the prooxidative reaction of these complexes with hydrogen peroxide to ultimately produce "warmed-over flavor."

30.2.2.1 Cured Meat Color

The color of cured meats is generally dependent on three factors: (1) the concentration of myoglobin in the muscle tissues, (2) the degree of conversion to the nitrosyl pigment, and (3) the state of the muscle proteins. Unlike fresh meats, cured meats retain their redness upon heating. It is inferred from this that the curing process changes the chemical nature of the chief muscle pigment myoglobin. Classical early work conducted by Haldane (1901) and Hoaglund (1914) suggested this difference in reaction of NO derivatives of the meat pigment toward heat is due to the formation of NO derivatives of the meat pigment as a result of the chemical interaction of the pigment with nitrite, either added directly in the curing brine or mixture, or obtained through microbial reduction of nitrate to nitrite (Urbain and Jensen 1940).

After the work of Haldane (1901) and Hoaglund (1914), the existence of a red pigment different from the blood pigment hemoglobin was demonstrated in muscle tissue by Kennedy and Whipple (1926). Haldane and Hoaglund assumed the pigment of meat to be identical with the blood pigment. Determination of amounts of muscle hemoglobin or myohemoglobin, by Shenk et al. (1934), occurring in the muscle tissue of different animals indicated that these pigments make up nearly all of the muscle tissue pigment. The slaughter process includes a bleeding period, which removes substantial portions of blood. Thus, it was rationalized that the pigments of cured meats are essentially NO myoglobin and nitrosyl-hemochrome.

Depending upon the type of meat product involved, there are several different methods used to cure meats. Usually they include

1. *Brine cure.* The meat is soaked in a solution containing salt and cure plus adjuncts. The period of cure is dictated by the size of the meat pieces, with adequate time being allocated to allow the complete diffusion of cure into the center of the meat. After this process, the meat pieces are usually cooked and smoked.
2. *Pump cure.* In this type of cure, multiple-needle pumping machines usually inject the brine solution throughout the piece of meat, thereby reducing the time required to fully distribute the curing solution into the meat pieces (2 pounds nitrite to 100 gallons pickle at 10% pump level "ingoing").
3. *Dry salt curing.* This is accomplished by rubbing the meat with a mixture of salt and cure and allowing sufficient time for the mixture to penetrate the product. Again, this is usually dependent on the size of the meat pieces. Smoking or other processing usually follows this type of curing.
4. *Cure for comminuted meats.* Coarse ground meat products that are to be cured involve the incorporation of the curing agents by direct mixing. Sodium nitrite may be included in coarse ground meats at levels up to 156 parts/million which equals approximately one-fourth ounce per 100 lb of meat.

All of these processes eventually lead to the formation of NO myoglobin. The general reaction is Myoglobin (purplish-red) + NO → Nitrosomyoglobin (dark red) + heat → Nitrosohemachrome (light pink, typical "cured meat color").

All of these reactions are nonreversible. Again, the source of the NO is nitrite. Nitrosomyoglobin is then the first pigment of cured meat. Upon the application of heat, it is converted into nitrosohemochrome, which is a denatured protein, and exhibits the characteristic pinkish-red color of cured meats.

30.2.2.2 Bacterial Inhibition

Nitrite addition to meat is responsible for the traditional and distinct color and flavor of products such as ham, bacon, bologna, and frankfurters (Sofos et al. 1979). An additional major benefit is the retardation of *Clostridium botulinum* growth and toxin production.

Botulism is a rare but serious neuroparalytic disease affecting humans and animals. It is caused by ingestion of a heat-labile protein neurotoxin produced by the vegetative cells of the microorganism *C. botulinum* (Tompkin and Christiansen 1976). Botulism may also be caused by wound infection. The disease has been recognized as a foodbome illness for more than 1000 years (Dolman 1964).

The incidence of the disease is worldwide. It can result from the consumption of a variety of foods, including canned meats, vegetables, fruits, and fish. Botulism was first reported in Europe as a disease caused by the consumption of sausage products. The word "botulism" was derived from the Latin botulus, meaning sausage (Sofos et al. 1979). Current research efforts have suggested possible inhibitory mechanism(s) by which nitrite inhibits *C. botulinum*. These include (a) formation of an inhibitory substance from nitrite and other meat components, (b) nitrite or intermediates acting as an oxidant or reductant on intracellular enzymes or nucleic acids, (c) restriction of iron or other metals essential to *C. botulinum* by nitrite, thereby interfering with the organism's metabolism or biological repair system, and (d) reaction of nitrite with cell membranes to limit metabolic exchanges or substrate transport. It is quite possible that more than one mechanism exists in a complex biological system such as meat (Sofos et al. 1979). Metal-sequestering agents such as ethylenediaminetetraacetic acid enhance the inhibitory activity of nitrite whereas excess iron caused a decreased inhibition (Tompkin 1978). Research has suggested that nitrite, probably via NO, reacts with the iron of ferredoxin in germinated cells (Woods et al. 1981). Inhibition of *Clostridium sporogenes* by reaction of NO with the nonheme iron of pyruvate: ferredoxin has been reported (Reddy et al. 1983). Another theory is the conversion of extracellular iron, which is essential to *C. botulinum*, to an unavailable form after reaction with NO (Tompkin 1978).

30.2.2.3 Flavor

Nitrite is a major contributor toward the development of the characteristic cured meat flavor (McDougall et al. 1975). This function of nitrite in cured meats has not been linked to any specific flavor components (Sebranek and Cassens 1973). Sodium nitrite alone is a very potent flavor enhancer in cured meat (Wasserman and Talley 1972). Salt is a catalyst for increased fat oxidation and rancidity (Rhee et al. 1983). Claims have been made that salt is the major factor responsible for cured meat flavor, rather than nitrite or the absence of oxidative rancidity. It should be noted, however, that salt pork and bacon have quite different flavors, suggesting a positive role of nitrite in flavor formation.

Bailey and Swain (1973) reviewed a very limited literature base and reported on the interaction of nitrite and meat constituents that influence flavor. Most studies that have reported on the effect of nitrite on meat flavors are related to sensory differences due to processing, or pertain to chemical reactions between nitrite and meat components (Sofos et al. 1979). Wasserman (1974) presented a review of these studies, but the lack of sufficient information prevented a complete discussion of the role of nitrite on cured flavor development. Cho and Bratzler (1970) and Wasserman and Talley (1972) reported that nitrite-containing products (longissimus dorsi muscle and frankfurters, respectively) possessed a more intense cured flavor. Improved color and sensory properties were reported by Kemp et al. (1975) in packages of sliced dry-cured hams containing nitrate and/or nitrite. Hustad et al. (1973) reported that as little as 25–50 μg of sodium nitrite per gram was adequate to give the typical color and flavor of cured meats in wieners.

30.2.2.4 Lipid Oxidation

Research on rancidity retardation by nitrite has been presented by Cross and Ziegler (1965), Watts (1954), and Herring (1973). The effect of nitrite on retarding the development of rancidity is probably due to the same reaction that is responsible for color development. The heme compounds of muscle contain iron ions that catalyze lipid oxidation reactions. When nitrite reacts to form cured pigments, iron is retained in the heme, usually in the reduced (Fe^{2+}) form, making it unavailable as a catalyst for lipid oxidation. This reaction therefore probably accounts for the prevention of "warmed-over" flavor in cured meats (Price and Schweigert 1987).

30.2.2.5 Nitrite Intake and Nitrosamines

Concern over nitrate and nitrite intake and its effect on human health dates back to the beginning of the twentieth century. Richardson (1907) stated that most of the nitrite ingested was from vegetables. Nitrite is considered toxic at high concentrations, and it is also implicated in carcinogenic nitrosamine formation. Under current regulations, nitrite is not considered a health hazard. Rare toxic episodes have been reported, mostly due to accidental overuse. The lethal nitrite dose is 300 mg/kg of body weight (Tannenbaum 1976). Nitrite can react as a vasodilator and hypotensive agent (Rubin et al. 1963). It can reduce the storage of vitamin A in the liver and it can interfere with thyroid function (Emerick et al. 1963). It is firmly established that nitrite can oxidize hemoglobin to methemoglobin, lowering the blood's ability to transport oxygen. This anomaly is called methemoglobinemia. It can be fatal and most commonly occurs in infants.

The FDA-USDA announced on February 5, 1972, that nitrosopyrrolidine, a nitrosamine, was formed in retail purchased and fried bacon. The fried product contained levels of this nitrosamine ranging from 30 to 106 parts/billion (ppb). Conversely, raw bacon was found to be free of nitrosopyrrolidine (Herring 1973). Many different nitrosamines have been found to be carcinogenic (Magee and Barnes 1967). Nitrosamines can be produced by a combination reaction of nitrite or nitrous acid and secondary or tertiary amines (Sebranek and Cassens 1973). Cured meats contain both nitrite and amines, thus there is potential for nitrosamines formation under appropriate reaction conditions.

Fried bacon is the cured meat product in which nitrosamines (mostly nitrosopyrrolidine) have been found. Bacon is high in the amino acids proline and hydroxyproline, and other nitrosopyrrolidine precursors. The most likely pathway for nitrosopyrrolidine formation is that proline is first nitrosated and then decarboxylated (Fiddler et al. 1974). In other cured meat products, nitrosamines have not been found or are only occasionally encountered. Researchers have detected nitrosopyrrolidine in bacon but not in other cured meat products. Hustad et al. (1973) tested frankfurter samples for 14 nitrosamines at the 10 ppb level and no positive results were obtained. Under controlled laboratory and normal heating conditions, no nitrosamines were formed in frankfurters formulated with nitrite levels up to 750 μg/g. A nitrite concentration of 1500 μg/g was necessary for dimethylnitrosamine formation at levels of 10–11 ppb. Research concluded that frankfurters conforming to U.S. federal regulations contained insufficient nitrite for nitrosamine formation (Fiddler et al. 1972).

Based upon the occurrence of nitrosamines, cured meat products can generally be divided into two groups. Bacon (mandatory ingoing nitrite at 120 parts/million), and ham, wieners, bologna, and similar products, which are less involved with nitrosamines. In bacon, the nitrosopyrrolidine concentration is higher in the fat cookout than in the cooked edible portion (Fazio et al. 1973; Fiddler et al. 1974). Nitrosamine formation in bacon is affected by ingoing nitrite concentration, cooking conditions, and postprocessing age of bacon. Several substances (ascorbate, ascorbyl palmitate, cysteine, glutathione, hydroquinone, alpha-tocopherol, and tertiary butyhydroquinone) have been shown to decrease nitrosamine formation in cured meat products (Gray and Dugan 1975; Mirvish et al. 1972).

Cured meats contribute little to the total human intake of nitrate and nitrite. Rubin (1977) reported some human nitrite intake comes from cured meats, and the rest comes from saliva, where it is formed from nitrate by the microflora of the mouth. Some common leafy vegetables such as spinach and lettuce and some root vegetables such as beets and radish are major nitrate sources. Additionally, some drinking

waters are high in nitrate (White 1975). Furthermore, White (1975) indicated that 10% of the ingested nitrate and 21% of the nitrite comes from cured meats whereas vegetables contribute 86% of the nitrate and saliva 77% of the nitrite. Therefore, Friedman (1973) noted, "Any possible risk to human health which could conceivably arise from the use of nitrite in food processing must be balanced against its value as an essential ingredient in the production of a host of well known traditional food products" (p. 537).

30.2.3 Curing Adjuncts

In addition to the commonly accepted curing agents, the meat processor in the curing process commonly includes several other adjuncts (ascorbates and erythorbates, phosphates, starches, and hydrocolloids). Problems over the decades associated with color and yields have been the primary motivation for inclusion of these ingredients.

30.2.3.1 Ascorbates and Erythorbates

Ascorbic acid ($C_6H_8O_6$), termed vitamin C, a water-soluble vitamin, is often used as a curing accelerator. That is, it helps speed the conversion of nitrite to NO to hasten the development of cured color in rapidly processed cured meats. One part of ascorbic acid is equivalent to one part erythorbic acid. Bauernfeind et al. (1954) reported the benefits of using ascorbic acid in cured processed meats: (1) curing time can be substantially reduced, (2) a more uniform color will result throughout the product, and (3) better color and flavor can be maintained during storage, distribution, and display. Similar results were reported by Watts and Lehmann (1952) by demonstrating that the addition of ascorbic acid with nitrite causes rapid development of the cured meat pigment. Additionally, salt, low acidity levels, high temperature, and freezing accelerated color fixation (Hollenbeck and Monahan 1955). Mirvish et al. (1972) reported another possible benefit of using ascorbate: the blocking effect that the compound appeared to have on the formation of *N*-nitroso compounds. However, the blocking effect depended on the particular nitro compound and the experimental conditions.

The USDA, for curing purposes, approves the following forms of ascorbates: ascorbic acid, sodium ascorbate, erythorbic acid, and sodium erythorbate. Sodium erythorbate is the most widely used form of ascorbate, due to its lower cost.

Federal regulations permit the addition of 550 parts/million (ppm) of ascorbic acid or in cooked processed meat products after erythorbic acid; or the equivalent molar level of sodium ascorbate or sodium erythorbate in each 100 gallons of curing pickle. For pumping pickle, this is 75 or 87.5 ounces/100 gallons when the pickle will be used at 10% of green weight. If the pickle is to be used in excess of 10%, appropriate reductions in the quantity of such substances are required.

30.2.3.2 Phosphates

The use of alkaline phosphates in meat curing is widely employed by the meat industry. Although claims have been made with regard to color retention, the various phosphates used by the meat industry have been used primarily to decrease shrinkage and purge in processed meats.

Phosphates appear to present a mode of action that is twofold: (1) elevating the pH of the meat, and (2) solubilization of muscle proteins. Polyphosphates contribute to the ionic strength of meat fluids, thus increasing protein hydration without increasing the apparent saltiness of the product (American Meat Institute 1960). Bendall (1954) postulated that pyrophosphate is effective in splitting actomyosin into its component parts, thus aiding in the extraction of more salt-soluble protein.

At the isoelectric point of meat, pH 5–5.5, fluid retention is at a minimum. Thus, any additive that significantly elevates the meat pH should increase the fluid retention of isoelectric meat. Early research suggested that large increases in pH favor fluid retention in meat (Mahon 1961). However, the addition of 0.2–0.5% sodium tripolyphosphate to meat only increases the pH by about 0.1–0.3 units. It is doubtful that by itself, such small increases in pH account for the pronounced effect of certain phosphates on the fluid retention of processed meats. However, use of more alkaline phosphate

blends that are currently available may have a more pronounced effect on fluid retention in certain applications.

Bendall (1954) pointed to differences between the volume of ground meat treated with polyphosphates alone and meat treated with polyphosphates and salt. He suggested the meat volume was a function of total ionic strength since the pH of both meat samples was essentially the same. Mahon (1961) presented research that explained the important synergistic role sodium chloride and sodium tripolyphosphate collectively play in water binding in cured meat systems. His conclusions were (1) salt concentration and not pH adjustment is the key to maximum cured meat volume, (2) low salt concentrations of the order of 0.5% are detrimental to cured meat volume, (3) salt and tripolyphosphate act synergistically to increase cured meat volume, (4) if used alone, high volumes of tripolyphosphate are required to induce maximum cured meat volume (well above the maximum limit of 0.5%), and (5) as salt concentration decreases, tripolyphosphate concentration must increase accordingly if cured meat volume is to be maintained.

Phosphates are essentially the salt forms of phosphoric acid. There are two recognized classes of phosphates: (1) the ortho (simple) phosphates containing a single phosphate anion, and (2) the poly (condensed) phosphates containing two or more phosphate anions.

All phosphates perform three basic functions in foods: (1) they provide some degree of buffering capacity, (2) they sequester metal ions (an important antioxidant effect), and (3) they act as polyanions to increase the ionic strength of solutions (McCormick 1983). These chemical functions of phosphate perform important functions in cured meat systems. Their beneficial functions include increased yields, retardation of oxidative rancidity, and color stabilization.

The following phosphates are approved for use in curing pickles: disodium phosphate, monosodium phosphate, sodium metaphosphate, sodium polyphosphate glassy, sodium tripolyphosphate, sodium pyrophosphate, sodium acid pyrophosphate, sodium hexametaphosphate, dipotassium phosphate, monopotassium phosphate, potassium tripolyphosphate, and potassium pyrophosphate. Tripolyphosphate and its blends with hexa-metaphosphate are the most commonly used phosphates for cured meats because, in most cases, these exhibit the most beneficial combination of properties.

The use of certain approved phosphates presents specific challenges that the meat processor must be aware of sodium tripolyphosphate, perhaps the most widely used phosphate in meat curing, must be the first component added to the curing pickle. It is practically insoluble once salt has been added. Phosphates are generally corrosive in nature, thus mandating the use of stainless steel or approved plastic equipment in handling brines and pumped meat. Another situation sometimes encountered in using phosphates has been the appearance of white crystals on the surface of cured products after processing. Commonly called “snow” or “whiskers,” this condition most often appears on product that has been allowed to dry out in holding coolers. These crystals have been identified as disodium phosphate and are believed to be caused by hydrolysis of the polyphosphates by the naturally occurring phosphatases in meat. This problem can be minimized by reducing the level of phosphate in the cure (maximum level in the finished product is 0.5%), and providing proper protection of the product during cooler storage.

Recognizing the fact that meat does contain phosphatases has brought some debate over the merits of using phosphates in preblends. Research has shown that the addition of phosphate to preblends increased soluble protein extraction by 15–20% and cooked stability (retained water) by 25–30%. There was no loss of protein functionality with time, even though phosphate hydrolysis was occurring. Therefore, phosphates constitute an important part of meat preblends (Eilert and Mandigo 1996).

30.2.3.3 Starches and Hydrocolloids

Starch is very abundant in nature, serving as a storage carbohydrate in many plants. Therefore, a wide variety of carbohydrates are available to meat processors. The challenge to the processor is to figure out what type of product is to be manufactured, what quality characteristics it is to possess, and what very specific benefit a particular starch or combination of starches should contribute to the product.

Starch is a multifunctional ingredient manifesting properties that can be applied to numerous food products. Starches contribute texture enhancement (allows for thin slicing), binding properties (usually water), and improved mouth feel to meat products. All of the characteristics are dependent on the origin of the starch, and its performance capabilities.

In its native form, starch is present as discrete granules that are insoluble in cold water due to hydrogen bonding between individual starch molecules, or directly through water bridges. Chemically, starch is composed of two glucose polymers, amylose and amylopectin. The relative proportions of these two polymers will differ depending on the source of the starch. Native starches exhibit optical polarity and display a "Maltese cross" diffraction pattern when viewed under plane-polarized light. When starch granules are heated in the presence of sufficient water, the cross pattern fades as starch granule swelling is initiated. This phenomenon is termed as gelatinization.

The initial swelling takes place in the amorphous (noncrystalline) regions of the granule, disrupting hydrogen bonds and then hydrating them. The viscosity proceeds to a maximum, which corresponds to the largest hydrated volume of the granules, which is then its peak viscosity. As heating, agitation/shear continue, the swollen granules rupture and collapse, thereby giving a viscous, colloidal dispersion of hydrated starch aggregates and dissolved molecules.

The most common starches used by meat processors are extracted either from cereals (corn, wheat, and rice), or roots and tubers (potato, konjac, and tapioca). Each type of starch has inherent characteristics that can be utilized to produce specific textural and sensory properties in a cured meat system. The contrast in physical properties of starch pastes can be attributed to the chemical composition and physical differences that exist between the various starch sources.

Differences in raw material sources play a vital role on the flavor profiles and swelling characteristics of starches. Almost without exception, meat processors must use a starch that is absolutely bland in flavor but is also capable of binding water and holding it throughout the product's commercial distribution. Again, the specific role the starch plays in a food product will dictate what type of starch the processor chooses.

Starch manufacturers are able to offer starches that will be stable under various conditions of pH, shear, heat, and freeze-thaw cycles. These characteristics are imparted to starches by three important modifications: (1) pregelatinization, (2) cross-linking, and (3) stabilization. Pregelationization involves cooking/drying the starch in heated drums or spray dryers to make it cold water swellable. This eliminates the necessity of the processor to precook the starch to achieve viscosity. Cross-linking is a method by which the starch granules are strengthened by the introduction of chemical bridges that link the hydroxyl groups on two different starch polymer molecules. This bridging of starch molecules controls the swelling of the starch and imparts stability to the starch to undergo further processing in high or low pH mediums and high shear or temperature processes. Additionally, it has the effect of increasing the gelatinization temperature. Stabilization decreases the extent of gelatinization of starch and thus increases its water-holding capacity. Chemical groups such as acetyl or hydroxypropyl groups increase the polarity of the starch, making it ionic, or by means of stearic hindrance cause repulsion of the polymer chains. This opens up the granules to improve the clarity and lowers the gelation temperature and increases the water-holding capacity. The tendency to retrograde or breakdown is reduced with a concomitant improvement of freeze-thaw stability (BeMiller and Huber 2008).

Carrageenan is a hydrocolloid produced from certain types of red seaweed. This product is particularly useful in its ability to bind and hold water. There are three types of carrageenan and they are classified by their gelling characteristics. κ- and ι-carrageenans form gels, and are therefore most often used in meat processing. λ is a nongelling carrageenan and is not commonly used by meat processors. Carrageenans are mostly used at levels below 1% and need to be heated to become fully functional. κ will form a firm gel whereas ι will produce a more elastic gel (Lamkey 1998). Use of both κ and ι carrageenan in cured meat products provide enhanced functionality due to their different gelling characteristics.

Konjac flour is refined from the root of the elephant yam (Amorphophallus konjac). It has tremendous water-binding capabilities and is used in relatively low levels in processed meats. Konjac is unique in its ability to form a heat-stable gel when treated with an alkali to remove acetyl side groups (Lamkey 1998). Konjac also provides enhanced textural improvement in low fat/fat-free sausage products.

30.2.4 Added Substances

Federal regulations pertaining to pickle-cured pork products, including hams, shoulders, picnics, butts, and loins, mandate that they comply with the minimum meat protein fat free (PFF) percentage requirements. These standards and labeling requirements for cured pork products are based on the minimum percentage of meat protein on a fat-free basis present in the finished pork product. The percentage is calculated as follows: (% meat protein/100-fat% × 100). For example, if the meat protein is 16.8 and the fat is 12.2, the resulting PFF is 19.13. Labels are based on the style and type of product (e.g., bone-in ham), and the minimum PFF percentage determines how the product can be labeled. For instance, cooked bone-in ham must have a minimum protein fat-free percentage of 20.5%; cooked ham with natural juices, 18.5%; cooked ham, water added, 17.0%; and cooked ham and water product-"X% of weight is added ingredients," <17% PFF. Shoulders, picnics, and butts are allowed one half percentage less in PFF values for each category than is required for ham and loin items. This program allows for a broad range of cured pork products to be marketed if they meet the proper standards and are accurately labeled (Price and Schweigert 1987).

30.3 Applications

30.3.1 Methods of Curing

The meat industry employs several methods of meat curing. However, they are all modifications of two basic procedures: dry salt curing and pickle curing. Dry salt curing is no doubt the oldest method. Evolution of the curing art eventually led to the more modern method of pickle curing.

30.3.1.1 Dry Salt Curing

Dry salt curing is a method dating back to prehistoric times and was the first curing method practiced by humans. The process uses salt alone, or sometimes in conjunction with nitrite or nitrate. The moisture is drawn out of the meat by the curing agents and drains off, leaving the meats drier and harder and leaving the flavor brackish. The product is usually laid skin side down, and all areas of exposed lean are plastered with the curing mix (Heller 1929). This process is commonly used for fatty cuts such as jowls and fat backs.

30.3.1.2 Dry Country Style Curing

Curing ingredients employed by dry curing generally are salt, sugar, nitrate, and nitrite. The common practice is to rub the mixture into the surface of the product (usually hams and bellies) and place the products on shelves in a curing room held at about 36–38°F. A general rule of thumb is 11/2–2 days/pound of ham; bellies up to 2 in. thick will cure in 14 days. It is also prudent to remove and "overhaul" or recoat the products with curing mixture half way through this process. Dry curing may also be used in conjunction with brine injection for some products. The product is usually pumped with about 10% of saturated brine, and the balance of the curing ingredients are applied as a dry rub. If dry-cured products are to be smoked, the smokehouse temperature should not exceed 100°F in order to prevent nitrate burn (green spots) in the products. Also, if these types of products are to be aged, care should be taken to prevent damage to the products from insects such as cheese skippers, mites, red-legged ham beetles, and larder beetles.

30.3.1.3 Brine Soaking

Brine soaking probably followed dry curing and was often used commercially for several years. Meat pieces are placed in curing brine, and the cure is allowed to penetrate the entire portion. This process is relatively slow, and spoilage may develop before the process is completed. Some items such as corned beef briskets and tongues may still be processed this way; however, the practice is becoming less frequent.

30.3.2 Curing Pickle Injection

The industry standard today is the injection of curing pickle directly into meat pieces. Internal injection of curing ingredients enhances efficiency and promotes a more rapid and uniform distribution of the cure throughout the product. There are three basic methods used to accomplish pickle injection: (1) artery pumping, (2) stitch pumping, and (3) multiple needle injection pumping. Smaller establishments may use artery and stitch pumping because these techniques are slow and labor intensive. Larger establishments that process thousands of pounds of product per day use multiple needle injection.

30.3.2.1 Artery Pumping

This technique was believed to have been developed by a New Zealand undertaker (Pearson and Tauber 1984). This method introduces curing pickle through the arterial system of the product. Hams are usually the only products cured in this manner. The single needle is inserted into the femoral artery, and the cure is pumped into the ham. Great care must be taken during fabrication to preserve intact the femoral artery. The process does not lend itself well to high speed, high volume production and is therefore seldom used today.

30.3.2.2 Stitch Pumping

Stitch pumping utilizes a single needle that has several openings. The operator inserts the needle into the meat piece in many different locations to deliver the appropriate amount of pickle. An experienced operator is required to evenly distribute the curing solution. After pumping, an equilibration period is often required to allow the cure to evenly diffuse throughout the product.

30.3.2.3 Multiple Needle Injection Curing

This method is widely used by the meat industry today. Due to its speed and effective distribution of curing agents throughout the tissues, it enables processors to manufacture large quantities of product daily (Figure 30.1). These injectors may be configured to pump either bone-in or boneless product of

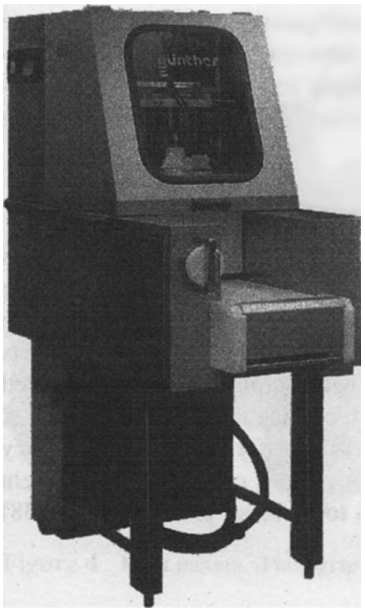

FIGURE 30.1 Multiple-needle curing pickle injector. (Photograph courtesy of Koch Supplies Inc.)

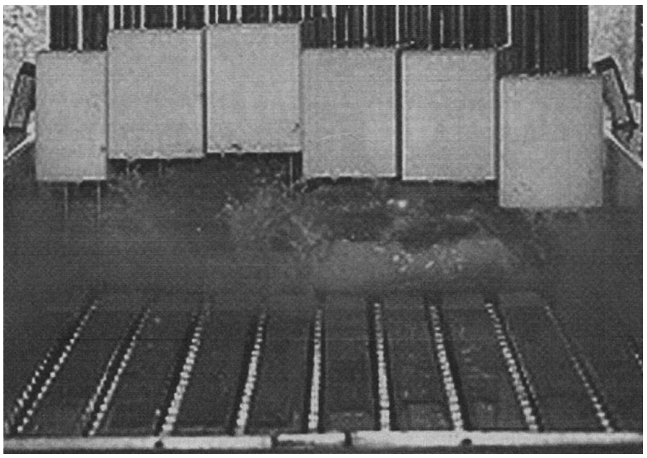

FIGURE 30.2 Ham injection. (Photograph courtesy of Nu-Meat Technology, Inc.)

various sizes and shapes. Pumping speed and the volume of pickle injected may be adjusted as needed. Pump head pressure must always be closely monitored. This should prevent excessive tissue disruption and formation of pickle pockets in the tissues (Figure 30.2).

30.3.3 Massaging, Tumbling, and Mixing

After the curing pickle has been introduced into the meat pieces, some type of mechanical energy is usually applied. Massaging, tumbling, or mixing the meat pieces for various lengths of time, usually under some degree of vacuum, may carry out this process. These physical processes are employed to extract salt-soluble protein and improve and accelerate the distribution of cure throughout the product.

30.3.3.1 Massaging

Massaging involves frictional energy resulting from meat pieces rubbing together (Addis and Schanus 1979). Meat massagers are vats that contain a mechanism for the slow stirring of meat pieces. The stirring arms or paddles made are set to various configurations. The agitation time can vary depending on the product but generally ranges between 3 and 6 h. The massaging process is a gentler form of mechanical energy input and works well with softer-textured product. This is especially important if the product is to retain a whole muscle appearance.

30.3.3.2 Tumbling

Conversely, tumbling is a more severe type of physical treatment. Tumbling involves the use of impact energy resulting from meat pieces falling and striking baffles or paddles contained in a rotating drum. Tumbling is usually carried out under vacuum to offset the potential problem of incorporating air into the protein exudates. Some products are loaded into tumblers immediately after injection with curing pickle and tumbled from 3 to 6 h. Longer cycles have been investigated, but due to severe limitations of time in commercial environments, this time frame works best for maximum efficiency and product quality. As with massaging, tumbling is carried out to extract protein for binding, to enhance tenderness and juiciness, and to increase product yields (Ockerman and Organisciak 1978) (Figure 30.3).

30.3.3.3 Mixing

Mixers generally have some type of paddles or ribbons that rotate around a metal shaft. They may or may not be equipped to hold vacuum, and they impart rather vigorous mechanical energy to the product.

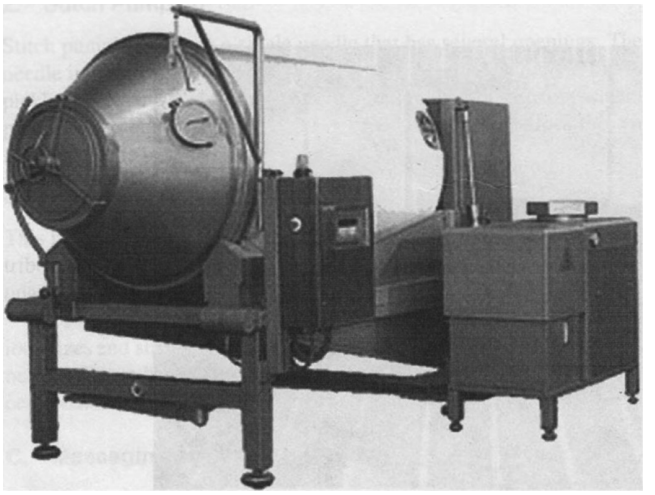

FIGURE 30.3 Meat tumbler. (Photograph courtesy of Nu-Meat Technology, Inc.)

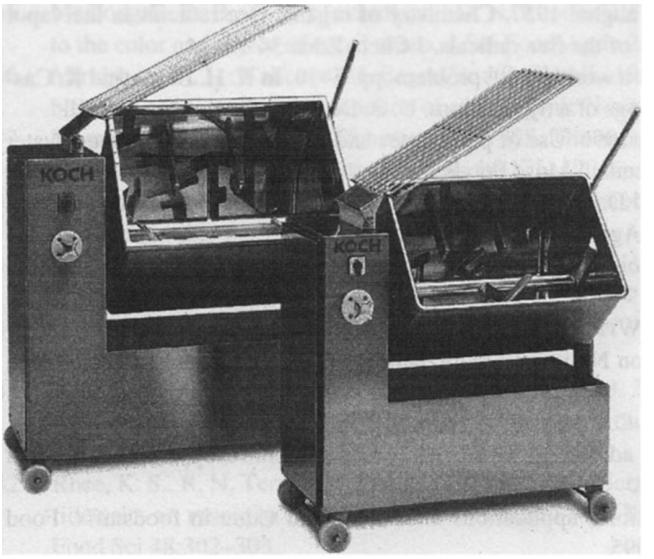

FIGURE 30.4 Meat mixers. (Photograph courtesy of Koch Supplies Inc.)

Short mixing times are usually the rule because longer mixes tend to both tear up whole muscle product and smear coarse ground product. Mixers are most often used in the manufacture of sausage products, but some whole muscle products are processed in this manner (Figure 30.4).

30.4 Summary

Successful meat curing is a complex art of scientific principles and good manufacturing practices. The constant emergence of new technologies, ingredients, and equipment has enhanced the meat processor's ability to produce a wide variety of products. Consumer demand for these products will continue to be driven by quality and consistency. Proper and consistent application of sound manufacturing techniques is of great importance in assuring these truly unique products remain an integral part of the food supply.

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31

Meat-Smoking Technology

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31.1 Introduction

Meat smoking began in prehistoric times, and was likely a by-product of an attempt to preserve meat. As with all scientific discoveries, people in prehistoric times may have studied how different types of wood imparted desirable, or more notably undesirable flavors, and how temperature (cold- versus hot-smoking) affected shelf-life. In the 1930s, Ostertag and Young (1934) hypothesized that the preservative effect of smoking was a result of muscle fiber shrinkage and an increase in interstitial space. Jensen (1943) and Draudt (1963) reasoned that the surface microbial load was reduced due to the bacteriostatic compounds from the smoke, such as resins, phenols, aldehydes, and aromatic hydrocarbons.

Smoke is a mixture of multiple wood combustion products (gases, tar, ash, carbonyls, phenols, etc.) that are visible as gases (carbon dioxide, water vapor, nitrogen, etc.) and carry unburned solid particles (resin, tar, ash, etc.) as they escape the combustible heat source. The exact composition or ratio of gases and solids within the smoke stream is defined by the type and moisture content of wood, rate and temperature of heating/burning, and other factors such as air flow. Smoke is imparted on the subjected meats at different rates depending on the air flow into the smoke house, surface moisture of the meat, temperature, and humidity of the smoke house.

Meat processors in many countries now incorporate smoke into their cooking process to impart desired flavors, aromas, and colors that enhances product acceptability. For processors that desire increased convenience in processing and reduced costs, liquid smoke is an excellent alternative to the use of wood smoke. Liquid smoke was approved for use in the United States (USDA-FSIS 1996) as an antioxidant and/or antimicrobial agent. Liquid smoke is generally referred to as natural smoke condensates and products including liquid smoke may carry the label designations of “Smoked” or “Naturally Smoked.” Liquid smoke also has the advantage of containing less polycyclic aromatic hydrocarbons (PAHs) and carcinogens that can be formed from incomplete wood pyrolysis.

Factors that affect meat smoking include preparation style, wood chips or sawdust, type of wood, wet or dry smoking, cold or hot smoking, and so on. This chapter will explain the various smoking processes that can be used to enhance flavor, shelf-life, and overall product quality.

31.2 Smoking Methods

31.2.1 Generation (Liquid, Friction, Burn)

Smoke generation is a product of the pyrolytic changes of wood. Pyrolysis (Greek-derived with pyr = fire and lysis = loosening) is a type of incineration that breaks down organic matter via chemical reactions in an anaerobic environment. These changes have been divided into two steps: (1) primary break down of wood via pyrolysis and (2) modification of the pyrolytic products via secondary break downs (polymerization, redox reactions, condensation, etc.). These chemical changes will be discussed later, but are mentioned here to convey that pyrolysis begins at 200–300°C (390–570°F) with typical occurrences being measured at 430°C (800°F) up to an ideal limit of 700°C (1292°F).

Smoke generation in meats processing is a scientific process that utilizes best management practices since smoke generation requires considerable knowledge of wood type, moisture content, air flow and temperature, smoke generators, and smoke house operations and their capabilities. Smoke generation is typically performed with the smoldering of wood chips, without a flame. Smoldering allows for steady smoke generation of desired concentration without the risk of higher temperatures or concentration changes if a flame is introduced by the ignition of the wood chips. A dampening chamber allows control of the smoldering rate by increasing or reducing airflow over the smoldering chips. A supplemental blower for the chamber or smoldering wood chips is not needed since the heated air leaving the smoke house will pull the smoke in from the smoke generation chamber and expose it to the meat. There must be a substantial volume of wood chips in the generator to maintain a continuous and consistent smoke stream. Chips should be maintained in a conical pile to reduce spreading since increased spreading will allow increased airflow over the larger surface area and increase the probability of ignition. Many smoke houses have chambers with motorized agitators that will turn in order to introduce wood chips into the heating chamber at set time intervals.

Friction is another method of smoke generation. In this process, a block of pressed sawdust or a wooden block is pressed against a rotating wheel. The resistance between the wheel and the block creates friction which in turn increases the temperature and causes smoldering to begin. This process is similar to sawing a block of wood with a dull blade on a circular saw. This process is seldom used due to inconsistency of the smoke stream generation and variation of the wood composition combined with varied moisture and oxygen levels exposed to the wood.

Commercial generators can be used to smoke meat products by passing super-heated steam through a wood chip pile to produce smoke with a greater moisture content in comparison to the burning of wood chips. This process has several advantages, including the use of less wood since there is no ignition, which leads to reduced emissions and cost. Another advantage is a reduced smoke generation temperature which may reduce carcinogen production. The major disadvantage is consistency. Utilization of steam requires a higher temperature than the temperature that is necessary for wood pyrolysis. This can lead to inconsistencies in flavor intensity and color deposition due to the type and moisture content of the wood. Also, the amount or inlet of oxygen into superheated steam will cause fluctuations in the smoke stream temperature.

In an effort to reduce emissions and increase consistency, many home and commercial operations use liquid smoke in processed meat products. Liquid smoke is produced through wood pyrolysis, similarly to the smoking of wood chips, yet at much more efficient and sustainable conditions. Liquid smoke also has the benefit of depositing less tar in ovens, therefore improving efficiency and reducing the amount of time and chemicals needed to clean ovens as compared with traditional wood smoking. During the manufacture of liquid smoke products, the smoke cloud is captured in water and through time and filtration, the condensate is processed to provide specific natural smoke condensate products. The following flow diagram (Figure 31.1) outlines the manufacturing steps of liquid smoke.

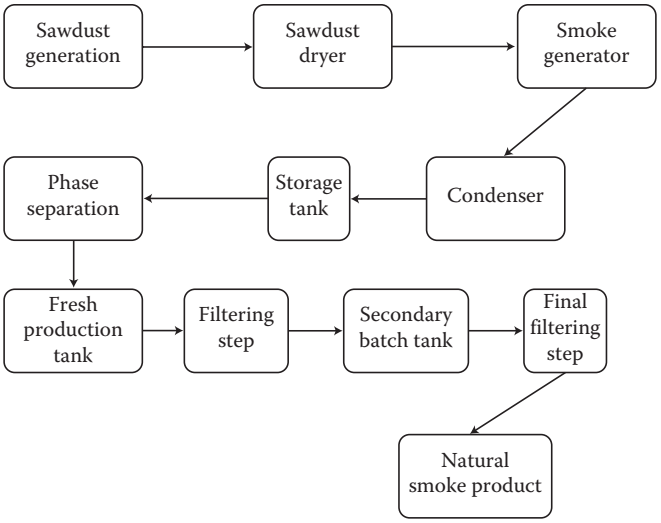

FIGURE 31.1 Basic flow diagram of the production of natural liquid smoke.

The smoke is cooled and condensed by the addition of water or organic solvents. Smoke condensates can be separated by distillation or gravity in the forms of a heavy precipitable oily resin phase, a low-density water-soluble phase (primary smoke condensates—PSC) and a high-density water-insoluble tar phase (primary tar fraction—PTF). The oily resin phase is not used in liquid smoke but producers are able to separate the components from the tar and water-soluble phases by function so that they are able to produce products with desirable flavor properties and color from the tar and water-soluble forms, while removing polycyclic aromatic hydrocarbons (PAHs) and other harmful or water-insoluble constituents. Benzo(a)pyrene is the most common PAH found in smoke and is present in the tar phase. The FAO/WHO (1987) established a limit of 10 ug/kg of benzo[a]pyrene in foods that have been smoked or treated with liquid smoke as it can serve as an indicator of carcinogens present in the meat. Liquid smoke producers are able to remove or reduce the concentration of these PAHs in liquid smoke using separation chemistry.

In a commercial setting, liquid smoke may be a mixture of PSC and PTF. The ratio is dependant upon the product identity and the desired smoke flavor and color in the finished product. Liquid smoke is most often incorporated into the meat by atomization of the liquid smoke into the air stream that is going through the smoke house. This application is very similar to the smoke stream that is generated by other methods, but has a higher moisture content and consistent composition, which leads to a more consistent smoke color on the meat products. Additional methods of liquid smoke application include direct addition to the meat formulation, especially when making comminuted or marinated meat products, drenching the products prior to thermal processing, or stuffing the products into presmoked casings and nets. In this fashion, liquid smoke should not be added at a rate more than 3.7 mL to 45 kg of product due to potency since higher concentrations may lead to the presence of bitter or astringent flavors. When adding liquid smoke to cured meat formulas, care must be taken to use a pH-neutralized smoke since acidic smoke products that are directly added to brines or meat formulations can promote rapid reduction of sodium nitrite to nitric oxide gas. Liquid smoke can also be used to dip meat products prior to heat processing. This method can be used in some marinated products and its use is dependent on the PSC and PTF content and the desired product flavor.

31.2.2 Parameters (Cold, Warm, Hot)

Cold smoking is typically performed inside the smoke house at 10–25°C (50–77°F) with approximately 70–80% relative humidity and the temperature outside the house being ±5°C to reduce the condensation

formation. Cold smoking is one of the oldest methods of preservation and is typically conducted in colder regions of the world. The relative humidity may be decreased in order to achieve the desired shelf-life as the biochemistry and function of cold smoking is to remove moisture, to lower the water activity, and prohibit microbial spoilage while attempting to prevent fat oxidation. Weight loss for these products is typically in the 15–20% range. Cold smoking is typically conducted with products that will require minimal thermal processing, such as cold-water fish, dried/fermented sausages, and cured bacon. Cold-smoked products typically have a lighter color (yellow to medium brown), and have a more pronounced and uniform flavor throughout the product when compared to warm and hot-smoked products, which is due to a longer smoking time (up to 14 days).

Warm smoking is typically conducted at 23–40°C (73–104°F) with approximately 75–85% relative humidity from 4 to 48 h. Time, temperature, and humidity are relative to the product dimensions and desired end product. Warm-smoked products have higher water activities than cold-smoked products and since their production occurs within the food's danger zone (4–60°C), these products require refrigeration. Warm-smoked products may have greater external smoke coating with less smoke penetration due to a shorter smoke time when compared to cold-smoked products. Warm-smoked products include salmon, mackerel, and other fish products.

Hot smoking can start at 40–60°C (105–140°F) but can continue as the smoke house temperature rises and can take place at temperatures as high as 90–100°C depending on the product and desired flavor with a relative humidity ranging from 60% to 85% for up to 4 h. Due to the shortened smoke time, this product must be refrigerated or fully cooked as shelf-life will not be significantly increased due to smoking. The following hot smoking process can be used to increase color deposition in fully cooked meat product's: (1) drying the surface of the meat, being careful to not raise the temperature too much or too fast since it will cause case hardening; (2) beginning to smoke the meat product between 45°C and 65°C (112–150°F) and possibly smoking at higher temperatures. For example, products such as frankfurters will not be smoked at a temperature above 60°C and 65°C (140–150°F), but larger diameter products such as hams or barbecue may continue to be smoked at temperatures as high as 77–110°C (170–225°F); and is followed by (3) fully cooking the product to an internal temperature between 60°C and 74°C (140–165°F). This is the most common form of smoking used.

Meat products need to be heated to remove the surface moisture which causes protein coagulation and development of a tacky surface which increases smoke deposition and adherence. Meat products should not be heated too rapidly as this will cause case hardening, which results in phenols and smoke compounds only being deposited on the outermost layer, and not penetrating the outer surface of the product. Also, if products are heated rapidly, fat will migrate prior to proteins setting by denaturation, which creates fat caps and weight loss, especially in comminuted products with small diameters.

In each form of smoking, the moisture of the wood and meat product must be taken into consideration. If the wood is too dry (moisture content below 20%), which only occurs with oven-dried wood or in more arid climates, the wood will not produce enough smoke and will be burned much faster. If the wood is too damp (moisture content above 25–30%), the wood may impart steam into the smoke house which will increase the amount of moisture on the meat's surface. If the meat's surface is too moist, the water-soluble components of smoke will mix with the condensate on the meat and drip off the meat, sometimes imparting a muddy color or streaks on the surface. Meat products are often cold showered at the end of cook cycles to begin rapid cooling. Smoke must be adhered to the product surface and have penetrated the surface to maintain the desired color and smoke flavor after the showering, cooking, and packaging processes.

31.3 Smoke Chemistry

Smoke flavor and color result from the direct deposition of the smoke particles onto the food's surface. In addition, smoke flavor and color penetrates the skin or product surface since smoke is able to penetrate organic surfaces. Maga (1988) reported that there is a chemical reaction between the carbonyls within the smoke vapor phase and the individual amino acids that make up the meat proteins, which undergo chemical reactions during the heating process. These chemical reactions cause nonenzymatic browning that is similar to the Maillard browning reaction. Modification of the amino groups to hydroxyl groups with

individual amino acids that are present in an organic casing will reduce the amount of chemical reactions, and therefore cause less smoke color deposition. Glycoaldehyde, glyoxal, and methyglyoxal are the carbonyls that are most noted to be reactive and associated with color formation in smoked foods. Other less reactive carbonyls include acetone, diacetyl, formaldehyde, furfural, and hydroxyacetone (Maga 1988).

31.3.1 Hardwoods/Softwoods

Any hardwood is suitable for smoking with oak, hickory, and maple being the traditional favorites. Softwoods (pine, spruce, etc.) will not burn as clean as hardwoods since they produce more soot, which imparts a darker smoke color (Stumpe-Vīksna and others 2008). Smoking with softwoods is further discouraged since they will impart more PAHs (Pagliuca and others 2003) and due to the higher levels of resin acids, the flavor from softwoods is reported to have a more acidic flavor which has been compared to turpentine (Ostertag and Young 1934). Hardwoods typically consist of 40–60% cellulose and 20–30% of both hemicellulose and lignin. This composition is important since pyrolysis of these components occurs at different temperatures.

Hemicellulose pyrolysis occurs from 200°C to 250°C (392–482°F). Cellulose pyrolysis occurs from 280°C to 320°C (536–608°F), and lignin pyrolysis occurs at 400°C (752°F) (Meier and others 2003). Yang and others (2007) measured the pyrolysis of these components on the basis of a thermogravimetric analyzer (TGA) with a differential scanning calorimetry (DSC) detector and a pack bed. Hemicellulose and cellulose pyrolysis occurred quickly, with the principle weight loss of hemicellulose and cellulose occurring at 220–315°C (428–599°F) and 315–400°C (599–752°F), respectively. Lignin pyrolysis was more difficult to isolate since decomposition by weight loss occurred between 160°C and 900°C (320–1652°F). The pyrolysis of hemicellulose depends on the structure of the wood. Hardwoods typically have a greater concentration of pentosans while softwoods have greater concentrations of hexosans. The chemical makeup of hemicellulose is heterogeneous β-(1,4)-linked pentose and hexose polysaccharides. The primary pyrolysis products of hemicellulose are carboxylic acids, furans, and furan derivatives. As these sugars and rings are degraded, aldehydes are formed which aid in the ability of smoke to cause non-enzymatic browning reactions on the product's surface. The high carboxylic acid content is one reason for the low pH of liquid smokes as well as the tart flavor. The hexosans of hemicellulose from softwoods also degrade to cellulose, a glucan polymer with linear β-(1,4) linkages of glucose, which further degrade to individual glucose molecules and subsequently 1,6-anhydroglucose and acetic acid with traces of furans and phenols. The degradation of hexosans to α cellulose causes increased concentrations of PAHs when softwoods are used for smoke production since hexosans do not undergo complete pyrolysis. Since lignin content has a linear relationship with the increase in age of the material that is used to create smoke, it may be possible to change the temperature of smoke generation, which would reduce PAH generation. Unlike cellulose and hemicellulose, the structure of lignin is not fully known since it is not a single compound. Lignin is an amorphous phenylpropane polymer with a disordered structure that typically contains three carbon chains that are attached to benzene rings. The structure of lignin is difficult to define, but it is the compound that imparts the strength to wood through binding cellulose and hemicellulose together through hydrogen bonding. The degree of binding and differences in the age of the material is displayed in the wide range of temperatures for pyrolysis. With the pyrolysis of lignin, phenolic compounds are released that are predominantly responsible for the smoked flavor in meat products.

Use of lower smoking temperatures is the primary parameter in the smoking process that is used to decrease PAH production. However, moisture content also affects PAH production. As the moisture content in the wood increases, PAH production decreases. Typically, the desired moisture content is from 20% to 30%. This causes reduced PAH production, slow wood combustion, and increased adherence of smoke to the product surface (Guillén and Ibargoitia 1996).

31.3.2 Compounds

Maga (1988) identified over 400 volatiles compounds in liquid smoke including acids, alcohols, carbonyls, esters, furans, lactones, and phenol. Various compounds from this list of over 400 are presented in Table 31.1 according to their classification as an acid, alcohol, carbonyl, ester, furan, lactone, phenol, or other.

TABLE 31.1

Various Compounds Identified in Wood Smoke

Acids	Alcohols	Carbonyls	Esters
Acetic	Amyl	Acetone	Cresyl acetate
Benzoic	Benzyalcohol	Benzaldehyde	Ethyl benzoate
Formic	Cyclohexanol	Cyclyopentanone	Methyl acetate
Glycolic	Ethyl	Diacetyl	Methyl crotonate
Isobutyric	Isobutyl	Hexanal	Methyl formate
Isovaleric	Methyl	Hydroxyacetaldehyde	Methyl isobutyrate
Sorbic	Propan-2-on-ol	Pentanone	Methyl palmitate
Valeric	Propyl	Propanal	Methyl propionate

Source: Adapted from Maga JA. 1988. *Smoke in Food Processing.* Boca Raton, FL: CRC Press.

Volatile compound generation is dependent upon temperature. Carbonyls and phenols are generated from 200°C to 600°C with phenol production decreasing as the temperature exceeds 500°C. Acid production is highest at 300°C, but decreases as the temperature increases over 300°C. Smoking is also known to produce polycyclic aromatic hydrocarbons (PAHs) (Karl and Leinemann 1996; Chen 1997; Larsson and others 1988). PAHs, which are produced during lignin pyrolysis, are generated over a wide temperature range (400–1000°C) due to water release and bond breakage. Their presence is typically higher for a food when the smoke vapor is generated at higher temperatures, with little oxygen, when the wood has too low of a moisture content, or is from a softwood tree (Maga 1988). While there are relatively few studies comparing hardwoods and softwoods (Potthast 1979; Larsson 1982; Guillén and others 2000; Stumpe-Vīksna and others 2008), researchers indicate that the PAH compounds that are produced from softwoods and hardwoods are similar, but also indicate that softwoods produce more PAHs. In addition, temperature and moisture content of the wood moisture can also alter the formation of PAHs. In addition to PAH formation, if smoking temperatures are high, burnt or charred food particles may contain heterocyclic aromatic amines that are formed due to protein pyrolysis.

31.4 Function

31.4.1 Color and Taste Formation

Smoking meat to enhance taste, texture, and flavor is widely accepted, but the intensity of smoke may change depending on the kind of meat being smoked. In 1912, Louis Camille Maillard discovered the browning reaction between reducing sugars and amino groups during an attempt to synthesize proteins (Davies and Labuza 1997). This reaction generally consists of three stages. The primary stage begins with the reducing sugar's carbonyl group from the smoke, condensing with the nucleophilic amino group of the amino acid in the meat product, to yield glycosylamine and water. The glycosylamine undergoes Amadori rearrangement to create an Amadori product, known as ketoseamines. While there are chemical reactions taking place in this stage, there is no color change. The ketoseamines are fragmented into deoxyosones. Deoxyosones are reactive and can create secondary hydrolytic fission products such as diacetyl, acetol, and pyruvaldehyde. This leads to aroma generation and color deposition with Strecker degradation as the amino acids form aldehydes that can also form aldols due to condensation. In the third stage, flavor and aromatic heterocyclic nitrogen compounds (furans, furanones, pyrones, etc.) are formed that impart pleasant caramel or fresh bread crust aromas and flavors or acrid, burnt aromas and flavors (Davies and Labuza 1997). While this reaction will take place at lower temperatures, it occurs more rapidly as the temperature increases and thus a darker or heavier smoke will be deposited. The humidity in the smoke house is a result of moisture in the wood or product, environmental conditions, and the relationship between the wet and dry bulb settings for the oven. Some humidity is needed in subsequent

steps after the initial smoke application to ensure that the process begins but should be monitored and reduced if condensates form to the point that the smoke does not adhere to the product's surface. If the humidity is too high, the product's color will be dark and muddy in appearance as opposed to a desirable color with mahogany or red tones.

Phenols and phenol derivatives that are deposited from the smoke vapor contribute a tangy smoked flavor to meat products. These compounds have been described as pungent, cresolic, burnt, and smoky (Fujimaki and others 1974; Baltes and Söchtig 1979). Volatile compounds that have been isolated from smoke vapor and smoked meats are listed in Table 31.2 with the aroma that they are likely to impart to the meat product.

31.4.2 Water Activity

A reduction in water activity (a_W) reduces microbial growth in meat products. Most bacteria will thrive if the a_W is 0.98 or greater (Frazier and Westhoff 1998). As the a_W decreases, the viability of bacteria decreases due to a reduction of nutrients flowing to the bacteria, a reduction in toxins moving away, and limited ability for the bacteria to move. While many bacteria will not be able to grow at a_W values below 0.86, some halophilic bacteria can grow in a matrix with a a_W that is as low as 0.75 (Troller and others 1984). Some yeast and molds will be able to grow at a_W values of 0.60. Thus, the surface of smoked meats or dried products may have some mold growth but will not support pathogenic bacteria. Below a a_W of 0.5, foods are generally considered stable in regards to microbial growth. In order to reduce the a_W in smoked meats, the relative humidity (RH) must be monitored throughout the process. Commercial processors monitor the dry bulb temperature, wet bulb temperature, and the RH. The dry bulb temperature is the air temperature and is the most commonly measured parameter. It is referred to as the "dry bulb" temperature since it is independent of the moisture in the environment. The wet bulb temperature is the temperature reported by a thermometer that is wet. This measures the amount of evaporative cooling, which is the extent of cooling that a product experiences as surface moisture evaporates. The RH is a measurement of the water vapor in the air of the cooking environment. RH is expressed in relation to full saturation. If the RH is too high, the product will retain moisture and prevent the meat product from drying. This will also create condensation within the house which may accumulate on the surface of the product and the smoke will not deposit as well. If the RH is too low, the product will experience case hardening. With case hardening, the product will form a thickened exterior layer that will be drier since the proteins have denatured and are unable to bind as much water. With the surface too dry, smoke will not adhere well and will not penetrate into the meat product, which will prevent uniform smoke flavor in the product. Lastly, if the product is not stored properly, a health hazard may exist because even though the surface is dry, the interior may have a greater a_W and promote microbial growth.

TABLE 31.2

Volatile Compounds That Have Been Identified from Smoke and Smoked Meat Products and Their Respective Flavor/Aroma

Compound	Flavor/Aroma	References
3,4-Dimethylphenol	Slight burnt flavor	Winter and others (1976)
2,4,5-Trimethylphenol	Phenolic odor	Winter and others (1976)
4-Ethylguaiacol	Smoky/roasted/burnt flavor	Winter and others (1976)
2-Methoxyphenol	Smoky flavor	Daun (1979)
2,6-Dimethoxyphenol	Smoky odor, woody/herby flavor	Zhu and others (1993)
2,4-Dimethylphenol	Cresolic odor	Kim and others (1974)
Pyrazine	Nutty, toasted flavor	Ames and others (1997)
Hexadecanal, 2-nonen-1-ol	Meaty flavor and odor	Huang and others (1991)
Geranyl isovalerate, octadecyl acetate	Fruity and fatty odors	Huang and others 1991

31.4.3 Antimicrobial and Antioxidative Compounds

Wood smoking or liquid smoke utilization can impart both antioxidative and antimicrobial properties to meat products (Anastasio and others 2004; Martinez and others 2004; Kjällstrand and Peterson 2001; Estrada-Munoz and others 1998; Fiddler and others 1967). Liquid smoke has USDA approval as a food additive for use as an antioxidant and/or antimicrobial agent (USDA-FSIS 1996). Phenols and carboxylic acids are the primary active antimicrobial and antifungal compounds from wood smoke. Carbonyls play less of a role and hydrocarbons do not play a role in the inhibition of microbial growth. The antimicrobial potential of smoke compounds has varied degrees of efficacy and if used as the sole antimicrobial step, smoke compound deposition is not adequate for ensured food safety (Suńen and others 2003). Pyrogallol and resorcinol are polyhydroxyphenolic compounds in smoke. These compounds are the most active antioxidants that are produced in smoked meats. Phenols can reduce oxidation by their ability to donate an electron to an oxidative radical while maintaining their structure. Guaiacol derivatives from methoxyphenols in the pyrolysis of lignin have less antioxidant activity than phenols since they generally have fewer OH groups.

31.5 Conclusions

Smoking can preserve meat products through drying, but preservation is no longer the primary reason for smoking meat. Various flavors can be imparted into meat products with smoking, and water activity can be reduced to preserve meat. The amount of phenols deposited on the surface of meat and that penetrates into meat causes the smoke flavor and imparts antioxidant activity in the meat. Humidity within the smoke house must be controlled to allow drying of the product as well as smoke deposition on the meat surface. Due to some concerns with PAH and other carcinogens or contaminants, inconsistencies with color and uniformity of flavor, liquid smoke is increasing in use as the primary means to impart antioxidative properties, uniform color, and smoke flavor.

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32

Meat Fermentation

Luca Cocolin and Kalliopi Rantsiou

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32.1 General Introduction

Food fermentations are microbial processes, often discovered by chance, that have played a fundamental role in nutrition and sustainability of human beings through the centuries. Cheeses and yoghurts have been discovered, for example, after transportation of fresh milk in containers made out of the stomachs of the animals, where the rennet (enzyme responsible for the hydrolysis of the caseins) was still present.

In general, fermentation of foods allows an extension of the shelf life of very perishable raw materials. Fermented products possess higher nutritional value with respect to the starting materials and they are characterized by different physicochemical properties and sensory profiles. Moreover, in Europe, they are bound to strong local traditions. This last aspect has to be connected to important microbial and meat enzyme contributions, which are responsible for the production of volatile compounds, such as alcohols, aldehydes, and ketones, from proteolytic and lipolytic activities. Finally, fermented foods are considered as safe products, since during production, acidification, often combined with drying processes, takes place, thereby creating an environment that does not allow the growth of pathogenic microorganisms.

Fermented meat products have a long history and tradition. It is believed that sausages were invented by Sumerians, in the modern Iraq, around 3000 BC. Chinese sausage *làcháng*, which consisted of goat and lamb meat, was first mentioned in 589 BC. Homer, the poet of the ancient Greece, reported a kind of blood sausage in the *Odysse*y (book 20, verse 25), and Epicharmus (ca. 550–460 BC) wrote a comedy called *The Sausage*. Evidence suggests that sausages were already popular both among the ancient Greeks and Romans (Lücke 1974).

32.2 Manufacturing Technology

For the production of fermented sausages, fresh meat (pork and other meats) and pork fat are first subjected to grinding and then mixed with salt and natural flavorings (including sodium nitrate and sodium

nitrite). Filling into natural or artificial casings follows and finally, a fermentation and ripening step, for variable periods, is carried out (Cantoni 2007).

The production process through which fermented sausages are prepared is detailed below:

Raw materials. Usually made of two-thirds of low-fat cuts (shoulder, loin, back meat and ham trimmed, low-fat parts, and bacon) and one-third of fat cuts (lard, hog-jowl, and hard fat). These meat cuts should present total microbial count below 10^5 colony forming units (cfu)/g, *Listeria monocytogenes* and *Salmonella* spp. have to be absent and the pH should be between 5.5 and 6.

Meat preparation. Tendons and connective tissues are removed from the meats and subsequently cut into pieces.

Refrigeration. The raw materials have to be stored under refrigerated conditions (0°C and 2°C) to reduce the growth of spoilage microorganisms. Moreover, the refrigeration will facilitate the following phase of the grinding.

Grinding. After storage at refrigeration temperatures, the meat is subjected to grinding. It is possible to differentiate fine grained (1–4 mm) and coarse-grained (4–14 mm) grinding, based on the type of sausage that has to be produced.

Flavoring mixture. Based on the type of sausages to produce, the mixture that is added is different. Several ingredients may be used

- Salt: influences the organoleptic characteristics of the product, but also possesses antibacterial properties. It lowers the water activity (A_w) and is able to solubilize salt-soluble proteins (mainly sarcoplasmatic and myofibrillar proteins), which are able to create a protein gel that assures the cohesion of the mixture during ripening of the product.
- Sugars: represented by dextrose, saccharose, and milk powder (as font of lactose). They serve as sources for the growth of lactobacilli, thereby enhancing the acidification step.
- Spices: influence the aroma of the product and their mix is different for each type of sausage.
- Additives: mainly represented by sodium nitrate and nitrite added together with salt as so-called curing salt. They have different roles; sodium nitrate can be reduced to nitrite, through the action of reducing enzymes (nitrate reductase) possessed by *Staphylococcus* and *Kocuria* spp., while sodium nitrite has an important double function on the color (It allows the maintenance of an intense red color.) and on the inhibition of specific spoilage and pathogenic microorganisms. As an example, nitrites are able to avoid the growth of *Clostridium botulinum* and germination of its spores during ripening.
- Ascorbic acid: has mainly an antioxidant activity and is thus involved in the red color formation together with nitrite.

As already indicated above, the type and quantity of flavoring mix depend on the fermented sausage to be produced. In the South of Europe, where the tradition is particularly rich of artisanal products, a vast number of sausages can be differentiated that differ slightly in their formulation. Moreover, at this stage, the addition of starter cultures can also be carried out.

Kneading. The goal of this procedure is to distribute homogeneously the ingredients and the fat in the meat portion. This phase has to be carried out at a low temperature and has to be rapid. One relevant risk associated with kneading is the rise in temperature and the subsequent liquefaction of the low-melting fats. In these conditions, the fats will cover the meat particles with an impermeable layer, hindering water loss and thereby priming growth of spoilage microorganisms.

Filling. After 24 h at refrigeration conditions (0–2°C), important for diffusion of the salt and for color formation, the meat batter is filled into casings. The stuffing machine works under vacuum, thereby avoiding the inclusion of air bubbles that can be responsible for oxidations and growth of spoilage microorganisms. Natural or artificial casings can be used for the filling process. In both cases, certain characteristics need to be taken into consideration: good porosity, which will allow a good exchange of water from the inside to the outside of the sausage; casings have to be soft and elastic to assure the adherence of the meat butter to the casing during all the process, and they have to allow a simple removal.

Tying. After filling, the sausage is tied. This step can be carried out manually (for traditional products) or automatically through specific machines, often associated with the filling equipment.

Fermentation. Conducted in temperature- and humidity-controlled rooms, this step lasts for 2–5 days. In the first 1–2 days of fermentation, the sausages are kept at 25–27°C and at a relative humidity (RH) of 65–75%. In these conditions, lactic acid bacteria (LAB) responsible for the acidification process are characterized by a fast growth. Thereby, the temperature used is dictated by the type of LAB that has been added as a starter culture. *Pediococcus* spp. usually prefer temperatures above 25°C, while *Lactobacillus* spp. grow better at temperatures below 25°C. A drying process follows for 2–3 days. At this stage, a decrease in the A_w is recorded and this allows a better preservation of the product. Also the lactic acid fermentation ends at this point. This process is conducted at temperature between 16°C and 22 °C and an RH of 55–65%. Ventilation can also be carried out to maintain homogenous RH inside the drying rooms. At the end of the fermentation process, the pH of the sausages is usually between 4.9 and 5.3. Another important event that takes place in the last stages of fermentation is the growth of molds on the surface of the sausages. This step is not obligatory; however, it is particularly important because the molds (more specifically *Penicillium nalgiovense* and *Penicillium caseiculum*) are enhancing the water loss process by creating microholes on the casings, they protect the sausages from oxidation because they create a protective layer against the light and at the end of the ripening, they are able to keep moist the product, avoiding excessive drying.

Ripening. It lasts for a couple of weeks to several months, depending on the dimensions of the sausages. During ripening, a weight loss of about 18–35% is foreseen. The conditions in which ripening is carried out are temperatures from 13°C to 15°C and RH of 75–90%. At the end of this step, the pH of the sausages increases to 5.6–5.7, due to proteolytic activities, and the products possess specific organoleptic characteristics, such as flavor and taste.

At the end of the production process, fermented sausages are characterized by a long shelf life at room temperature. This important characteristic is due to the acidification during the fermentation and the decrease of A_w during ripening. Acidification is also essential for consistency, color, and flavor. Decrease in A_w results in an increase of the ratio salt/A_w, thereby negatively influencing the development of spoilage and pathogenic microorganisms during ripening. The organoleptic profile of the fermented sausages is the result of a complex set of parameters, such as the equilibrium in the spices, the acidification process, and the enzymatic reactions on the lipids and proteins (Cantoni 2007).

32.3 Microbial Ecology

Meat fermentations are complex microbial ecosystems, in which bacteria, yeasts, and molds are involved. The study of their microbial ecology highlights the extreme interspecies heterogeneity, described by the isolation of several genera and species, but also underlines an important intraspecies biodiversity, represented by the coexistence, in the same niche, of diverse biotypes belonging to the same species. For instance, Urso and others (2006a) have shown that during the natural fermentation of Italian sausages, a number of different *Lactobacillus sakei* types could be identified, contributing in a different way to the characteristics of the final product.

The fermentation of sausages is a microbial process that has been investigated since 1960 (Lerche and Reuter 1960, Lücke 1974, Niinivaara and others 1964, Reuter 1972) and these studies showed that the main microorganisms responsible for the transformation are LAB (mainly *Lactobacillus* spp.) and coagulase-negative cocci (CNC, *Staphylococcus*, and *Kocuria* spp.). These evidences have been repeatedly confirmed by several researchers during the last 20 years (Bacha and others 2010, Comi and others 2005, Coppola and others 2000, Drosinos and others 2005, Samelis and others 1994a, Villani and others 2007). The application of modern approaches, based on molecular biology, has further supported the importance of LAB and CNC as the main species during sausage fermentations (Albano and others 2008, Aquilanti and others 2007, Aymerich and others 2003, Cocolin and others 2001b, Fontana and others 2005, Rantsiou and others 2005c, Silvestri and others 2007). Moreover, in some fermented sausages, especially those produced in France, Italy, and Spain, the characteristics of the final products are influenced by the activity of molds and yeasts that are developing on the surface of the product (Lücke 2000).

The type of microbiota that develops during sausage production is often closely related to the fermentation technique utilized. Sausages with a short fermentation time have more lactobacilli from the early stages of fermentation, and an "acid" flavor predominates in the products, which are commonly sold after <2 weeks of ripening. The intensity of the flavor depends on the pH value, but at a given pH, a high amount of acetic acid gives the product a less "pure" and more "sour" flavor (Montel and others 1998). Longer fermentation times and greater activity of microorganisms other than LAB, such as CNC and yeasts, lead to higher levels of volatile compounds with low sensory thresholds. Lipids and peptides are precursors of most of these substances. Tissue enzymes are the main agents of initial lipolysis and proteolysis processes (Lücke 2000); however, later in ripening, bacterial enzymes play a role in the degradation of peptides and free fatty acids being released.

In naturally fermented sausages, there is an even more evident and strong connection, between the microbiota that develops during fermentation and the sensory characteristics of the final product, with respect to sausages added with starter cultures. As a consequence, the study of the autochthonous microbial ecology is an important parameter to consider in sausage fermentation and for this reason is a subject of intense study.

Apart from the contribution of the raw materials to the initial contamination with the technologically important microorganisms, it should be pointed out that, in the past years, it has been repeatedly demonstrated that the processing plant is playing a crucial role in the enrichment of important biota for the production of fermented sausages. This aspect has been reported at least for plants in Greece (Gounadaki and others 2008), France (Lebert and others 2007, Leroy and others 2010), and Denmark (Sørensen and others 2008). A possible connection of high numbers of the "house-biota" with the presence of pathogenic microorganisms in the sausages, such as *L. monocytogenes*, *Salmonella* spp., and *Staphylococcus aureus*, has also been reported; however, this evidence could not always be demonstrated (Gounadaki and others 2008).

In the 0X's, scientific evidences highlighted that the use of methods that are relying on the cultivation of the microorganisms (culture-dependent techniques) do not properly profile the microbial diversity present in a specific ecosystem (Hugenholtz and others 1998). Indeed, populations that are numerically limited or microorganisms that are stressed or in a sublethal state cannot be recovered, and thereby they are eliminated from consideration. Moreover, viable but not culturable cells that are not able to form colonies on agar plates, but possess metabolic activity, will not be picked up by culture-dependent methods. Methods that do not depend on cultivation (culture-independent techniques) have attracted the attention of many scientists in different domains of investigation, spanning from the environmental microbiology to food fermentations. Soon this trend also reached the field of fermented sausages and a new generation of studies started to be published exploiting approaches based on the direct extraction of DNA and/or RNA from the sausage matrix (Rantsiou and Cocolin 2006a). Culture-independent methods are able to profile the microbial populations in complex microbial ecosystems without any cultivation. Once the DNA and RNA are available, they can be subjected to several types of analyses that can be preceded by a polymerase chain reaction (PCR) step. The culture-independent method that has been more extensively applied to sausage fermentation is the denaturing gradient gel electrophoresis (DGGE) (Figure 32.1). The method is able to differentiate DNA molecules based on their denaturation behaviors. When the method is used for microbial population profiling, the PCR is carried out with universal primers, able to prime amplification for all the microbes present in the sample. After this step, a complex mixture of DNA molecules will be obtained, which can be differentiated if separated in gels with denaturant gradients. Every single band that is visible in DGGE gels represents a component of the microbiota. The more bands are visible, the more complex is the ecosystem. By using this method, it is possible not only to profile the microbial populations, but also to follow their dynamics during time. DGGE analysis has been applied mainly to Italian fermented sausages (Cocolin and others 2001b, Rantsiou and others 2005c, Silvestri and others 2007, Villani and others 2007), but studies on the fermentation dynamics of Argentinean sausages are available as well (Fontana and others 2005). However, this method is not quantitative. Recent technological improvements allowed the PCR to become a quantitative method, and thus, direct enumeration of technologically important species during fermentation of sausages can be achieved. Such an approach was described for the first time by Martin and others (2006), which optimized a quantitative PCR (qPCR) protocol for the rapid quantitative detection of *Lb. sakei* in fermented sausages.

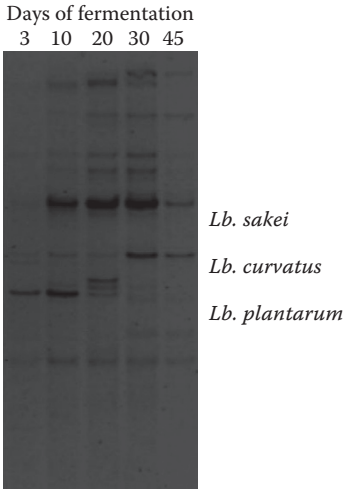

FIGURE 32.1 DGGE profiles of DNA extracted directly from a natural fermented sausage produced in Italy, on different days of the fermentation and ripening. The initial dominant populations of *Lb. plantarum* are accompanied by *Lb. sakei* and *Lb. curvatus* from the 10th day of fermentation. In the last two sampling points, these populations took over the fermentation process.

Finally, a promising culture-independent method, which has not yet been efficiently exploited to study the microbial diversity in fermented sausages, is the fluorescence *in situ* hybridization (FISH). In this case, a set of specific probes are used to target different microorganisms, directly in the sample. The probes are labeled with different fluorophores, thereby allowing a detection of several species simultaneously. Since the probes are generally designed on the ribosomal RNA, only alive cells are detected by FISH (Bottari and others 2006). One of the most fascinating features of FISH is the possibility to localize the microorganisms directly into the food matrix. This method was first applied to dairy products (Ercolini and others 2003). The only application of FISH to fermented and fresh sausages, in order to profile their microbial populations, has been described by Cocolin and others (2007).

32.3.1 Lactic Acid Bacteria

LAB are mainly responsible for acidification. They are able to reduce the pH of the sausages by production of lactic acid from carbohydrates (Hammes and Knauf 1994, Hammes and others 1990). Moreover, they influence the sensory characteristics of the fermented sausages by the production of small amounts of acetic acid, ethanol, acetoin, pyruvic acid, and carbon dioxide (Bacus 1986, Demeyer 1982), and they are able to initiate the production of aromatic substances, thanks to their proteolytic activity of muscle sarcoplasmatic proteins (Fadda and others 1999). Finally, a relevant function, which in the past years has been investigated more intensively, relates to the capability of certain strains on LAB to produce antimicrobial compounds, defined as bacteriocins, able to inhibit the growth of pathogenic and spoilage microorganisms (Stiles 1996). LAB are the fastest-growing microbial group during the production of sausages. From the initial counts of 10^2–10^3 cfu/g, they reach values of 10^7–10^8 cfu/g in the first 3 days of fermentation (Aymerich and others 2003, Cocolin and others 2009, Drosinos and others 2005, Mauriello and others 2004), and this situation establishes both in the core and in the external layers of the sausage (Coppola and others 2000). Their counts remain quite stable in number throughout the ripening period. Concerning the LAB ecology, it is apparent that *Lb. sakei*, *Lactobacillus curvatus*, and *Lactobacillus plantarum* are the best-adapted species of *Lactobacillus* spp. to meat fermentations (Urso and others 2006a). These species are described as main LAB obtained from fermented sausages produced in different countries and usually they are isolated together from the same product. Only in few cases, papers report the lack of detection of one of the species mentioned. For instance, Samelis and others (1998) and Coppola and others (1998, 2000) did not isolate *Lb. curvatus*. Other species of *Lactobacillus* have also

been identified. Samelis and others (1994b), Papamanoli and others (2003), and Parente and others (2001) described the isolation and identification of other members of LAB (i.e., *Lactobacillus farciminis, Lactobacillus coryniformis, Lactobacillus casei* subsp. *pseudoplantarum, Lactobacillus paracasei* subsp. *paracasei, Lactobacillus buchneri, Leuconostoc carnosum, Leuconostoc gelidum*, and *Leuconostoc pseudomesenteroides*). Moreover, representatives of the genus *Weissella* have also been identified. It is interesting to point out that only in few cases, wild strains of *Pediococcus* spp. were found during the fermentation process (Kaban and Kaya 2008, Parente and others 2001, Santos and others 1998). *Pediococcus* spp. are among the most common starter cultures used in fermented meat products in the United States, while in Europe, *Lactobacillus* spp. are used more often (Bacus and Brown 1981). This fact is raising the issue of whether or not *Pediococcus* spp. should be used as starter cultures for the production of traditional products in Europe. As a matter of fact, it has been demonstrated that starter cultures isolated from one product have failed to lead fermentation when used to produce a different product (Marchesini and others 1992). In the late 1990s, the advancement in molecular biology offered a number of new methodologies that could be used for a molecular identification of isolated LAB from fermented sausages. Rebecchi and others (1998) used randomly amplified polymorphic DNA (RAPD) analysis to group LAB that were subsequently subjected to the 16S rRNA gene sequencing resulting in the identification of *Lb. sakei* and *Lb. plantarum*. The RAPD-PCR approach was also used by Andrighetto and others (2001) that identified *Lb. sakei* and *Lb. curvatus* from traditional salami produced in the Veneto region, Italy. Versatile methods, recently extensively used for strain identification, are DGGE and temperature gradient gel electrophoresis (TGGE). Since the separation between strains is based on differential migrations, these techniques can be used for screening and grouping the isolates, thereby reducing the number of cultures to identify by 16S rRNA gene sequencing. This approach was used to identify LAB during production of Italian, Greek, and Hungarian fermented sausages (Cocolin and others 2000, Comi and others 2005, Rantsiou and others 2005a, 2006b). It is interesting to note that the application of PCR-DGGE and 16S rRNA gene sequencing allow the identification of a large number of strains in a quick and fast way. Finally, the use of species-specific primers and restriction analysis of amplified DNA segments are also advantageous approaches that researchers have used to identify sausage LAB isolates (Albano and others 2009a,b, Bonomo and others 2008, Cocolin and others 2009).

32.3.2 Coagulase Negative Cocci

Also, CNC (mainly represented by *Staphylococcus* and *Kocuria* spp.) contribute to the final characteristics of the product due to the production of proteolytic and lipolytic enzymes responsible for the release of low-molecular-weight compounds, such as peptides, amino acids, aldehydes, amines, and free fatty acids, which influence the aromatic profile of the final product (Demeyer and others 1986; Schleifer, 1986). CNC also have a fundamental role in the development and stability of the red color through the formation of nitrosomyoglobin by nitrate reductase activity, possibly involved in the limitation of the lipid oxidation, as well (Talon and others 1999). As described for the LAB, CNC also show a rapid increase in the first days of fermentation starting from as low as 10^3 cfu/g reaching counts of 10^5–10^6 cfu/g after the first few days of fermentation (Comi and others 2005). Growth of CNC is also favored by the availability of oxygen, thus cell counts inside differ from that on the surface. However, in some cases it has been reported that fast growth of LAB, with the consequence of deep acidification of the substrate, could result in inhibitions toward CNC that exhibit a slow growth (Papamanoli and others 2002).

Taking into consideration, the CNC microbiota, the species always isolated, independently of the country of production, is *Staphylococcus xylosus* (Iacumin and others 2006a). It often dominates the CNC populations and this is justifying its wide use as a starter culture. Another species, among *Staphylococcus* spp., that was frequently isolated from the fermentation process is *Staphylococcus saprophyticus* isolated from Greek sausages (Samelis and others 1998, Papamanoli and others 2002), and from Naples-type salami (Coppola and others 2000, Mauriello and others 2004). *Staphylococcus carnosus*, *Staphylococcus simulans*, *Staphylococcus equorum*, *Staphylococcus kloosii*, *Staphylococcus sciuri*, *Staphylococcus warneri*, and *Staphylococcus lentus*, as well as *Kocuria* spp. were identified at lesser extent. As reported for LAB identification, also in the case of CNC, an increased application of molecular methods has been observed in the past years. PCR-DGGE was exploited to identify CNC from Italian

sausages (Cocolin and others 2001a) and 16S–23S rRNA genes intergenic PCR and species-specific PCR were used, as well (Blaiotta and others 2003, 2004, Bonomo and others 2009, Rantsiou and others 2005b, Rossi and others, 2001).

32.3.3 Yeasts and Molds

Yeasts are present during sausage fermentations in lower numbers with respect to LAB and CNC. Cocolin and others (2006a) showed that they can reach values of 10^6 cfu/g during production of Italian-fermented sausages at low temperature, confirming results obtained previously by other authors (Metaxopoulos and others 2001, Samelis and others 1993). Coppola et al. (2000) found yeasts to be a predominant flora, together with LAB and CNC, in Naples-type salami. Studies carried out on different yeast starter cultures in fermented sausages have demonstrated their participation in the development of the color (by removing the oxygen) and flavor, due to their ability to degrade peroxides, lipolytic activity and, to a lesser extent, proteolytic activity (Lücke 2000). Moreover, yeasts, together with molds, protect the sausages from lipid oxidative reactions primed by light, due to the creation of a physical barrier that does not allow its penetration. Several studies have investigated the ecology of yeasts during fermentation of sausages and the species most often isolated is represented by *Debaryomyces hansenii*, followed by other species isolated with lower frequencies such as *Candida zeylanoides, Rhodotorula mucilaginosa*, and *Yarrowia lipolytica*. This evidence became available from studies considering sausages from different countries, such as Italy, Spain, and Portugal (Cocolin and others 2006a, Encinas and others 2000, Flores and others 2004, Gardini and others 2001, Saldanha-Da-Gama and others 1997). Interestingly, molecular characterization of *D. hansenii* by RAPD demonstrated a shift in its population from the beginning to the end of the maturation of the Italian sausages (Cocolin and others 2006a). Strains present during the early stages of the fermentation were grouped in clusters that differed from those isolated in the final phases of the maturation, underlining the genetic differences between these two populations of *D. hansenii*. However, all the isolates were able to grow in the presence of 3.5% sodium chloride and at 10°C, evidence that these parameters did not select the species present at the end of the maturation period.

Also molds, in particular species belonging to the genus *Penicillium*, are involved in the development of the organoleptic profile of fermented sausages and it is their lipolytic activity that is mainly involved in the aroma formation process. Since molds are strictly aerobic organisms, they grow only in the surface of the sausages, where they create a homogenous white mycelium that characterizes certain productions in the south of Europe (López-Díaz and others 2001). *P. nalgiovense* and *Penicillium chrysogenum* are the most important species during sausage production and they are often used as starter cultures (Leistner 1990).

32.4 Safety Aspects

Fermented sausages can be considered an excellent example of food product produced by following the philosophy of the hurdle technologies (Leistner and Gorris 1995) in which the intelligent use of combinations of different preservation factors or techniques results in mild but reliable preservation effects. In fermented sausages, from raw materials and without the use of heat treatments, the growth inhibition of pathogenic microorganisms can be guaranteed by a series of factors, such as pH, A_w, organic acids, bacteriocins, sodium chloride, and nitrates/nitrites. Foodborne pathogens that can be found in the raw materials, thereby being present in the sausages at the beginning of the fermentations, are represented by *Salmonella* spp., *L. monocytogenes*, *S. aureus*, and shiga toxin-producing *Escherichia coli* (STEC). Growth of salmonellae is inhibited by low pH, low temperature, and low A_w while staphylococci are retarded in their growth by the combined action of low pH and low temperature. Listeriae, on the other hand, can grow at low temperature and low pH and STEC can survive, for a long time, these conditions that normally destroy many other foodborne pathogens (Incze 1998). An important differentiation, in terms of safety of sausages, must be made between short-ripened products and long-ripened and dried sausages. Short-ripened sausages represent a substantial risk for human health since specific pathogenic microorganisms, such as STEC *E. coli, L. monocytogenes*, and *Yersinia enterocolitica* (Lindqvist and

Lindblad 2009), may survive the physicochemical conditions of the final product, thereby considering heating as a suitable treatment to guarantee their absolute safety (Incze 1998). Conversely, long-ripened and dried sausages possess low-safety risks. During the long ripening time, the synergistic effect of different parameters allows the control of pathogenic bacteria; also, if they are present in abundance in the raw materials, they most likely die off during ripening and drying (Heir and others 2010, Montet and others 2009). Several studies investigated the presence of *L. monocytogenes* and *Salmonella* spp. during different stages of production. It has been defined that generally these foodborne pathogens are absent in 25 g of the product (Aymerich and others 2003, Comi and others 2005, Rantsiou and others 2005c). *L. monocytogenes* can contaminate the fresh sausage mix, but commonly it is undetectable at the end of the fermentation (Drosinos and others 2005; Samelis and others 1998; Metaxopoulos and others 2001).

Another aspect of safety concern in fermented sausages is represented by the production of biogenic amines (BA). These compounds are produced by the decarboxylation of aminoacids, thereby enhancing the production in fermented foods produced from raw materials particularly rich in proteins, such as sausages and cheeses. Microorganisms, responsible for BA production in fermented sausages, are represented by *Staphylococcus*, *Enterococcus*, and to a lesser extent, *Lactobacillus* (Ansorena and others 2002). *Enterobacteriaceae* can also be involved in their production; however, they decrease in number and activity immediately at the beginning of the fermentation process, thereby not representing a major risk for BA accumulation. Several papers have been published on the role of different microorganisms in the production of BA and the influence of the microbial population dynamics, starter culture adjunction, ingredients, sausage diameter, and time of ripening (Ansorena and others 2002, Bover-Cid and others 2009, Gardini and others 2002, Komprda and others 2009). Generally, it can be concluded that the control of BA in fermented sausages can be reached by the addition of strains with negative amino acid-decarboxylase activity, although contaminations, during production, with BA producers, such as *Enterococcus* spp., seem to be inevitable. Also, the use of appropriate mixes of spices and the modulation of the casing diameter may have an impact on the reduction of the BA content (Komprda and others 2009).

Finally, an increasing concern in sausage fermentation is represented by mycotoxins. Proteinaceous foods have been shown to stimulate the production of mycotoxins, such as ochratoxin A (OTA) (Larsen and others 2001); thereby sausages can represent a vehicle for mycotoxin ingestion. *Aspergillus ochraceous, Penicillium nordicum*, and *Penicillium verrucosum*, isolated from meat products, are able to produce OTA. A recent study on the OTA contamination of artisanal and industrial dry sausages highlighted the contamination of about 45%, in a total of 160 sausage samples, with OTA, with concentrations that were spanning from 3 to 18 μg/kg. The OTA content was reduced below the limit of quantification if sausages were brushed and washed prior to sale, highlighting that OTA accumulation is taking place only in the surface and it is not able to diffuse inside the sausage (Iacumin and others 2009).

32.5 Biopreservation of Fermented Sausages

Nowadays the consumers demand for more natural products, with a reduction of chemically synthesized preservatives. In this context, a new approach to food stabilization, called biopreservation, based on the antagonism displayed by one microorganism toward another, was established. According to Stiles (1996), biopreservation refers to extended storage life and enhanced safety of food using natural or controlled microbiota and (or) associated antibacterial products. Microbial interference by LAB can also arise due to the production of bacteriocins (peptides, ribosomally synthesized by bacteria, which have the capability to interfere with the growth of many foodborne spoilage and pathogenic bacteria) (De Vuyst and Leroy 2007). Adding a pure culture of a viable bacteriocin-producing LAB strain, during fermented sausage production, represents an example of biopreservation. This indirect way of incorporating bacteriocins in meat products depends on the capability of the added strain to grow and produce the bacteriocin during the fermentation process. Moreover, synthesis of a bacteriocin under laboratory conditions does not guarantee its effectiveness during real production conditions. When evaluating a bacteriocin-producing culture for sausage fermentation or biopreservation, it is important to consider that meat and meat products are complex systems, therefore, the influence of formula and fermentation technology on the performance of bacteriocin-producing strains needs to be tested (Hugas 1998). An integrated

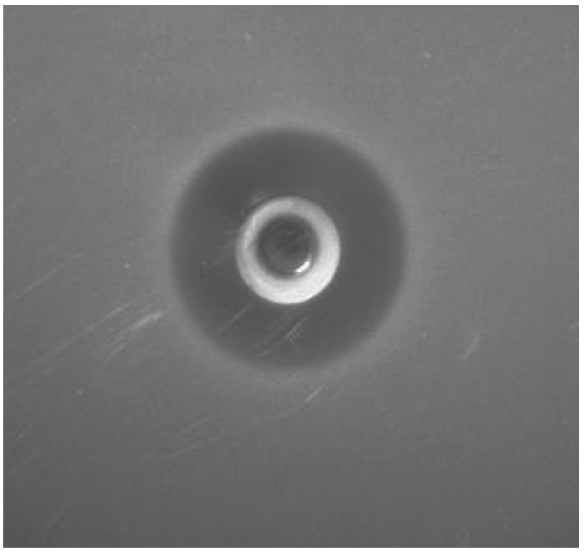

FIGURE 32.2 Agar well diffusion assay of *Lb. sakei* I151 producing sakacin P active against *L. monocytogenes*. The halo around the well is produced by the inhibition of the bacteriocin on the pathogenic microorganism inoculated in the layer of agar.

selection approach of a bacteriocinogenic *Lb. sakei* (named I151) has been described by Urso and others (2006b,c) (Figure 32.2). After the sequencing of the bacteriocin-encoding gene, which revealed the presence of the *sakP* gene, responsible for the production of sakacin P, the strain was inoculated in a sausage fermentation and the expression of the gene, together with the quantification of the bacteriocin, was carried out in order to assess the capability of the strain to produce the antimicrobial compound during the transformation process. Bacteriocin gene expression and production were detected throughout the fermentation, emphasizing the potential of the strain to be used as a starter culture (Urso and others 2006b). In a subsequent study, *Lb. sakei* I151 was used and compared with a commercial starter culture for sausage manufacturing. Sausages made with the I151 strain had an overall better microbiological quality, with low levels of enterococci and total bacterial count, compared to the sausage produced with the commercial starter, and a sensory profile that was preferred by a team of panelists (Urso and others 2006c). Biopreservation of fermented sausages is an approach that has been repeatedly described to reach products with enhanced safety. *Lactobacillus* spp. (Liu and others 2010, Papathomopoulou and Kotzekidou 2009) and *Pediococcus acidilactici* (Albano and others 2007, 2009a, Nieto-Lozano and others 2010) are the main LAB investigated *in situ* for their efficacy. Moreover, the purified bacteriocins, enterocin AS-48 (Ananou and others 2010) and enterocins A and B (Jofré and others 2009), in combination with high pressure, have been used to improve the safety of low-acid-fermented sausages. The use of bacteriocinogenic LAB has shown a good applicability in the fermentation of sausages, although differences in reduction of the foodborne pathogens are observed.

32.6 Starter Cultures in Meat Fermentations

Starter cultures are mixtures of specific strains of microorganisms, which with their activity are able to produce relevant transformation during the sausage production process. Usually, they consist of mixtures of LAB (*Lactobacillus* and *Pediococcus*) and CNC (*Staphylococcus* and *Kocuria*); however, starter cultures of yeasts and molds are also available in the market. Starter cultures are added to meats for different purposes: enhancement of the safety aspect by reducing or eliminating pathogenic microorganisms; to improve the stability, by controlling spoilage microbiota; to provide diversity at sensory level; and to provide health benefits, if starters are also probiotic microorganisms (Lücke 2000). By the

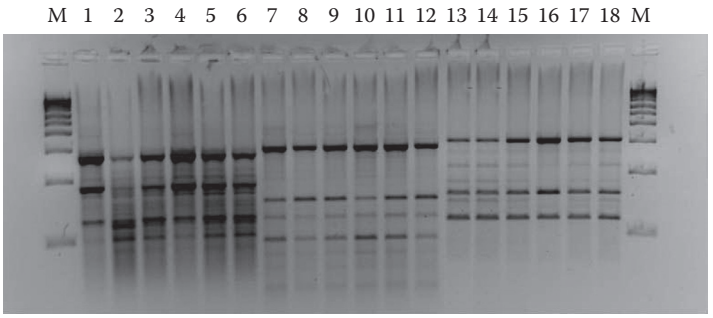

FIGURE 32.3 RAPD profiles of *Lb. curvatus* isolated from a commercial starter culture. It is obvious how the different isolates show a different pattern underlining the intraspecies variability within *Lb. curvatus* contained in the starter culture. At least, three different biotypes can be observed. M, molecular weight; 1–18, *Lb. curvatus* isolates from the starter culture.

addition of starter cultures, fermentation of meat turns out to be a process that is easier to control and with reduced fermentation time; moreover, the final organoleptic characteristics of the product are more standardized, thereby offering to the consumer a constant quality throughout the year (Hugas and Monfort 1997). On the other hand, one of the biggest criticisms addressed to the use of starter cultures is the risk related to the flattening of the sensory properties of different fermented sausages. Especially in South of Europe, where the tradition of fermented sausages is particularly colorful and vivid, the use of starter cultures may jeopardize the unique organoleptic characteristics of these products. In order to protect and promote the unique aspects of these products, researchers have dedicated efforts to develop indigenous starter cultures for typical fermented sausages (Talon and others 2007). These starters are combinations of strains isolated from naturally fermented sausages or from processing plants that do not use commercial starter cultures. The selection process is not a simple task and should comprise molecular identification of the isolated strains, their technological characterization, definition of the production of BA and the resistance to antibiotics (Ammor and Mayo 2007) and finally they have to be tested *in situ*, and their capability to take over the fermentation process should be proven by molecular-typing methods (Figure 32.3). This last aspect is becoming an important step in food fermentations because it is often demonstrated that strains inoculated as starter cultures are not able to dominate; thereby the process is guided by wild microbiota. One such example was described by Cocolin and others (2006b) during a study to assess the performances of a commercial starter culture for sausage production, declared to contain *Lb. plantarum* and *S. carnosus*. Isolations were carried out from the lyophilized culture and during fermentation LAB and CNC were first identified and then subjected to molecular characterization to compare the profiles obtained by RAPD. The results obtained showed that the starter culture did not contain *S. carnosus*, instead *S. xylosus* was the main species isolated. Moreover, three *Lb. plantarum* RAPD-types were detected and only one was able to persist and dominate the fermentation process. The use of autochthonous starter cultures resulted to be a successful approach at least in the case of fermented sausages produced in Greece (Baka and others 2011), France (Talon 2006), and Italy (Bonomo and others 2010).

32.7 Final Remarks and Future Perspectives

Fermented meats are foodstuffs that have been investigated for the past 50 years. The transformation process, as well as the technology, is well described and established. The modern meat fermentation industry has been capable of translating ancient procedures in up-to-date processes in which the safety of the products is guaranteed and the quality is kept at high level. Looking at the past half-century, the main advancement that was achieved in this field was the development of starter cultures that could be used to improve the safety and to standardize the quality of traditional products, as already described above. Considering the technology, it should be pointed out that no big changes in the traditional way to

produce sausages have taken place. Upscaling was carried out in the industrialized countries, resulting in the necessity to process higher quantities of sausages. This traditional sector of food fermentations has seen a particular interest from the researchers, thanks to the new possibilities given by the molecular biology field. New techniques have been produced and made available to scientists, who used them to confirm, or not, what was done from the 1940s with the conventional microbiological techniques. Nowadays, there are powerful methods that could analyze the microbiota without the need of its cultivation; moreover, isolates can be identified without any biases if sequencing of the ribosomal RNA is performed. The picture of the fermented sausage microbiota that can be observed with the application of molecular methods agrees with the one produced using conventional identification. Once more, the predominance of *Lb. sakei* and *Lb. curvatus*, and of *S. xylosus*, is emerging. Other lactobacilli could be identified, but their number was found to be significantly lower than that of *Lb. sakei* and *Lb. curvatus.* *Leuconostoc* and *Weissella* spp. were identified at lesser extent underlining possible pitfalls in their identification by traditional methods, or more simply, their low presence in the type of sausages studied. Also in the case of CNC, apart from *S. xylosus*, other species were described. *S. carnosus, S. simulans, S. condimenti, S. pulvereri/vitulis, S. equorum*, and *S. saprophyticus* were identified among the CNC isolates, and it is interesting to note that some species were identified only when molecular methods were applied. One of the future challenges that research would face is the study of the intraspecies biodiversity of the strains involved in the fermentation, in connection with their capability to produce a specific sensory profile. It is pretty amazing that fermented sausages produced in different countries, or different regions of the same country, may possess different organoleptic profiles, only partially due to the ingredients used in the manufacture. It has been demonstrated that during natural fermentation of sausages, a complex ecosystem is created, in which several biotypes of the same species can be identified. The balance of those biotypes, together with biotypes belonging to other species active in the fermentation (e.g., *Lb. sakei*, *Lb. curvatus*, and *S. xylosus*), is directly influencing the sensory profile of one specific sausage. Studies on the biodiversity of LAB (Urso and others 2006a), CNC (Iacumin and others 2006b), and yeasts (Cocolin and others 2006a) have underlined that there may be a progression during fermentation of several biotypes, or, on the opposite, one or two types can take over the process and totally define the organoleptic characteristics. The study of strain interactions will be extremely important to fully understand their behavior during fermentations. In the postgenomic era, transcriptomics, proteomics, and metabolomics are becoming the experimental approaches to go for in order to better comprehend meat fermentations (Hufner and others 2007). Moreover, interactomics, defined as the study of the interactions of the different microorganisms, will have to be considered, as well. The modern sequencing technology (i.e., pyrosequencing) gives a good opportunity to scientists interested in trying to investigate the physiological response of microorganisms at transcriptional level. Of course, it should not be forgotten that meat fermentation is a complex mixture of different microorganisms; therefore the study of single populations in laboratory conditions will not help at all the comprehension of the phenomena taking place during the production of fermented sausages.

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33

Nonmeat Ingredients and Additives

Youling L. Xiong

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33.1 Introduction

"Food additives" is a general term referring to anything added to food to achieve specific functions, for example, to aid in food processing, to increase the product's nutritive value, to enhance the product's palatability through regulating its physicochemical properties, and to extend the product's storage stability. In muscle food processing, both synthetic and natural, generally recognized as safe (GRAS) chemical compounds and ingredients are used as functional nonmeat additives to assure quality and safety of finished products.

The main ingredients in a typical meat product are raw muscle and fat. Nonmeat ingredients include a variety of additives that are small molecules (salt, phosphate, antioxidant, and antimicrobial compounds present in plant-derived spices and seasoning, etc.) or large molecules (starch, gums, nonmuscle proteins, etc.). All these additives can be referred to as chemical ingredients (Table 33.1). Nonmuscle proteins and protein hydrolysates (peptides), such as, soy protein, soy protein hydrolysate, and sodium caseinate, are used in meat processing to improve textural characteristics and water-binding capacity in finished products. Common small ingredients influence meat product quality through interaction with proteins and lipids in meat processing. Examples include monobasic salts (NaCl, KCl, etc.), divalent cationic salts ($CaCl_2$, $MgCl_2$, etc.), various alkali and acid compounds, and different phosphates. Lipid and nonlipid free radicals, which are commonly generated in meat processing, can also have a profound impact on the quality of meats because they not only cause off-flavors but also impair products' texture by reducing muscle protein functionality. To control oxidation, antioxidant compounds are widely used. Moreover, the cross-linking enzyme microbial transglutaminase (MTGase) is beneficial in improving gelling properties of muscle proteins under both low- and high-salt conditions and has been used in the manufacture of restructured meatloaves, meatballs, steaks, and nuggets. Overall, the intended roles of many of the

TABLE 33.1

Common Ingredients Utilized in Meat Processing

Ingredient	Example	Main Function
Salts	NaCl, KCl, LiCl, $CaCl_2$, $MgCl_2$	Flavor; protein solubility; protein functionality; meat hydration
Phosphates	Na_3PO_4 (ortho-), $Na_4P_2O_6$ (pyro-), $Na_5P_3O_8$ (tripoly-), $(NaPO_3)_n$ (hexameta-)	pH stability; protein extraction; water-holding; meat hydration
Alkalis	$NaHCO_3$, alkali phosphates	Protein extraction; meat hydration
Acids	Citric acids, malic acid, lactic acid, acetic acid	Protein extraction; meat hydration
Curing agents	Nitrate, nitrite, erythorbate	Cured meat color (pinkish-red); flavor; antimicrobial
Nonmuscle proteins	Soy protein, sodium caseinate, protein hydrolysates	Extender; filler; texture; water-binding
Polysaccharides	Carrageenan, xanthan, Konjac flour, starch	Water-binding; emulsification
Enzymes	Bromelain, papain, ficin, transglutaminase	Meat tenderness; meat-binding; texture of restructured meats
Seasoning	Paprika, black pepper, fennel, cumin, garlic	Flavor; antioxidant; antimicrobial
Health ingredients	Vegetable oils, protein hydrolysates, rice bran	Nutrition; health

large- and small-molecular chemical ingredients are affected by the specific meat processing conditions.

In this chapter, the roles of different ingredients used in the processing of comminuted as well as whole-muscle meat products, particularly those with a low- or reduced-fat content, are discussed. Emphasis is placed on the fundamental mechanisms by which different ingredients affect the physicochemical characteristics of cooked products as related to palatability, for example, gelling, emulsifying, texture-forming, water-binding, and hydration properties. For additional reading, authors are referred to several excellent reviews (Keeton 1996; Rogers 2001) and a comprehensive book edited by Tarté (2009) on similar or related topics.

33.2 Salts

Salt can be defined as any compound that results from replacement of part or all of the hydrogen atoms of an acid by a metal ion(s). Thus, salts are ionic compounds composed of cations (positively charged) and anions (negatively charged). Although salts are electrically neutral compounds, in aqueous solutions, such as the aqueous phase of meat products, cations and anions in many salts are dissociable, making them chemically active substances that can react with proteins and other muscle constituents.

The most commonly used salt in meat products is NaCl. The main functions of NaCl, other than providing flavor, are to solubilize proteins thereby eliciting their functionalities, to improve meat dehydration, and to alter osmotic pressure so as to inhibit bacterial growth and subsequent spoilage. Salt can be applied to meat directly in its dry crystal form or dissolved in solution (pickle or marinade) before incorporation into meat. The exploitation of meat protein functionality by the use of NaCl is accomplished through two distinct mechanisms: aiding in myofibrillar protein extraction and modifying protein–protein interactions. When used as a protein extraction agent, NaCl at an application level of ~2% or higher raises the ionic strength of the sarcoplasm to above 0.5, enabling myosin filaments to depolymerize and myofibrils to swell resulting in improved hydration and water-holding capacity (Hamm 1986). When salted raw meat is finely chopped in the presence of NaCl, myofibrils disintegrate and dissociate, leading to the extraction of solubilized actomyosin, myosin, actin, and most other myofibrillar proteins (Offer and Trinick 1983; Xiong and others 2000), a process known as "salting-in." Solubilization is achieved through ionic interactions of cations (Na^+) and anions (Cl^-) with oppositely charged side-chain groups of proteins (COO^- and NH^+) to enhance electrostatic repulsions between proteins and promote protein–water interaction. The concentration of NaCl affects the thickness of the hydration layer, which is closely related to the electric double layer surrounding a protein, hence, solubility of the protein (Figure 33.1).

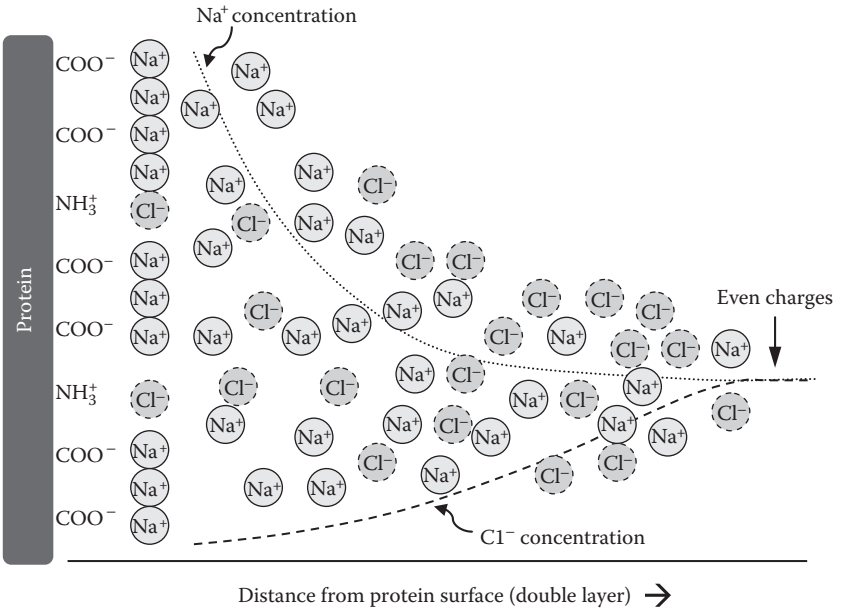

FIGURE 33.1 Schematic representation of an electric double layer created by the presence of salt (NaCl) in hydrated meat.

Because an excess dietary intake of sodium is linked to hypertension, there has been considerable effort to partially replace NaCl with nonsodium salts in meat products. Gordon and Barbut (1992) compared four chloride salts (KCl, LiCl, $MgCl_2$, and $CaCl_2$) as potential substitutes for NaCl in reduced-sodium meat emulsion products. A similar protein extraction pattern was noted between NaCl, KCl, and LiCl (1.5%) treatments. $CaCl_2$ and $MgCl_2$ were less capable of stabilizing fat and binding water, which was evidenced by the porous structure in cooked batters as revealed by the electron microscopy and excessive amount of water released. This was probably due to the amount of the divalent cation salts used being too high (ionic strength 0.43), because at a much reduced concentration (<5 mM), both $CaCl_2$ and $MgCl_2$ promoted the extraction of myofibrillar proteins, including myosin (Xiong and Brekke 1991).

In fact, low concentrations (<5 mM) of divalent cations, notably Ca^{2+}, enhance the gel-forming properties of chicken muscle myofibrillar proteins (Xiong and Brekke 1991). Similar cation effects have been reported for turkey muscle protein extraction (Nayak and others 1996). The efficacy of cations varies depending on muscle fiber types. As shown by Xiong and Brekke (1991), while the extractability of chicken breast myofibrillar proteins (white) was enhanced by <5 mM $CaCl_2$ or $MgCl_2$, it was lowered at >10 mM $CaCl_2$. However, the extractability of red muscle myofibrillar proteins (red) increased 40–70% upon treatment with 10 mM $CaCl_2$ or $MgCl_2$ and remained unchanged within the 10–100 mM concentrations. The presence of <10 mM Ca^{2+} promoted protein–protein interaction, leading to a more rigid gel with a stronger water-holding capacity. However, when the Ca^{2+} concentration exceeded 10 mM, gelation was suppressed for white myofribrillar proteins. It appears that subtle structural differences in myosin isoforms (Pette and Staron 1990) affect carboxyl-Ca^{2+} association.

33.3 Phosphates

Phosphates are common functional ingredients in meat processing due to their unique capability to interact with myofibrils to enhance water-binding and extract myosin. In the United States, the amount of phosphates added to meat cannot exceed 0.5% of the product. The most widely used phosphates are pyrophosphate (PP), tripolyphosphate (TPP), and hexametaphosphate (HMP). Monophosphate (orphophosphate) is rarely used except when a buffering agent is needed. The chemical structures of these phosphates

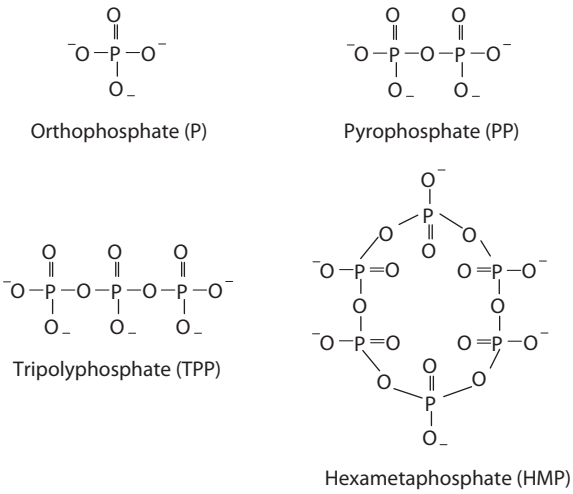

FIGURE 33.2 Chemical structures of phosphates commonly used in meat processing.

are displayed in Figure 33.2. PP is a diphosphate; it exists in acidic, neutral, or basic forms, depending on the number of metal ions (Na^+ or K^+) attached to the molecule. TPP has three phosphorus atoms; it, too, varies in the number of bound metal ions to form acidic, neutral, or basic compounds. On the other hand, ring-structured phosphates, generally referred to as metaphosphates, are condensed phosphates. They are composed of up to 15 or more orthophosphate units connected via oxygen atoms to form bulky cyclic compounds. Among them, sodium HMP is commonly used in meat products. Commercial HMP usually is a mixture of metaphosphates with different numbers of phosphate units.

Open-chain phosphates can readily react with divalent cations; hence, they have a tendency to precipitate in aqueous solutions containing high amounts of calcium, for example, hard water. Even in the absence of calcium, PP has a relatively low solubility when compared with TPP. Thus, PP is often used in conjunction with TPP or HMP, which are more tolerant of Ca^{2+}, in injection or marination brines. Possible mechanisms by which phosphates enhance water-binding ability and meat hydration in marinated muscle foods include the ionic effect and pH alteration contributed by phosphates and the ability to sequester Ca^{2+}, thereby reducing myosin aggregation. For PP and TPP, the ability to dissociate the actomyosin complex also contributes to water-binding in meat. The dissociation of actomyosin enables myofibril lattices to expand, thereby allowing increased water entrapment (Offer and Trinick 1983).

The mechanism underlying the action of PP and TPP to promote hydration and water-binding in fresh meat has been elucidated using myofibrils as a model. Significant hydration of salt-marinated meat begins at ~0.6 M (i.e., 2.1% in meat) NaCl, which parallels significant enlargements of the myofibril diameter. When 10 mM (0.3% in meat) PP is also added, the required level of NaCl for meat hydration is reduced to ~0.4 M (1.6% in meat). This effect is of importance for reduced-salt meat products. Because of its actomyosin dissociation capability, the presence of PP facilitates the extraction of myosin, which occurs preferably at both ends of the A-band of the sarcomere, namely, the A–I junction (Xiong and others 2000). Interestingly, marination in the presence of orthophosphate or metaphosphate does not induce the above extraction pattern, which is similar to that of NaCl-only marination. In general, the ability of sodium phosphates to facilitate water-binding in meat follows the order of PP ≥ TPP > HMP > orthophosphate. The improvement in protein extraction by the presence of phosphates leads to better protein functionalities, including gelation and emulsification, which, in turn, improves the textural properties of comminuted, cooked meat, such as sliceability and smoothness.

The efficacy of phosphates is sensitive to pH and ionic strength. In general, phosphates improve water-binding and meat product texture only if the NaCl concentration is <2% (equivalent to an ionic strength of ~0.5) under the typical meat pH condition (pH 5.50–6.0). If the salt content exceeds 2% or the pH of the meat product is sufficiently high (>6.3), the effect of phosphates tends to diminish. With the combination of high levels of salt and pH, NaCl alone is capable of causing myofibrils to swell and myosin to solubilize.

Thus, the exact efficacy of phosphates in modifying protein functionality is directly related to the solubility status of myofibrillar proteins, which is influenced by the processing conditions as indicated above.

33.4 Alkalis

Because phosphates at elevated concentrations can produce off-flavors and pose health concerns to some individuals who are sensitive to phosphorous compounds, there have been attempts in recent years to use alkali and acids to replace phosphates as water-binding agents in meats. Sodium bicarbonate ($NaHCO_3$) has been used to enhance the textural quality of meat products through alterations of physiochemical properties of muscle. Chicken, beef, and pork treated with sodium bicarbonate showed increased juiciness and overall palatability (Sheard and others 1999; Sen and others 2005). Sodium bicarbonate has also been used to alleviate the inferior-water-binding problem associated with pale, soft, exudative pork (Kauffman and others 1998).

The efficacy of bicarbonate is attributed to the ability to partially solubilize myofibrillar proteins and enhance their electrostatic repulsion through raising the pH. Sodium bicarbonate lowers the hydrogen ion concentration and shifts the pH of the intramuscular aqueous phase away from the isoelectric point of myosin (pH ~ 5.2). This leads to transverse expansion of myofibrils, allowing more water pickup and retention. Because the expansion increases the protein surface, it further promotes hydrogen bonding and electrostatic interactions between water and individual muscle proteins. Thus, swelling of the myofibrils leads to an osmotic compression of water molecules, thereby enhancing juiciness of cooked products and increasing consumer acceptance. At comparable application levels, sodium bicarbonate has been found to be at least as effective as sodium PP in increasing hydration and reducing cooking loss in marinated fresh pork meat (Bertram and others 2008). In fact, bicarbonate-marinated pork has more extensive myofibril swelling, and consequently, reduced space between the myofibrils than samples treated with PP.

Moreover, the addition of sodium carbonates to meat and meat emulsions can decrease drip loss. Dolata and others (1999) reported that sausage produced with carbonate additives (0.075–1.7%) had an increased cooking yield when compared with control sausage. The addition of the carbonate preparation OPREN (0.075%) yielded the highest pH value and lowest thermal drip loss, indicating an inverse relationship between the two factors. A 0.5 U increase in pH would lead to markedly enhanced water-holding capacity of meat batters. Sen and others (2005) showed that chicken breast meat marinated with 3% bicarbonate had a reduced cooking loss than the control (treated with 2% NaCl) or breast meat marinated with 3% TPP.

33.5 Acids

Weak acids are also used in meat processing, for example, in marination. Acid marination is especially desirable for lower-grade meat cuts. The marination creates a tenderized meat by affecting muscle proteins and fiber structures through a variety of processes. When the pH is lowered to a level far below the isoelectric point of myosin, muscle fibers and connective tissue will swell due to charge repulsion. Ke and others (2009) reported a significant increase in water-binding capacity of bovine muscle at pH 3.52 upon the addition of citric acid (0.2 M). Burke and Monahan (2003) noted increased moisture uptake, fiber swelling, and collagen solubility along with a reduced cooking loss of shin beef upon marination treatments with 31% citric juices (orange and lemon). The substantial reduction in the pH (from 5.7 to 3.1) was thought to be responsible for the improved water-binding capacity in the muscle tissue.

In addition, a reduced pH level can activate cathepsins, thereby promoting proteolytic degradation of myofibrils and increasing the solubility of collagen fibrils (Berge and others 2001; Aktas and others 2003). The type of acids is important in the overall pH reduction, moisture retention, and cooking yield of final meat products. For example, lactic acid yields a lower pH value compared to citric acid in marinades and can produce greater moisture uptake. This difference is attributed to the buffering properties of citric acid, a weak organic acid with three ionizable carboxyl groups ($pK_{a1} = 3.06$; $pK_{a2} = 4.74$; $pK_{a3} = 5.40$), compared with only one ionizable group ($pK_a = 3.86$) for lactic acid that gives rise to stronger acidification of the muscle fibers.

33.6 Nitrate, Nitrite, and Cure Adjuncts

Nitrate (NO_3^-) and nitrite (NO_2^-) are used exclusively in the meat-curing process to develop desirable red/pinkish color of cooked meats. Sodium nitrate was originally approved for color fixation in cured meats but now is largely replaced by nitrite. This is because nitrate is reduced to nitrite either by organisms or reducing compounds before curing reactions take place, and direct application of nitrite can be more easily controlled. Nitrate is now used only in a few products, such as country-cured hams and dry sausage.

Nitrite is a multifunctional compound: it induces and stabilizes the pinkish color of lean meat, contributes to the characteristic flavor of cured meat, inhibits the growth of spoilage microorganisms, and retards development of oxidative rancidity. No other compounds that we know can uniquely serve all these functions simultaneously. Another benefit of using nitrite in cured meats is its ability to prevent the growth of *Clostridium botulinum*, a pathogen that produces neurotoxins. The chemistry of nitrite curing has to do with the binding of nitric oxide (NO), converted from NO_2^- through reduction, to heme iron (Fe^{2+}), forming nitrosylmyoglobin that appears pinkish red (Figure 33.3). Upon cooking, nitrosyl myoglobin changes into nitrosylhemochrome or dinitrosylhemochrome due to the denaturation of the protein moiety (globin) or displacement of protein by NO at one of the coordination sites of heme iron. To facilitate the conversion of NO_2^- to NO, and ferric ion to ferrous ion, erythorbic acid or sodium erythorbate, which has a strong reducing power, is commonly used as a cure adjunct. Erythorbate also functions as an antioxidant to stabilize both cured color and flavor and decrease the formation of nitrosamines. Other cure adjuncts are sugar, salt (NaCl), and phosphates. Salt is needed to impart taste and to enhance firmness of meat through dehydration, and sugar is used to mainly counter hardness of meat caused by NaCl. On the other hand, phosphates are used to balance the water-binding and dehydration of cured meats.

The level of nitrite or nitrate allowed in cured meats, both ingoing and residual, is strictly regulated by the United States Department of Agriculture (USDA). For instance, the maximum level of ingoing $NaNO_2$ in pumped bacon is 120 ppm, and the residual level (in the finished product) shall not exceed 40 ppm. The restriction of nitrite levels is based on the concern that nitrite can form carcinogenic nitrosamines by reacting with secondary amines in cooked-cured meat as well as in the intestines of the human body. Amine compounds are degradation products from proteins that can be formed in meat and in the gastrointestinal tract. The formation of nitrosamines is greatly facilitated at high temperatures. For this reason, less nitrite is permitted in cured meats that are normally cooked at high temperatures, bacon (≤120 ppm nitrite), for example, than products cooked at relatively low temperatures, ham (≤200 ppm nitrite). Nitrosamines are known to be carcinogenic based on animal testing, but their carcinogenicity is quite variable among species. For an average meat consumer, the amount of nitrite ingested from cured meats is extremely low and should not be a concern.

In fact, many nonmeat products, including a variety of leafy vegetables, are excellent sources of nitrite or nitrate compounds. For example, the nitrate content is 2400–3000 ppm in radishes, 1600–2600 ppm in celery, 1000–1400 ppm in lettuce, and 600 ppm in zucchini. Other nitrate-rich fresh produce include cabbages, potatoes, and spinach. These vegetables contribute much more nitrite to the human body than do cured meats, but they have been known to be safe for hundreds of years. In fact, total dietary intake

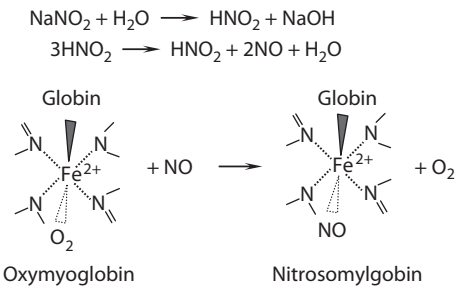

FIGURE 33.3 Meat-curing reactions.

of nitrite or nitrate from cured meats is only about 2–4% of the nitrite entering the human body. The vast majority is produced by the human body itself in the intestine (80–130 mg per day) and during salivation (8 mg per day) (Romans and others 1994).

The consumer concern with the potential toxicity and carcinogenicity of nitrite and its derivatives has promoted the meat industry to develop alternative curing methods. For example, "No-Nitrate/Nitrite Added" cured meats are produced by some meat processors. Instead of using pure nitrate or nitrite, vegetable extracts such as radish juice, cabbage juice, celery powder, and spinach extract, which contain up to 2000 ppm nitrite, are used to develop cured meat color and flavor (Sebranek and Bacus 2007). These products must be labeled with the statement "No nitrate or nitrite added except that found in radish juices or (other)." Another alternative method to produce pink, cured color is through microbial conversion of myoglobin. Several lactic acid bacteria have the ability to reduce $Mb(Fe^{3+})$ to $Mb(Fe^{2+})$ and change the muscle color from brown to bright red. Among them, *Kurthia* spp. and some strains of *Lactobacillus fermentum* are capable of converting $Mb(Fe^{3+})$ to cured meat pigment $NO\text{-}Mb(Fe^{2+})$ (Arihara and others 1993).

33.7 Nonmeat Proteins

A variety of nonmeat proteins are used as fillers, extenders, or water-binding agents in processed muscle foods, of which, soy proteins and dairy proteins (nonfat dry milk, sodium caseinate, whey protein concentrate, etc.) are most widely used. A general application level of up to 2% for protein isolates and 3.5% for protein concentrates is allowed. Meat products containing these proteins have improved texture and sliceability as well as reduced formulation costs in most cases. A technical challenge for using these nonmeat proteins is their thermoincompatibility with muscle proteins. Processed meats are usually cooked to a final temperature of 65–73°C for palatability. However, these final temperatures are not sufficient to denature main constituents of most plant proteins intended as meat binders or extenders. Consequently, interactions are limited between plant and animal proteins needed for the production of a viscoelastic composite product structure upon cooking. For example, 7S and 11S soy globulins denature around 75°C and 90°C, respectively (Scilingo and Anon 1996). High-salt concentrations (2–3.5% NaCl), which are usually present in processed meats, shift the denaturation of these soy proteins to even higher temperatures (Nagano and others 1996). This explains why native soy protein does not contribute to meat batter gelling properties (McCord and others 1998). For this reason, commercial soy proteins for meat product applications are usually subjected to preheat treatment in order to dissociate protein subunits as well as induce partial structural unfolding. Preheat treatments of plant proteins would enhance their performance in meats, including meat batter emulsion stability and bind strength (Feng and Xiong 2003). When slightly destabilized by brief heat treatment, soy globulins can promote muscle protein gelation and water-holding capacity.

Similarly, partially denatured whey proteins through brief heating can promote myofibrillar protein gelation and emulsification, thereby improving the textural properties of comminuted meats (Hung and Zayas 1992). As much as 50% denatured protein can be found in commercial whey protein ingredients intended for meat processing. Partially destabilized whey proteins, notably β-lactoglobulin, can synergistically interact with myosin when a meat batter is cooked to 65–70°C. The ensuing hydrophobic aggregation and disulfide cross-linking lead to the formation of a composite gel system of high rigidity and binding strength (Beuschel and others 1992). It is also possible that heat-denatured whey proteins can act as active fillers in comminuted products, that is, they can interact with surrounding meat proteins to reinforce the gel matrix (Barbut 2006).

Exposures of nonmeat proteins to extremely low or high pH conditions can also enhance the functional performance of proteins. For example, when subjected to pH 1.5 or 12 to induce structural unfolding, followed by pH 7 to refold (a process known as "pH-shifting"), a native soy protein isolate (SPI) exhibits drastically improved emulsifying activity (Jiang and others 2009) (Figure 33.4). The pH-shifting process has a larger influence on 11S globulins than on 7S. In addition to structural changes in protein monomers, the dissociation of native soy protein complexes contributes to the emulsifying property enhancements. Because pH-shifting treatments greatly increase the solubility of proteins at pH 6, the process seems to promote the overall functionality of soy proteins (Jiang and others 2010; Wagner and Guéguen 1999).

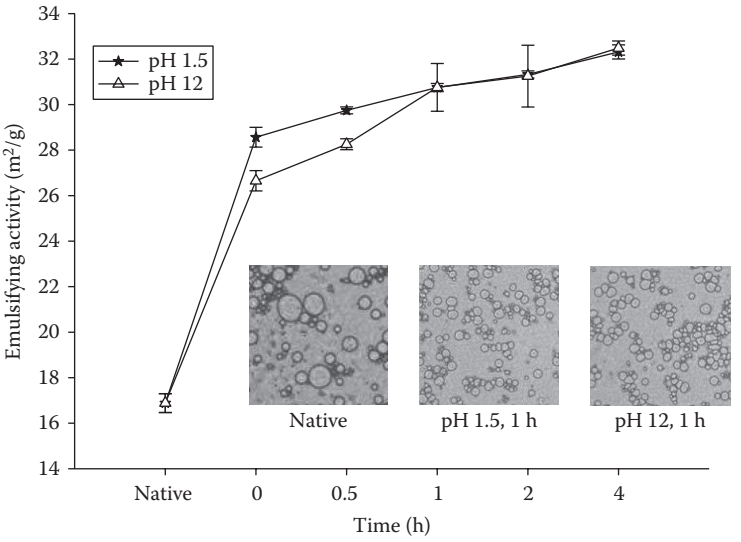

FIGURE 33.4 Improvements in the emulsifying activity of pH-shifting-treated soy protein isolate. Data from pH-shifting treatments up to 4 h, and the micrographs of emulsions stabilized by proteins of 1 h pH-shifting treatments, are included.

33.8 Polysaccharides

Various plant-derived polysaccharides are used in meat processing to bind water and modify texture in low- and reduced-fat products, such as sausage, bologna, and deli-type boneless hams. Polysaccharides are long-chain carbohydrates possessing polyhydroxyl groups. Some of them contain carboxylic and sulfate groups, hence, are negatively charged when dissolved in water. Because of their high affinity for water molecules, polysaccharides exhibit hydrocolloid characteristics, that is, they can absorb moisture rather efficiently and will swell, thicken, or gel in aqueous solutions. Many polysaccharides when applied to comminuted meats also have the ability to emulsify fat, encapsulate fat particles, and stabilize emulsions. Polysaccharides used in low-fat and restructured meats include alginate, carrageenan, locust bean gum, guar gum, gellan gum, xanthan gum, konjac flour, curdlan, cellulose derivatives, and starches. Excluding starches, these polysaccharides are nondigestible. They are generally applied at a level <0.5% to avoid excessive slippage and gastrointestinal upset.

Alginic acid (β-D-mannuronic acid and α-L-guluronic acid) forms irreversible gels in cold water in the presence of calcium, and hence, is used in the formation of restructured fresh meat steaks (Means and Schmidt 1986). The heat stability of the gel makes alginate useful in low-fat meat products that will be reheated. Carrageenan (sulfur-containing polygalactose) consists of three major types: κ-carrageenan, ι-carrageenan, and λ-carrageenan. κ-Carrageenan forms firm gels that can undergo syneresis, while ι-carrageenan forms weak gels that are not subject to syneresis. Both gels are thermally reversible. To control syneresis and firmness of gels, blends of κ- and ι-carrageenan are often used. κ-Carrageenan has been used in restructured turkey rolls, ham, and sausage, and ι-carrageenan is used in low-fat beef patties. When used in low-fat sausage, κ-carrageenan not only improves water-binding, but also textural characteristics when compared with other polysaccharides (Xiong and others 1999). κ-Carrageenan also shows a high efficacy in increasing water-binding and texture and reducing cooking loss in structured beef rolls (Shand and others 1994). λ-Carrageenan does not form a gel and is not normally used in meat products. Carrageenan solubilizes as meat products are heated. A gel is formed when the cooked product is chilled to below 60°C. Carrageenan interacts with proteins to stabilize fat particles, bind water, improve sliceability, and increase tenderness of finished products. In addition, it reacts synergistically with starches, konjac flour, and locust bean gum, and therefore, can be used in conjunction with these polysaccharides in low-fat sausage.

Locust bean gum (polygalactomannan) is used in canned meat, low-fat bologna, and salami. It is insoluble in cold water but soluble when heated. It is added to meat to impart a smooth texture, reduce syneresis, and provide a fat-like mouthfeel. Locust bean gum does not gel by itself, but when mixed with xanthan or carrageenan, they form cogels and improve meat-cooking yield. Guar gum, another polygalactomannan, is soluble in cold solutions. Its use in low-fat meat is mainly to increase viscosity and cream-like mouthfeel of the product. Xanthan gum, which is a fermentation product by the bacterium *Xanthomonas campestris*, is a heteropolysaccharide consisting of glucose, mannose, and glucuronic acid. It does not gel in aqueous solutions. However, its remarkable thickening effect enables it to function as an excellent water-binding agent in comminuted meats, such as frankfurters (Fox and others 1983). Konjac flour, which contains water-soluble glucomannan polymers, can form thermostable gels. This gelling ability allows Konjac flour to complement the texture- or gel-formation by proteins in meat products (Chin and others 2009). Furthermore, because Konjac gels are firm, they can be used to physically mimic ground fat, which is removed from meat product formulation.

Different starches, generally at up to 3.5% application levels, are used to improve texture and water-binding capacity in meat emulsion products, low-fat sausage, and other water-added meats. Starches suited for cooked meat should have a gelatinization temperature (i.e., swelling and dissolution of starch granules) within the range of meat products' final cooking temperatures (65–75°C). However, due to their susceptibility to freeze-thaw conditions, instability to shear and acids, and potential retrogradation when meat products are refrigerated, native starches are of limited use in meat products. Therefore, starches intended for meat processing are usually modified, for example, through cross-linking, to minimize negative impacts associated with excessive swelling of starch granules, unwanted gelatinization, or other problems as indicated above.

Skrede (1989) compared different types of starch (potato flour, wheat starch, corn starch, tapioca starch, and modified potato starch with acetylated distarch phosphate) for their effects on textural and sensory properties of sausage. Total quality index, defined as sensory quality index (firmness, juiciness, etc.) minus percent cooking loss and liquid exudation, and long-term storage stability were found to be the highest for sausage with modified potato starch cooked to 75°C. This sausage also had a relatively large freeze–thaw tolerance. Pietrasik (1999) also noted reduced cooking loss and increased hardness of sausage formulated with 3–5% modified potato starch when compared with untreated sausage. Joly and Anderstein (2009) evaluated five modified starches for their influence on cooking yield, purge, and firmness of chicken rolls injected with 45% brine. The results showed that modified waxy corn starch was most effective in increasing the cooking yield while modified potato starch produced the greatest firmness in cooked products.

33.9 Enzymes

Several enzymes are used to improve palatability of fresh meat or textural characteristics of processed meat products. Bromelain, papain, and ficin are three proteases widely used to tenderize meat. They are extracted from the fruits of, pineapples, papayas, and figs, respectively. These plant protease extracts are commonly used in a marinade or directly sprinkled on uncooked meat prepared in restaurant and institutional settings. The enzyme will penetrate the meat and hydrolyze both myofibrillar and connective tissue (collagen) proteins, tendering meat upon cooking. Plant proteases are particularly useful for tenderizing tough muscles, such as beef chuck, and meat from mature animals in general. However, because these proteases are activated at cooking temperatures (50–70°C), the texture may become too "mushy" for slowly cooked meat due to overwork of the enzymes. Hence, care must be taken to avoid excessive proteolysis, for example, through controlling the marination and cooking times.

Other enzymes that have been approved for use to tenderize meat include those from fungi (*Aspergillus oryzae* and *Aspergillus niger*) and bacteria (*Bacillus subtilitis*). Actinidin, a main protease present in kiwi fruit extracts, shows considerable activity against myofibrillar and connective proteins (Christensen and others 2009). The enzyme is activated at a lower temperature than is bromelain, ficin, or papain, making it easier to control the tenderization reaction in a marinade even at refrigerator temperatures. Ginger extracts also contain protease activity that is useful for tenderizing tough meat. Like bromelain,

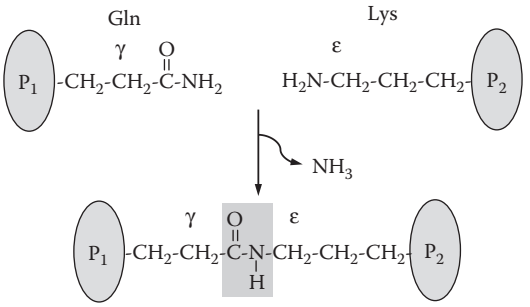

FIGURE 33.5 Cross-linking reaction catalyzed by transglutaminase.

ginger rhizome protease degrades collagen more than myofibrillar proteins and has an optimum temperature of about 60°C (Lee and others 1986).

Transglutaminase, an enzyme that catalyzes an acyl transfer reaction (Gln ε Lys) to form ε-(γ-Glu)-Lys isopeptide bonds within and between proteins (Figure 33.5), is used to promote protein functionality and bind meat particles in restructured products (Kuraishi and others 2001). The enzyme also catalyzes the hydrolysis of the γ-carboxyamide group in glutaminyl residues, resulting in deamidation. In muscle foods, where protein lysine residues (acyl acceptors) are abundant, the Gln-Lys cross-linking reaction prevails. While the enzyme can be extracted from a variety of natural sources, MTGase produced from *Streptomyces mobaraensis* has attracted the most attention. MTGase was introduced initially by Ajinomoto Co. to meat processing to facilitate the production of muscle protein gels and the "bind" in restructured raw meat, including fish fillets and nuggets. MTGase suits processed meats well as it maintains high activity over a broad pH, temperature, and salt concentration range.

A soft gel that can bind meat particles in raw, restructured meat can be formed by incubation of myofibrillar proteins with MTGase at refrigerator temperatures. To obtain a stronger gel, a small amount of caseinate (an excellent MTGase substrate) is generally added to aid in meat particle binding (Kuraishi and others 1997). When heated to 30–50°C, MTGase becomes highly activated. Therefore, for cooked meat products, nonmeat protein additives are generally unnecessary as long as a small amount of salt is added to ensure a minimal amount of soluble proteins (substrate) is available for the enzyme. With MTGase, myofibrillar protein thermal gels can reach an overall rigidity of as much as 10 times that of control (MTGase-free) gels (Ramirez-Suarez and others 2005) or MTGase-treated cold-set gels. This is true to both low- and high-salt protein or meat gels (Chin and others 2009). The enhanced gel rigidity (storage modulus) can be explained by the formation of thread-like cross-linking in MTGase-treated protein samples (Ahhmed and others 2009). MTGase-treated myofibrillar protein and meat batter gels also exhibit improved water-holding capacity. Moreover, emulsifying activity of myofibrillar proteins, emulsion characteristics, and emulsion stability are markedly enhanced following MTGase treatment (Ramirez-Suarez and others 2005). This would explain why MTGase treatments *in situ* improve meat emulsion firmness and stability (Kawahara and others 2007).

33.10 Seasonings

Seasonings are ingredients that impart flavor to food and food products. Seasonings used in meat products, besides salt, include various spices (usually seeds) and herbs, for example, fennel seeds, paprika, cumin, nutmeg, oregano, mace, clove, garlic powder, onion, ginger, rosemary, sage, parsley, and white, black, and red pepper. Fresh sausage is the main user of seasonings, but the specific application depends heavily on the type of sausage, which varies between countries, regions, and cultures. For example, Italian fresh sausage is seasoned with red pepper to give it a hot, spicy taste, and Chinese winter sausage is seasoned with soy sauce to give it a umami savory taste. Spice and herb extracts are also used in many processed meats, as is liquid smoke.

Studies have shown that many spices and herbs possess antioxidant and antimicrobial activity, which is largely attributed to phenolic compounds. Kong and others (2010) compared 13 common spice extracts for their antioxidant activity, of which clove, rosemary, and cassia bark, which had the greatest total phenolic content, were strongly inhibitory of lipid oxidation in fresh pork during storage. Rosemary and licorice extracts were also shown to inhibit *Listeria monocytogenes* in fresh pork, cooked ham (Zhang and others 2009), and Swedish-style meatballs (Fernandez-Lopez and others 2005). Eugenol (from clove) was reportedly effective against *L. monocytogenes* on beef slices (Hao and others 1998). Spoilage microbes, particularly Gram-positive species, are also susceptible to antimicrobial spice and herb extracts. Therefore, spice and herb extracts can be used as alternatives to synthetic antioxidants due to their equivalent or greater effect on the inhibition of lipid oxidation as well as microbial growth in meat and meat products.

33.11 Nutritional and Health Ingredients

33.11.1 Vegetable Oils

Vegetable oils have been used as partial substitutes for animal fat in frankfurters and other comminuted meats to produce cooked products with nutritionally balanced fatty acid profiles and reduced cholesterol content. Ambrosiadis and others (1996) replaced fat (20% of product weight) in beef frankfurters with vegetable oils (soy, sunflower, cotton, corn, or palmine), reporting acceptable sensory properties in finished products. Youssef and Barbut (2010) indicated that for such formulated products, the stability of meat emulsion was critically influenced by fat/oil type and the level of soluble proteins. Unlike animal fat, vegetable oils are fluids, thus, difficult to immobilize. A common approach to producing a stable meat batter is to add preemulsified oils. Bloukas and others (1997) demonstrated that up to 20% of pork backfat could be replaced by olive oil in the form of an emulsion without negatively affecting the processing and quality characteristics of dry-fermented sausage. Muguerza and others (2001) also succeeded in manufacturing traditional Spanish sausage by substitution of preemulsified olive oil for up to 25% of pork backfat. The sensory characteristics, including texture and color, were comparable with those of traditional products. Rheologically, preemulsified vegetable oils, stabilized with a protein membrane, can be readily incorporated into the gel matrix in comminuted meats where the oil droplets are stabilized through protein–protein interactions and function as fillers to enhance the viscoelastic properties of cooked products (Wu and others 2009).

33.11.2 Natural Antioxidants

Because meat products are susceptible to lipid and protein oxidation due to the high-fat and prooxidant (heme, free iron, copper, etc.) contents, antioxidants have long been used in meat product formulations. Traditionally, butylated hydroxyanisole, butylated hydroxytoluene, propyl gallate, and tertiary butyl hydroquinone (TBHQ), at an application level not exceeding 0.01% of fat content when used individually or 0.02% if used in combination, have been used in sausage and other meats. However, the increasing concern with the safety of synthetic compounds has prompted the meat industry to replace synthetic antioxidants with those of natural origins. Of different plant-derived antioxidants, mixed tocopherols and rosemary extracts are the most widely used, and a myriad of basic and applied studies that consistently illustrate oxidative protection of sausage and meat emulsions by these natural ingredients have been published in scientific literature. In addition, as discussed earlier, antioxidative compounds present in many spices contribute to the oxidative stability and shelf life of cooked pork, beef, chicken, and turkey meat products.

Many peptides and protein hydrolysates have also exhibited antioxidant activity, hence, have been applied to muscle foods to improve oxidative stability. Carnosine, a dipeptide with the sequence of β-alanyl-histidine, and its related dipeptides anserine (β-alanyl-1-methylhistidine) were shown to strongly inhibit lipid oxidation and preserve the red color of fresh, salted ground pork (Decker and Crum 1991). Hydrolyzed casein (Diaz and Decker 2004) and potato protein (Wang and Xiong 2005)

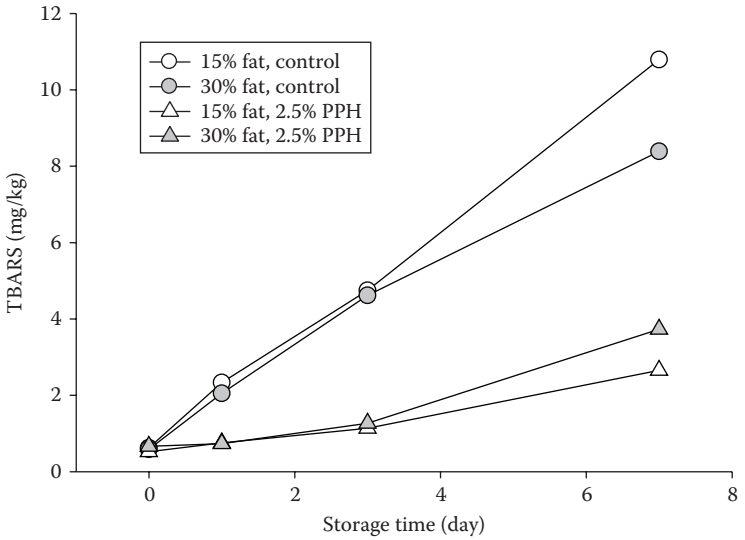

FIGURE 33.6 Inhibition of lipid oxidation (TBARS) in cooked, refrigerated pork emulsion sausage by potato protein hydrolysate (PPH).

were excellent inhibitors of lipid oxidation in raw and cooked beef patties. Similarly, when applied to cooked pork meat emulsions containing 15% or 30% fat, hydrolyzed potato protein at a 2.5% application level reduced lipid oxidation in the products by two- to four-fold (Figure 33.6). Because peptides and protein hydrolysates are water soluble, they can complement fat-soluble antioxidants, such as tocopherols and phenolic extracts from spices, to improve the oxidative stability of meat emulsion products.

33.11.3 Dietary Fibers

Plant ingredients rich in dietary fibers have been used in cooked meat products to improve textural properties and impart health benefits. Rice bran, rye bran, wheat bran, and oat bran added to comminuted and emulsified meat products have the ability to bind water and stabilize fat particles (Fernandez-Gines and others 2005). These cereal fibers are composed primarily of cellulose, hemicellulose, and lignin, which are insoluble polysaccharides. Claus and Hunt (1991) reported that oat fiber-containing bolognas were grainy and somewhat dry, but they had reduced purge when compared with nonfiber control. In addition, by-products of fruit juice processing, for example, citrus peel, and apple, peach, and orange pomaces, which are rich in pectin-type soluble fibers, are potential ingredients to modify the texture of sausage (Lantto and others 2006). However, because high amounts of dietary fibers increase the hardness of cooked sausage, their application level should be kept to low levels, for example, <2%.

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Part VI

Secondary Processing: Products Manufacturing

34

Meat and Meat Products

Robert Maddock

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34.1 Introduction

"Meat and meat products" is a broad topic that encompasses a multitude of foods and processes. This chapter provides a general overview about the use of meat and meat products in secondary product manufacturing. Other chapters in this volume discuss more specific examples and processes.

In order to better understand this chapter, some clear definitions are required. Different definitions or variations thereof may be found elsewhere in this book, but for the purposes of this chapter, the following definitions are used.

Meat: Meat is the flesh, which is muscle, fat, and connective tissue, of mammals used for food as differentiated from organs, and also from poultry and fish.

Variety meats: Edible organs from mammals used for food.

Poultry: The edible portions of avian species.

Fish: The edible portions of aquatic animals, including vertebrates and invertebrates.

Secondary processing: Altering the characteristics of meat, by changing the shape, size, or chemical properties of meat, often by the addition of ingredients, to enhance flavor, functionality, or value.

Meat products: Any product in which meat, including varieties of meats, poultry, and fish, is the primary ingredient. For example, a breaded chicken breast would be considered a meat product, whereas a sausage pizza would not.

Product manufacturing: The process of adding ingredients and/or processing meat, poultry, or fish.

34.2 Whole Muscle Products

In general, there are two major classifications of meat and meat products: (1) whole muscle, and (2) those that have in some way been reduced in size via cutting, grinding, or are otherwise comminuted. It is important to note that many meat products may include a combination of processes. For example, precooked ground beef patties are ground, formed, and cooked before reaching consumers, and precooked

TABLE 34.1

Examples of Meat and Meat Products of Secondary Processing and Major Characteristics of Each

Item, Item Category, or Process	Typical Meat Block (Species or Source)	Whole Muscle or Comminuted (Ground)	Added Ingredients (Yes or No) and Typical Added Ingredients	Cured (Nitrate or Nitrite Added) or Uncured
Tenderized meat	All, usually beef, some pork	Whole muscle	No	No
"Enhanced"	All, especially beef, pork, and poultry	Whole muscle	Yes, water, salt, phosphates, some antioxidants	No
Precooked	All	Typically whole muscle, some ground	Yes, water, salt, sweetener, phosphates, flavorings, some antioxidants	No
Ham	Pork	Whole muscle	Yes, water, salt, sweetener, phosphates	Yes
Bacon	Pork	Whole muscle	Yes, water, salt, sweetener	Yes
Other cured meat	All, beef, pork	Whole muscle	Yes, water, salt, sweetener, phosphate	Yes
Jerky/dried meat	All, commonly beef	Whole muscle or ground	Yes, salt, sweetener, spices, seasonings	Yes, some possible without cure
Fresh sausage	All, especially pork and beef	Ground	Yes, salt, sweetener, spices, and seasonings, other nonmeat food items becoming more common	No
Cooked sausage	All, especially pork and beef	Ground	Yes, salt, sweetener, spices	Yes, some possible without cure
Formed products	All, especially poultry	Ground	Yes, salt, seasonings, some binders	No

roast beef is usually injected before cooking. Table 34.1 provides a summary of several types of meat products and processes.

Whole muscle products and processing can be further divided into cured or uncured products, where cured meats have been exposed to salt and some sort of curing agents, usually nitrite or nitrates, and uncured products have been altered from their original state in some physical form but have not had curing ingredients added.

Several different processing techniques are used to add value to uncured, whole muscle meats, including tenderizing, "enhancing" (also known as "pumping" or injection) fresh meat, precooking, preseason/marinating, and many others. Information and examples of the application of some of these techniques are presented below.

34.2.1 Tenderized Whole Muscle Products

34.2.1.1 Factors Affecting Tenderness

Tenderness is the most important "taste" factor influencing enjoyment of meat. Meat tenderness is defined as the amount of force taken to bite into and chew a piece of meat (Tornberg 1996). Tenderness has two different phases: initial tenderness and sustained tenderness. Initial tenderness is the force required to make the first bite in a piece of meat, and sustained tenderness is how many chews it takes for a bite of meat to completely disintegrate (Miller and others 1997). Tenderness of meat is influenced by the following four primary factors which that are affected by secondary processing (Savell and Cross 1988).

Proteins. Proteins that allow living muscle to do work are called contractile proteins. The integrity of contractile proteins in meat is a major factor in tenderness. Meat proteins become disrupted over time during postmortem aging, mostly due to the action of muscle enzymes called calpains. The disruption of proteins is the reason that aging is an effective method for tenderizing meat.

Sarcomere length. Muscle proteins are able to perform work by sliding past each other to contract and relax. When muscles are contracted, they have a shorter sarcomere length and meat is tougher. After slaughter, meat gets "stuck" with the sarcomere length when rigor mortis sets in.

Connective tissue. The amount and type of connective tissue are very important in meat tenderness. Great amounts of connective tissue result in tougher meat. Also, as animals age, connective tissue becomes tougher due to cross-linking between collagen fibrils.

Composition of the meat. The amount of fat, water, and protein in meat will affect tenderness. While not always a major factor, in general greater amounts of intramuscular fat (marbling) are associated with greater tenderness.

34.2.1.2 Tenderization Methods

There are several methods to improve meat tenderness. Besides natural aging (postmortem storage), mechanical tenderization, enzymatic degradation, and chemical treatments are three common techniques used to tenderize tough meat.

Mechanical tenderization. An almost foolproof way to improve tenderness of almost any cut is mechanical tenderization. Needles penetrating the cut disrupt muscle fibers and connective tissue. The primary issue with mechanical tenderization is a possible reduction in the safety of steaks and roasts, which may not be cooked to temperatures high enough to kill bacteria introduced by the needles during tenderization. Therefore, mechanical tenderization is most appropriate for those cuts that will be fully cooked. Mechanical tenderization is the best option for cuts with large amounts of connective tissue (Loucks and others 1984).

Enzymatic tenderization. Enzymes from tropical plants such as pineapple (bromelain), papaya (papain), and figs (ficin) are extremely effective in tenderizing meat. These enzymes degrade muscle proteins very rapidly. The enzymes must be injected into the cuts, or be allowed to soak into meat as a marinade. In general, higher value cuts from young beef do not need enzymatic tenderization, but cow beef and tougher cuts may benefit (Ashie and others 2002).

Chemical tenderization. Chemical tenderization is a general term for tenderization that is caused by the addition of acids, such as vinegar, to fresh meat. The acids degrade both myofibrillar proteins and connective tissue, thereby improving tenderness (Chang and others 2010).

34.2.2 Enhanced or Injected Fresh Meat

Enhancing meat, usually via pumping or injecting, involves the addition of an aqueous solution to fresh meat to improve palatability, especially juiciness and tenderness (Robbins and others 2003). This is accomplished by using an injector to add a solution of water, salt, phosphates, and sometimes other compounds as well directly into the whole muscle cut (Lawrence and others 2003). Various labeling rules must be followed with producing enhanced meat products; some examples from the United States Department of Agriculture (USDA 2005) are included here:

> **Marinated**: a product must use a marinade that is a mixture in which food is either soaked, massaged, tumbled, or injected in order to improve taste, tenderness, or other sensory attributes, such as color or juiciness. Marinade should be that amount necessary to affect the finished product, and limited to 10% pickup in red meat.
>
> Solutions intended to impart flavor (not extend the product) may be added in any amount to uncooked, cured and uncooked, uncured red meat products including those that have been chunked, ground, wafer sliced, etc., and formed/shaped.
>
> For products marinated (i.e., soaked, steeped, massaged, tumbled, or injected in order to improve taste, texture, tenderness, or other sensory attributes, such as color or juiciness) and identified as "marinated," the solution added is limited to 10%. For other products, the qualifying statement must include the percentage of solution contained in the product, e.g., "Marinated with up to 12% of a Solution of Water, Salt, and Sugar." For uncooked products, the percent added substances for the label statement is determined by subtracting the fresh (green) weight of the article from the weight of the finished (total) product, i.e., after injecting, marinating, etc., dividing by the weight of the fresh article, and multiplying by 100. (USDA 2005, p. 104)

A typical enhancement solution contains water, salt, and phosphates. Water is required to dissolve other ingredients and provide weight to the final product. Salt, along with water, are the only two ingredients necessary for deep marination of fresh meat. Salt improves flavor and helps meat bind and hold water. Phosphates, usually alkaline phosphates such as sodium tripolyphosphate, are commonly used in marinades. Phosphates are used to improve the water-binding capacity and have some antioxidant properties. It is important to know that salt and phosphate together will improve water-holding capacity than either would alone (Siegel and others 1978).

Other ingredients that may be used in the injection solution include organic acids, such as lactic acid, diacetate, or citric acid, which improve shelf life and enhance flavor (Jensen and others 2002). Antioxidants can be added to improve display life and prevent the formation of off-flavors during storage and display. A common antioxidant is rosemary extract, which can improve the shelf life of enhanced fresh meat (Djenane and others 2003). Flavorings and flavor enhancers, such as spices, monosodium glutamate (MSG), and vegetable proteins, may also be added to marinades. Finally, natural tenderizers, for example, ficin, bromelain, and papain, can be incorporated into enhancement solutions to improve the tenderness via degradation of both muscle fibers and connective tissue by these proteases.

Guidelines for the application and use of enhanced fresh meat include

1. Deep marinades are appropriate for almost all meat cuts, but naturally tougher cuts may be better suited for enhancement. For example, there is less potential improvement in tenderness

when tenderloin is deep marinated versus top sirloin. Tougher cuts will benefit more than tender cuts. On the other hand, adding weight via deep marination to high-priced cuts results in greater profit margins (Hoffman 2006).

2. Quality of raw materials is an important consideration. Lower quality grades of beef, for example, USDA Select or no-roll, will have a greater improvement due to injections when compared with USDA Choice or Prime. Other meats, for example, pork from older animals, will show more improvement from injecting than pork that is of high quality.
3. Cuts that will be cooked to higher degrees of doneness will benefit more from deep marination as higher cooking temperature tends to reduce the moisture content of the finished product.
4. Typical injection levels for fresh meat range from 7% to 15% with little difference in consumer ratings of tenderness and juiciness between the high and low levels. Higher injection levels result in greater purge loss during shipping and display (Boles and Shand 2001).
5. Formulating salt and phosphate levels to as low as possible to adequate bind water in fresh meat is desirable from a taste and label standpoint. A common beef deep marinade formulated for a 10% injection level will often have 2–3% salt, and 1–3% phosphate, giving rise to 0.2% and 0.1% phosphate in the beef. Less phosphate will result in more purge, but using the USDA limit (0.5%) can cause off-flavors (Wierbicki and Howker 1976).

34.2.3 Preseasoned and Marinated Products

The simple addition of spices or flavoring to whole muscle cuts is another common industry practice and is referred to as "preseasoning." The production of preseasoned meats is often coupled with the addition of a marinade that is either injected into raw product, or added via soaking or tumbling. One example of preseasoned and marinated product is pork tenderloins that have been marinated and have flavors such as pepper and citrus added, which are common at many markets in the United States. Flavorings or marinades can be added as part of an injection or added via tumbling. Flavorings can also be simply added to a container and allowed to be absorbed by meat over time. Seasonings added to whole muscle can be spices, herbs, and other flavors, and the marinades can be simple salt and water solutions. However, seasonings often have an acid component, such as dilute acetic acid (vinegar) or citric acids added to improve tenderness. The addition of acids can improve tenderness considerably (Chang and others 2010) by disrupting proteins, including collagen in the meat structure.

A typical process for the production of preseasoned and/or marinades meat would include selecting the meat product to be processed, trimming and portioning the product, developing the seasonings or marinade, and applying the marinade to the meat. The addition of seasoning and marinades is often most advantageous to meat that is of lower palatability, or to products that have the potential for increased value due to the addition of ingredients. Whole muscle pork loin and tenderloin, and poultry, especially chicken breasts, are among the most commonly presasoned and marinated products. Surface fat and connective tissue are often removed before the addition of seasoning and marinades to improve visual appeal and to allow seasonings to be better incorporated into the product. Different flavor combinations of seasoning and marinades can be developed; there are endless flavor combinations that can be developed for different markets. Applying the seasoning/marinades to the product can be accomplished most rapidly by tumbling to quickly coat the surface of the meat, or by hand or mechanical application. Finally, allow the seasonings/marinades to coat the meat. This can be accomplished in the package for most meat products. It may be necessary to use packaging designed to seal in the presence of seasonings and marinades to prevent packaging failure.

34.2.4 Precooked Products

Precooking is a common method to add convenience and value to whole muscle meat products. It is possible to precook almost any meat product and achieve customer satisfaction with product quality if done properly. Fully cooked meat products are a growing segment of meat processing and offer potential for increasing profit by utilizing low-valued muscles to make higher-valued items. Classically, deli-style

roast beef is the product made by precooking beef; however, there is great potential to precook other types of beef and pork.

Precooking is often accomplished in large batches using many different types of technology and processes. For example, precooked ground beef patties are often cooked using convection ovens, and precooked bacon is often cooked at the processor level using large microwave ovens. To process for precooking can vary greatly, but usually involves preparing the product to be cooked by trimming, portioning, or addition of ingredients, batching product or placing on a conveyor, and then cooking. After the product is cooked, it will be temperature stabilized (chilled) and packaged. To ease packaging, a precooked product that is not whole muscle may be frozen. For the purposes of this discussion, precooking does not refer to cooked sausages or other common products such as ham.

Precooking whole muscle products can involve several technical hurdles, the most common being to avoid introduction of off-flavors or other product defects. The most common off-flavor associated with precooked meats in general, and beef in particular, is warmed-over flavor (WOF). WOF is often described as the "cardboard-like" flavor that occurs when meat is cooked and reheated (Gene and Pearson 1979). It is possible to minimize off-flavors in precooked beef products by knowledge about factors that cause off-flavors in beef. Preventing WOF and other defects requires several quality control procedures noted here.

34.2.4.1 Degree of Doneness

Most off-flavors in precooked beef items are the result of the oxidation of fat, causing rancidity (Gene and Pearson 1979). The addition of heat during cooking is the primary source of oxidation. Lesser doneness results in less fat oxidation and the potential for less off-flavors. Depending on the product, meat should be cooked to the lowest degree of doneness that is feasible.

34.2.4.2 Ingredients

Many ingredients are strong antioxidants and prevent the formation of WOF in cooked beef. Most notably, nitrite (cure) is a very effective antioxidant, which is why hams and sausages generally do not have problems with oxidation flavors. However, nitrite is not an option for precooked fresh meat items. Some common ingredients are also good antioxidants, including phosphates, organic acids (lactic acid, citric acid, and lemon juices), and several other products commercially available specifically to prevent oxidation. WOF development can also be decreased by the addition of Vitamin E or other antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT).

34.2.4.3 Raw Product Handling

It is not possible to start with abused or low-quality raw product and end with a high-quality finished product. Temperature abuse allows for the rapid growth of bacteria, and bacteria are able to cause oxidation during growth. On the other hand, a product that has freezer burn is also highly oxidized and will result in many off-flavors. The simple practice of maintaining proper cold storage of meat and preventing temperature abuse can improve the flavor of cooked beef products.

34.2.5 Breaded or Coated Products

The addition of breading or coatings is a common meat process not only for poultry, but also for some other meats. Breading and coatings are usually starch or flour based and the product is often cooked using oil or hot air convection. A common problem with the addition of breading and coatings is the loss of adhesion of the coating during handling and cooking. Breading losses from poor adhesion of coating to meat products is a major concern because final product quality and appearance can be greatly affected by poor-coating adhesion. There are some tried and true methods of improving batter and coating adhesion, and a significant amount of current research being conducted investigating the problem of poor batter

adhesion. The commonly used methods include predusting that is often used to maximize adhesion between the meat and the subsequent coating, and generally consists of finely ground crackermeal, fine breadcrumbs, or straight flour. A high adhesion predust may have additional flavors added, and should include powdered eggs, starches, or other ingredients that work to enhance the substrate coating.

34.2.5.1 Ingredients

Ingredients that help with batter adhesion include flour, starches, hydrocolloids, proteins, and leavening systems. Wheat flour is the main component of most batters and breading. Flours promote adhesion by providing the structure for other ingredients to bind. The typical dry batter mix contains a significant amount of flour. Flours from other sources can also be used to provide different flavors or textures, but are more expensive. Starches, which are long chains of carbohydrate (sugars), are used in batters and breading to improve crispness, batter adhesion, and moisture control. Modified starches, often having a higher amount of long-chain sugars, are better at forming film and picking up fat during frying. Modified corn starches are used most often, and others used include potato, wheat, and tapioca. Starch levels of 5–30% of the dry mixture are common.

34.2.5.2 Proper Handling of Ingredients

Proper equipment and handling of prepared batters and breading are also important: batter type (convention versus tempura) greatly affects the equipment needs and handling of the product. Being aware of the handling needs of each type of batter will result in better adhesion. For example, conventional batters can be pumped and made continuously, whereas tempura batters must be made in batches and cannot be pumped. Trying to use similar equipment for both types of batters will not work. Breading type is also important, as free-flowing breading is often easier to work with, but if the product type requires a coarse-type breading, special equipment and processes may be needed.

34.2.6 Cured Whole Muscle Products

Cured whole muscle products are a broad class of meats involving secondary processing. Curing involves adding salt and cure, usually nitrite or nitrate, to fresh products to allow the curing action to take place. The cure action does many things: providing benefits to color, taste, and preservation of many products. More extensive discussions on cured meats are presented in other chapters; the following descriptions are brief. Figure 34.1 shows a typical processing flow for whole muscle-cured meat products.

Cured meat includes ham, bacon, cured beef, and any other meat product that is whole muscle and had nitrite or nitrates as an ingredient. Meats are cured using the following common ingredients: water, salt, sugar, and nitrite/nitrate. In addition, it is common for cured meats to have phosphates, and erythorbates/ascorbates as ingredients too. Sometimes, spices or flavorings, such as black pepper and honey, are added either to the surface of the meat or to the brine to change the flavors or characteristics of products.

Methods of curing meat include the following (Pearson and Gillet 1996):

1. *Dry curing.* This is the oldest method of meat curing. Dry curing is obtained by rubbing the surface of the meat (usually ham or bacon) with salt, sugar, and cure (usually nitrates). This process takes a long time (3 months to 1 year) and results in a great amount of shrinkage and loss of product to drying out the surface. Examples of dry-cured products are country-cured hams and bacons, prosciutto hams, and country-cured bacons. Dry curing has become a very specialized industry and is limited in scope in the United States. This type of product has a very intense flavor and is highly valued by chefs.
2. *Cover curing.* Cover curing involves mixing of a brine containing salt, sugar, and nitrites in water, placing the meat in a large vat, and covering the meat with the cure. This type of curing also takes a long time (up to 6 weeks).

1. Select meat ingredients
2. Formulate recipe with salt, sweetener, cure, desired flavors, and other ingredients
3. Trim or otherwise prepare meat
4. Mix ingredients
5. Inject or add ingredients to meat
6. Allow time for curing
7. Net or prepare meat for smoking and cooking
8. Smoke
9. Cook
10. Shower
11. Chill
12. Package
13. Ship
14. Display
15. Market

FIGURE 34.1 Typical processing flow for cured whole muscle meat products.

3. *Injection curing*. Injection curing involves injecting brine directly into the meat. This greatly reduces the amount of time it takes to cure meat and is the most common type of curing in the United States. Injection curing is further broken into single-stitch or multiple-stitch injection. Single-stitch injection uses a small hand-held injector, whereas multiple-stitch injection uses a large injection machine with many needles.
4. *Artery injection*. Some meat-processing plants inject hams by placing a needle into the femoral artery and using the existing blood vessels to distribute the cure. This method is uncommon in modern curing.
5. *Combination curing*. This is a combination of injection and dry curing or cover curing. Typically, the meat is injected and then rubbed with a dry cure. By injecting the meat, time taken for curing is decreased.

34.2.7 Jerky and Dried Meats

Jerky and dried meats are an important meat category that has grown in recent years. Jerky can be either whole muscle or ground and formed, with the process being substantially different depending on the product. Some important considerations about beef jerky are as follows:

1. All Jerky products must have a moisture-to-protein ratio (MPR) of 0.75:1 or less. This ensures that bacterial growth is very limited and it allows the product to be stored at room temperature. Jerky products may be cured or uncured, dried, and may be smoked or unsmoked, and air or oven dried.
2. Beef Jerky is produced from a single piece of beef.
3. Beef Jerky Chunked and Formed is produced from chunks that are molded and formed and cut into strips. This allows for the addition of flavorings and for more precise portioning.
4. Beef Jerky Ground and Formed or Chopped and Formed is produced as described, where the meat block may be finely ground before being molded and formed and cut into strips.
5. Jerky may be dried at any stage of the process.

The process of making whole muscle jerky is fairly simple. Large pieces of meat, usually beef round or shoulder, is sliced into thin strips and mixed with a marinade. The marinade typically contains salt, sweetener, and other flavors. In addition, the marinade may contain cure (nitrite) to prevent bacterial

growth and to serve as an antioxidant. The meat may simply be allowed to soak in the marinade for several hours, or the meat and marinade may be tumbled to decrease production time. After the meat has absorbed the marinade, it is laid out and smoked or dried. After drying, the jerky can be portioned and packaged.

34.3 Comminuted Products

Comminuted products are meats that have been reduced in size via some sort of grinding, cutting, or other processes. The most common product of this type of secondary processing is sausage. There are literally thousands of methods of comminuted meat manufacturing; only some common themes are covered in this chapter. Note that comminuted products with no other ingredients added, such as fresh ground beef or ground pork, are not discussed here as they are considered part of primary processing.

Sausages include a very large range of comminuted meat products, with the only commonality being that meat is ground, and salt is added. There are three major classifications of sausage: fresh, cooked, and dry/semidry. Each classification has different characteristics and processes, but they share a common start. For sausage manufacturing, meat is comminuted by grinding, finely chopping, or emulsifying. To this comminuted meat, salt is added. In fact, the only requirement for a sausage is ground meat and salt. However, several other ingredients are commonly used in sausage manufacturing, including sweetener, cure (nitrite), spices/seasonings/flavorings, phosphates, lactates, erythorbates/ascorbates, starter cultures, and nonmeat food ingredients.

34.3.1 Fresh Sausage

Fresh sausage is a very common type of processed meat product and is the easiest to make. What makes a product a "fresh" sausage is the fact that no cure (nitrite) is added, and the sausage is not cooked during processing. Fresh sausages may be frozen to improve shelf life and quality and still be referred to as "fresh." Fresh sausages must be kept refrigerated and cooked by the consumer before consumption. Sometimes, fresh sausages are cooked at the processing plant before sale; these sausage are called "fresh-cooked" sausage and have a unique appearance as they do not have any cure (nitrite) added.

Here is a summary of the characteristics of fresh sausages:

1. Fresh sausages must not contain any cure (nitrate or nitrite).
2. Fresh sausages should not contain any phosphates.
3. Fresh pork sausage typically has a high-fat content, as much as 50% total fat.
4. Fresh sausages, and especially fresh pork sausages, typically have antioxidants added. The antioxidants BHA, BHT, or propyl gallate are commonly added to fresh pork sausages.
5. Fresh beef sausage typically has a lower fat content than pork, usually around 30% fat.
6. Small amounts of water or ice may be added to fresh sausages to assist with mixing and ingredient dispersion. In the United States, the amount of water added to fresh sausages is limited to 3%.
7. Whole hog sausage is a fresh sausage made from the meat of an entire hog in the proportions normally found in a hog carcass.

Fresh sausage manufacturing is among the simplest of all secondary meat processing. A meat source is mechanically chopped into small pieces, often using a grinder, subsequently, salt and other ingredients are added. The meat and ingredients are mixed, which can be done by hand for small home applications, or by larger mixers for commercial meat production, and then either stuffed into a casing, or packaged in bulk. After stuffing or packaging, the fresh sausage is kept refrigerated until cooking. Figure 34.2 shows a typical processing flow for fresh sausages.

1. Select meat ingredients
2. Formulate recipe with desired flavors, fat percentages, and other ingredients
3. Grind meat
4. Mix ingredients
5. Stuff or package
6. Chill or freeze
7. Package
8. Ship
9. Display
10. Market

FIGURE 34.2 Typical processing flow for fresh sausages.

34.3.2 Cooked Sausages

Cooked and/or emulsified sausages are the most widely consumed sausage products (hot dogs). Cooked sausages are fully cooked prior to sale, but still must be kept refrigerated. Cooked sausages are sometimes reheated, for example, smoked sausages, but are also consumed without further cooking (bologna).

Summarized below are the characteristics of cooked sausages:

1. Cooked sausages almost always contain cure (nitrite), which is added at a level of 156 ppm nitrite according to USDA standards.
2. Hot dogs, also called frankfurters, contain a maximum fat of 30%, and maximum added water plus fat of 40%.
3. Cooked sausages will often have phosphates added to prevent excessive cooking loss.
4. Fully cooked sausages must be cooked to a minimum of 50°C, with most cooked to around 55°C.
5. Water or ice is added to most cooked sausages. Ice is added to emulsion-type sausages to keep temperatures low during emulsification.

Producing cooked sausages starts in a similar way to fresh sausage, as meat is ground or chopped and ingredients are added. Many cooked sausages are emulsified products. Emulsified sausages are produced by chopping the meat very finely using a bowl chopper or emulsifier. Emulsified sausages have a distinct texture, and are capable of holding large amounts of fat and water. Cooked sausages are normally stuffed into some sort of casing before cooking. Cooked sausages differ from fresh sausages in that cure (nitrite) is commonly added, and the sausages are cooked at the processing facility. There are numerous methods to cook sausages, including ovens, smokehouses, infrared, steam, and hot water. After sausages are cooked, they are rapidly cooled to improve quality and prevent microbial growth. Figure 34.3 shows a typical flow for cooked sausages.

34.3.3 Fermented Sausages

Fermented sausages are the oldest type of sausages produced. Some facts about fermented sausages:

1. Fermented sausages fall into two broad categories: semidry, which includes summer sausages (cerveleat, thuringer, etc.) and snack sticks; and dry, which includes pepperoni and salamis.
2. Fermented sausages are generally shelf stable and may or may not be cooked at all before consumption.
3. Dried sausages are generally never cooked; they rely on fermentation and drying to prevent microbial growth.
4. If pork is used in dried sausages, it must be "certified" to ensure the destruction of trichina worms.

1. Select meat ingredients
2. Formulate recipe with desired flavors, fat percentages, and other ingredients
3. Grind meat
4. Mix ingredients
5. Stuff or form
6. Link or tie
7. Hang
8. Smoke
9. Cook
10. Shower
11. Chill
12. Package
13. Ship
14. Display
15. Market

FIGURE 34.3 Typical processing flow for cooked sausages.

5. Sausages are fermented by the addition of starter cultures or using the natural microbes in the meat. Using starter cultures provides a much more consistent product than natural microbes. Old-time sausage makers would use a few pounds of an existing batch to spread microbes into a new batch. This was called "backslopping" and is very dangerous as it can spread pathogens as well as other microbes.
6. The pH of meat is around 5.6; the pH of fermented sausages will often decrease below 4.5 due to lactic acid production.
7. When making fermented sausages, simple sugars (e.g., dextrose) are added to provide an energy source for the microbial culture.
8. Sausage also can be acidified by the addition of encapsulated acids, such as lactic or citric acid or by the use of glucono delta lactone.

Dried sausage manufacturing is truly the highest art of meat processing. As with any sausage, the process starts by reducing meat particle size, adding ingredients, mixing, and stuffing the meat into casings. At this point, the production of dried sausages differs from that of fresh or cooked ones. Typically a starter culture of lactic-acid-producing bacteria is added to promote fermentation and a pH decline. To promote the growth of these bacteria, the sausages are subjected to various levels of temperature and humidity. After the pH has declined, the sausage may be dried by hanging in a drying room, or sometimes the sausages are cooked or partially cooked to add smoke and other flavors. Dried sausages are often shelf stable due to pH and low water activity.

34.3.4 Formed Ground Meat Products

Formed products are generally defined as comminuted meat, given a specific shape or size using mechanical means. Meat formed as patties, meatballs, nuggets, and several other shapes and sizes makes up a large amount of the total meat consumed in the United States and is especially important to foodservice. In fact, over 44% of all beef consumed is ground and a large portion of that is formed (Davis and Lin 2005). Hamburgers account for over 75% of beef servings consumed away from home (Davis and Lin 2005). Forming high-quality meat products requires using quality meat and the proper application of technology.

The process of forming meat products starts with selecting high-quality meat. The quality of formed meats can be defined as the ability to hold shape through further processing such as breading, freezing, in-plant cooking, and packaging. Other important quality factors are consistent size, shape, and weight, cooking yields and color, and consumer acceptability. In order to produce high-quality formed products,

some important things need to be considered, for example, source of trimmings and collagen content of meat. Trimmings affect nearly all the other quality measures. Market animal trimmings (steers, heifers, barrows, and gilts) have different characteristics than trimmings from cull animals such as bulls, cows, or sows. The types of trimmings are also important. Typically, trimmings from market animals will have less connective tissue, more fat, and a lower total microbial content than trimmings form cull animals. Collagen is the primary component of connective tissue. Collagen has a unique structure that allows it to stretch and contract. This structure causes collagen to shrink when heated. Formed meats containing high amounts of collagen will shrink during cooking, changing the shape of the product. For this reason, high-collagen meats such as shanks and beef cheeks should be limited to <10% of most formed products. Collagen content can be limited by limiting use of shank meat, or by using low connective tissue meat such as primal and subprimal trimmings or bull meat and boneless cow meat along with shank meat.

After selecting the ground product, it is ground, and often ingredients are added to the ground meat. Depending on the final product, salt, phosphates, flavorings, binders, and spices may be added. For example, formed chicken patties will have salt and binders added before forming. After ingredients are properly mixed, the meat is placed through a forming machine. Meat formers vary widely depending on application, but generally fall into one of two types: those that have a mold and those that extrude meat. Meat formers that use a mold are often used for products that are flat, such as ground beef patties, whereas extruding formers are often used for products such as meatballs. Once the meat is formed, other processes such as batter/breading, cooking, and seasoning may occur. Most formed meats are individually quick frozen before packaging to improve quality and handling properties.

34.4 A Brief Comment on Food Safety

Safety considerations are essential in the production of any meat product. Even though safe meat processing is discussed elsewhere in this book, it is important to always put safe production of meat at the beginning of any process. What follows is a brief review of important safety considerations when producing any meat or meat product.

1. *Temperature*. Proper cold and hot temperatures are the most effective method of controlling bacterial growth and ensuring food safety. Meat should be processed at temperature below 4°C, properly cooked, and rapidly chilled.
2. *Microbiological quality*. Meat is routinely tested at processing plants, and it is important to have thresholds for routine microbiological testing. There is essentially a zero tolerance for pathogenic bacteria, but total plate counts that serve as quality control (QC) markers may have a limit as well. Excessive microbial growth is an indicator that meat was not produced in a sanitary manner, was subjected to temperature abuse, or was contaminated at some point. High microbial counts are a good indication that raw meat should be redirected to fully cooked products.
3. *Foreign materials*. Some foreign material is a quality concern, and some is a food safety concern. Make sure that they are differentiated in all cases. For example, a stray meat hook that ended up in a combo of beef trim is more of a quality concern, but glass or metal fragments found in raw meat are food safety concern.

There are, of course, a number of other food safety concerns that need to be addressed, including packaging, personnel, rework, and so on. These concerns are generally based on processor practices and experience. The safe production of meat products is addressed elsewhere in this book.

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35

Meat and Commerce in the United States: Label Requirements and Purchase Specifications[*]

Y. H. Hui

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[*] The information in this chapter has been modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. Used with permission.

35.1 Introduction

The title of this book is *Handbook of Meat and Meat Processing*. Traditionally, a book of this nature is devoted to the basics in the science and technology of processing meat and meat products. The goal of all this knowledge is to produce products that will attract consumers to buy them. Consumers buy meat and meat products because they are a source of quality protein and because they offer superior sensory attributes such as appearance, taste, aroma, and so on, when compared with other categories of food and beverage products. In a modern society, fraud can exist in any level of the food chain of commerce before the product is offered for sale at a grocery store. Obviously, every country has its own requirements and specification governing the commerce of meat and meat products to protect the consumers. In the United States, the concerns of the government revolve around four major premises in the buying and selling of meat and meat products:

1. To assure the safety of the products.
2. To minimize or reduce fraud, with an assumption that fraud will exist with or without government intervention.
3. To offer quality assurance of the products.
4. To facilitate the selling and buying of meat and meat products at two levels: institutional purchases and consumer markets.

35.2 Labeling Policy

Any food label covers: identity, ingredients, nutrition, cooking instruction, safety warning, and other items of concern. The information on a food label achieves two objectives: safety and avoid economic fraud. The safety of processed meat and meat products is discussed in several chapters. This chapter provides some discussion on the standard of identity of the product on the label of a package (can, etc.) of meat product. By giving a standard of identity for a particular name of a meat product, the same language prevails in the commerce of meat and meat product. This facilitates trade for domestic manufacturers and importers.

The complete labeling document can be obtained from the website of the United States Department of Agriculture (USDA), Food Safety and Inspection Service (FSIS), http://www.fsis.uda.gov. The following section discusses the labels or standards of identity for selected meat and meat products.

35.2.1 Beef Burgundy or Bourguignonne

The product must contain at least 50% beef. Product contains beef cubes, mushrooms, onions, and red wine or burgundy gravy. May include other vegetables, for example, carrots, shallots, tomato paste, or potatoes. Other acceptable names include “Beef a la Bourguignonne,” “Beef Burgundy Style,” “Beef Burgundy,” and “Burgundy Beef.”

35.2.2 Breakfast Links or Patties

The names “Breakfast Links” and “Breakfast Patties” can be considered fanciful names, which must be followed by a descriptive product name. Such products are acceptable without compliance with the fresh pork sausage or breakfast sausage standard. If the names “Breakfast Links” or “Breakfast Patties” are used without further qualification, the products must meet either the fresh pork sausage standard or the breakfast sausage standard.

35.2.3 Canadian and Canadian Style Bacon

“Canadian Bacon” and “Canadian Style Bacon” are synonymous and should not be considered geographical terms.

The term "Canadian Style Bacon," when featured on the label as a product name or part of a product name (i.e., as a description, etc.), may stand alone without an additional qualifier indicating the true geographical origin of the product.

"Chunked and Formed" and "Water Added" products are permitted, provided proper labeling is applied.

Uncooked and/or unsmoked "Canadian Style Bacon" is also permitted, provided labeling describes the product as uncooked and/or unsmoked.

Product which is identified as "Canadian Style Bacon" is made from a trimmed boneless pork loin. On the shoulder end, the cross section of the longissimus dorsi muscle shall be equal to or larger than the combined cross-sectional areas of the splenius and semispinalis capitis muscles. The ham end shall be removed anterior to the ilium. The exposed faces shall be approximately perpendicular with the skin surface. The dorsal and ventral side on each end of the "Canadian Style Bacon" shall not be more than 1.0 in. different in length. The belly is removed adjacent to the longissimus dorsi muscle. All bones and cartilage shall be removed. The tenderloin and the flesh overlying the blade bone are excluded. The surface fat (and false lean when necessary) shall be trimmed to 0.3 in. thick at any point. The fat on the ventral and dorsal sides is neatly beveled to meet the lean.

35.2.4 Chili

1. "Brick Chili" or "Condensed Chili" requires 80% meat. Cereal is limited to 16%.
2. Chili with reconstitution directions should meet the chili standard when reconstituted.
3. When beef heart meat, cheek meat, or head meat is used in excess of 25% of the meat block, it must be reflected in the product name, for example, "Chili with Beef and Beef Heart Meat."
4. When beef appears in the product name, *beef may be the only meat source used*. Beef Chili may not contain beef fat or other beef by-products.
5. "Chili Gravy with Meat" requires at least 40% fresh meat and no more than 8% cereals.
6. Cured meats are not an expected ingredient in chili; when used, they must be shown as part of the product name.
7. The terms "Chili" or "Chili con Carne" may be used interchangeably.
8. Since "con carne" means "with meat," products labeled as chili con carne should include only red meat and not poultry. Products which meet the chili standard and include poultry may be labeled "beef and chicken chili," "beef chili, chicken added," and so on, as appropriate. The binder and extender limitation of 8% is based on total formulation.

35.2.5 Chinese Pepper Steak

A Chinese main dish, usually served with rice, must contain at least 30% cooked beef. Beef steak is cut into thin strips, browned in fat or oil, and added to a soy-flavored sauce. Vegetables are also added to the sauce. Green pepper strips are always used and other vegetables may be included.

35.2.6 Chipped Beef

Beef that is dried, chipped, or sliced and may be cured or smoked. An MPR 2.04:1 is required. It may be chunked, ground, chopped, and formed. If so, the product name must be qualified, for example, "Chipped Beef, Chunked and Formed."

Acceptable fill:

1. 2 oz. in a 4 fluid oz. glass, or
2. 2 1/2 oz. in a 5 fluid oz. glass, or
3. 5 oz. in a 9-5/8 fluid oz. glass.

35.2.7 Cutlet, Pork

"Pork Cutlet" may consist of pork temple meat, inside masseter muscles, and small pieces of lean from the tip of pork jaws. These are flattened and knitted together in "cutlet" size products by means of "cubing" or "Frenching" machines, or by hand pounding with "cubing hammers." The term "cutlet" relates to thin slices of meat. They can be identified as sliced pork meat product when the designation clearly states the specific part of the carcass from which the meat in the product is derived (e.g., "Pork Loin Cutlets"). All the terms should be conspicuously displayed on labels.

35.2.8 Eggs Benedict

The product must contain at least 18% cured smoked ham, and a poached egg on a toasted English muffin, topped with a slice of ham, and covered with hollandaise sauce.

35.2.9 Fajitas

The Spanish translation is "little belts" or strips of meat. Fajitas are strips of seasoned or marinated red meat or poultry meat, which have been cooked. Red Meat Fajitas require labeling in accordance with the current policy memo on added solutions. Fajitas may also be sandwich-like product, requiring 15% strips of cooked meat or poultry meat (excluding the solution above green weight), topped with onions, peppers, and sauce, and rolled in a flour tortilla. Fajita, including the name of the meat or poultry, may stand alone, for example, "Beef Fajita," "Chicken Fajita."

35.2.10 Ground Beef Chuck and Round

Product to be labeled "Ground Beef Chuck" or "Ground Beef Round" must comply with the following guidelines:

1. "Ground Beef Chuck" must be derived from all or part of the primal part of the beef carcass commonly referred to as the "Beef Chuck," except as provided for in 3. The product must comply with the fat requirements of 9 CFR 319.15(a).
2. "Ground Beef Round" must be derived from all or part of the primal part of the beef carcass commonly referred to as the "Beef Round," except as provided for in 3. The product must comply with the fat requirements of 9 CFR 319.15(a).
3. Generally, shank meat may be added but may not exceed the natural proportion of the beef carcass, which is considered to average 6%. Higher quantities of shank meat may be used if the shank meat remains attached during the cutting and boning of the boneless chuck or round, or if the processor can demonstrate that a higher percentage is applicable.

35.2.11 Ham, Fresh (or Uncured)

Ham that does not contain a cure must be labeled either "Fresh" or, if the ham meets the requirements of 9 CFR 319.2, "Uncured." This also applies to cooked product, and must be labeled cooked product "Cooked Uncured Ham."

35.2.12 Honey Cured or Sugar Cured

"Honey Cured" may be shown on the labeling of a cured product if:

1. The honey used contains at least 80% solids or is U.S. grade C or above.
2. Honey is the only sweetening ingredient or when other sweetening ingredients are used in combination with honey, they do not exceed one-half the amount of honey used; and honey barbecue touch of honey.

3. Honey is used in an amount sufficient to flavor and/or affect the appearance of the finished product.

Traditionally, cured products that are labeled to indicate the presence of honey, for example, Honey ham, must meet the parameters prescribed herein.

"Sugar Cured" may be used on the labeling of a cured product if:

1. The sugar used is cane sugar or beet sugar.
2. Sugar is the only sweetening ingredient or when other sweetening ingredients are used in combination with sugar, they do not exceed one-half the amount of sugar used.
3. Sugar is used in an amount sufficient to flavor and/or affect the appearance of the finished product.

"Honey and Sugar Cured" or "Sugar and Honey Cured" may also be used on labeling if:

1. The honey and sugar are of the nature described above.
2. The honey and sugar are the only sweetening agents or when other sweetening ingredients are used in combination with the honey and sugar, they do not individually exceed either the amount of honey or sugar used and collectively do not exceed one-half the total amount of honey and sugar.
3. The honey and sugar are used in amounts sufficient to flavor and/or affect the appearance of the finished product.

35.2.13 Jambalaya

The product must contain at least 25% cooked ham and one other meat or seafood must be included. A New Orleans dish includes rice and ham, and usually tomatoes (shrimp or other shellfish, other meat or poultry), together with seasonings. The true product name, for example, "Ham and Shrimp Jambalaya" must be shown.

35.2.14 Kielbasa

A sausage that is cured, cooked, and usually smoked. Kolbassy is Czechoslovakian spelling; other variations include Kielbassy, Kolbasa, and Kolbase. Kielbasa is made from coarsely ground pork or coarsely ground pork with added beef or mutton. "Hungarian Style Kolbase" is finely ground product, seasoned and stuffed into casings. The 70/30 rule can be used; however, pork must always be the predominant meat ingredient. "Beef Kielbasa" is prepared with only beef as the meat ingredient. By-products are not permitted ingredients in these sausages.

An uncured (fresh), uncooked variety, with not more than 3% water exists. "Fresh" shall be used in the name when the product is uncured. When fresh Kielbasa is cooked or smoked, then cooked or smoked is required in the product name. The requirements of Policy Memo 110 apply when these perishable, cooked, uncured products are packaged in hermetically sealed containers.

35.2.15 Lasagna

Sauce is an expected ingredient of lasagna products and its declaration in the product name is optional.

Cheese lasagna with meat: 12% meat

Lasagna with meat and sauce: 12% meat

Lasagna with meat sauce: 6% meat in total product

Lasagna with poultry: 8% poultry meat

Lasagna with tomato sauce, cheese, and pepperoni: 8% pepperoni

Meat lasagna: 12% meat
Poultry lasagna: 8% poultry meat

35.2.16 London Broil

The name can only be applied to a cooked product. Products including the expression "London Broil" on labels must be prepared with beef flank steak. Uncooked product must be labeled to indicate this, for example, "Beef Flank Steak for London Broil." If prepared from another cut, the identity of that cut must accompany the term "London Broil," for example, "Sirloin Tip London Broil."

35.2.17 Luncheon Meat

1. "Luncheon Meat" cannot contain livers, kidneys, blood, detached skin, partially defatted pork or beef tissue, or stomachs.
2. On the label, the meat components of "Luncheon Meat" are identified in the ingredients statement as "beef," "pork," "beef tongue meat," "pork tongue meat," "beef heart meat," and "pork heart meat."
3. In the ingredients, statement "Beef" and "Pork" means lean meat with overlying fat and the portions of sinew, nerve, and the blood vessels which normally accompany muscle tissue and which are not separated in the process of dressing but not including bone and skin. Up to 10% of the meat portion of the formula can consist of cured and smoked meat trimmings, which does not require special declaration in the ingredients statement except included under "pork" and "beef."
4. Heart or heart muscle, tongues, or tongue meat and cheek meat can be included in "Luncheon Meat" under the following restrictions:
   a. Hearts or heart meat or tongues or tongue meat must be declared individually by species in the ingredients' statement on the label.
   b. No restriction on the percentage limits of hearts, heart meats, tongues, and tongue meats in the formulation.
   c. The terms "heart meat" and "tongue meat" refer to the muscle tissue remaining after heart caps, glands, nodes, connective tissue, and so on are trimmed away.
5. Water added to "Luncheon Meat" during manufacture cannot exceed 3% by weight of the total ingredients, and this is controlled by weighing ingredients and not by analysis. Care must be used to see that water is not added indirectly through the use of undrained hearts and tongues.
6. The only ingredients permitted in "Luncheon Meat" are curing ingredients, sweetening agents, spices, and flavoring. All these substances must be declared in the ingredients statement by name, except for the various "flavorings" and "spices" which need not be named individually. "Spices" refer to natural spices and not to extracts.

35.2.18 Meatballs

Uncooked or cooked pork, beef, veal, and lamb, and other ingredients in a ball form.

1. The product must contain at least 65% meat.
2. The binders and extenders are limited to 12% of the total product. A 6.8% of isolated soy protein is considered equivalent to 12% of the other binders or extenders. The permitted binders and extenders include, but are not limited to, cereal, bread crumbs, cracker meal, soy flour, soy protein concentrate, isolated soy protein, and textured vegetable protein.
3. Cheeks, hearts, and tongues are not allowed, but product may contain head meat, cheek meat, heart meat, and tongue meat when declared in the ingredients statement.
4. Partially defatted chopped (PDC) (species) may be used up to 25% of the meat block. PDC (species) can be identified as (species) in the ingredients statement. (See entry for PDC species.)

35.2.19 Pepperoni

A dry sausage prepared from pork or pork and beef. Combinations containing more than 55% beef are called beef and pork pepperoni. Pepperoni made with beef must be called beef pepperoni. Pepperoni must be treated for destruction of possible live trichinae and must have an MPR of 1.6:1 or less. Antioxidants are permitted in pepperoni. The casing, before stuffing, or the finished product, may be dipped in a potassium sorbate solution to retard mold growth. Extenders and binders are not permitted in pepperoni. Hearts, tongues, and other by-products are not acceptable ingredients.

35.2.20 Polish Sausage

A sausage that is cured, cooked, and usually smoked. Pork and pork by-products shall comprise at least 50% of the meat and meat by-product ingredients. To have beef as a predominant ingredient, the product name would be "Beef and Pork Polish Sausage." Green peppers are permitted up to 4% in total formulation.

An uncured (fresh), uncooked variety with not more than 3% water also exists. "Fresh" shall be used in the name when the product is uncured. When Fresh Polish Sausage is cooked or smoked, then the product name is either "Cooked Fresh Polish Sausage" or "Smoked Fresh Polish Sausage." The requirements of Policy Memo 110 apply when these perishable, cooked, uncured products are packaged in hermetically sealed containers.

35.2.21 Salisbury Steak

Finished product must contain at least 65% meat. Fat is limited to 30%. Other requirements are

1. It is an unbreaded cooked product.
2. The meat block may contain 25% pork, with the remainder being beef. Or, the meat block may contain up to 12% PDC beef and pork.
3. Extenders are permitted up to 12%. When isolated soy protein is used, 6.8% is the equivalent of 12% of the other extenders. Those extenders include, but are not limited to: cereal, bread crumbs, cracker meal, soy flour, soy protein concentrate, isolated soy protein, and textured vegetable protein.
4. Meat by-products are not permitted. Beef heart meat is permitted.
5. Permitted liquids include, but are not limited to: water, broth, milk, cream, skim milk, and reconstituted skim milk [9 parts water to 1 part non fat dry milk (NFDM)].
6. Product not cooked, which conforms to the above, may be labeled "Patties for Salisbury."

35.2.22 Soup Products

Bean & Ham Shank: When soup is made from ham shanks, they must be shown in the true product name, for example, "Bean and Ham Shank Soup."

Blood: Product must contain at least 1% blood and be made under inspection. Chowders: Follow standard for soups.

Consomme: A broth cooked with vegetables and then strained. Must have an MPR of 135:1.

Consomme Instant: Dehydrated—not amenable.

Cream: Condensed cream soups may be made from various creams, whole milk, or dry milk powder. The amount of cream, whole milk, or dry milk powder should provide a minimum of 0.45% butterfat to the final product. Examples:

1. A cream containing 18% butterfat should make up the product formulation; this provides 0.45% butterfat to the product formulation.
2. Dry milk powder containing 27% butterfat should make up 1.67% of the product formulation.

Dried Meat Soup Mixes: Not amenable.

Italian Style Minestrone: Soup must contain zucchini. Identify meat in the true product name.

Pepper Pot: Soup must contain at least 20% scalded tripe.

Petite Marmite (FR): A soup made with meat, chicken, and vegetables.

Scotch Broth: Soup must contain at least 3% mutton in a thick mutton broth.

Vegetable: Vegetable soups made with soup stock are not considered amenable.

35.2.23 Swiss Steak

Swiss steak and gravy: Contains not <50% cooked beef.

Gravy and swiss steak: Contains not <35% cooked beef.

Product labeled "Swiss Steak" must be floured or dusted before searing, or may have flour added to gravy.

35.2.24 Veal Parmigiana

The following categories of products exist

1. "Breaded Veal Parmigiana" is the product name for a solid piece of veal that is breaded and topped with cheese and tomato sauce. Breaded cooked veal must represent 40% of the finished product.
2. "Breaded Veal Parmigiana, Chopped and Formed Beef (or Beef Fat) Added" is the product name for chopped veal with up to 20% beef and/or beef fat added that is formed, breaded, and topped with cheese and tomato sauce. The chopped and formed beef added statement is shown to be one-third the size of "Veal" contiguous to the product name. Breaded cooked patty must represent 40% of the finished product.
3. "Breaded Veal Parmigiana made with Veal Patties, Beef (or Beef Fat) Added" is the product name for a veal patty containing at least 70% fresh meat (in unbreaded patty) of which 20% may be beef or beef fat. The patty is breaded, topped with cheese and tomato sauce. The entire qualifying statement in the product name is to be shown one-third size of "Veal" contiguous to product name. The breaded cooked patty represents 40% of the finished product.
4. Breaded Veal and Beef Patty Parmigiana. The patty may be prepared in proportions as governed by 9 CFR 317.2(f)(1)(v) of the regulations; the minimum meat patty requirement is 50%. If the product is breaded, the name must reflect this fact. The cheese component of the product does not have to be shown in the name of the product. A specific kind of cheese is not required, although Romano, Mozzarella, and Parmesan are the usual types used. No specific spelling of the word "Parmigiana" is required. Name applies to a "Cooked Product Assembled, Ready to Heat and Eat."

The labeling of Veal Parmigiana made from a veal patty shall include veal patty in the product name, for example, "Breaded Veal Parmigiana made with Veal Patties" or "Breaded Veal Patty Parmigiana." The ingredients of the veal patty do not have to be part of the product name.

35.3 Institutional Meat Purchase Specifications

35.3.1 Guidelines

To facilitate the buying and selling of meat between institutions (schools, hospitals, restaurants, prisons, etc.) and meat companies, the USDA through its Agricultural Marketing Service (AMS) develops and maintains the Institutional Meat Purchase Specifications (IMPS) for meat and meat products. These

TABLE 35.1

IMPS Series

General requirements
Quality assurance provisions
Fresh beef: Series 100
Fresh lamb and mutton: Series 200
Fresh veal and calf: Series 300
Fresh pork: Series 400
Cured, cured & smoked, and fully cooked pork products: Series 500
Cured, dried, and smoked beef products: Series 600
Edible by-products: Series 700
Sausage products: Series 800
Goat and goat products: Series 11

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specifications have become the language of commerce of meat and meat products in the United States. They also serve frame of reference for countries exporting meat to the United States. The IMPS series includes those described in Table 35.1.

These specifications are recommended for use for any meat product procurement activity. For assurance that procured items comply with these detailed requirements, the USDA, through its Meat Grading and Certification Branch (MGCB), provides a voluntary Meat Certification Service. For labeling purposes, only product certified by the MGCB may contain the letters "IMPS" on the product label. In each document, the names and definitions of portion cuts are also provided. These portion cuts are the names we see in grocery stores and restaurant menus.

The purpose of each document is to provide the purchaser with a variety of options for meat handling, packing and packaging, and certification that conform to good commercial practice. Contractors are responsible for delivering products that comply with all contractual and specification requirements and are advised to set up a quality control system designed to assure compliance. Thus, when a restaurant buys meats, for example, beef or pork, it can tell the seller, following specification of IMPS series 100—Beef, or IMPS series 400—Pork. Or, it can ask the USDA to serve as a neutral party to assure it will receive what it buys.

Each IMPS series is government by two approaches: general requirements and product specifications. The general requirements are given in Table 35.2.

A brief discussion of information in Table 35.2 follows.

For the purposes of determining acceptance of any IMPS item, the following definitions are provided.

Chilled: Meat product having an internal temperature >28°F (–2.2°C) and is held under refrigerated storage temperatures.

Continuous operation: A processing method that proceeds without excessive or unnecessary interruptions.

Fresh: Product, that has not been canned, cured, smoked, or cooked.

Fresh-chilled: Fresh product that has never been previously frozen.

Frozen: Product that has an internal temperature <28°F (–2.2°C) and is stored at <0°F (–17.8°C).

Stockpile: Product that is produced and stored according to IMPS requirements without a contract or purchase order.

All meat and meat products will originate from animals slaughtered in establishments regularly operated under the Meat and Poultry Inspection Operations (FSIS) regulations of the FSIS, or under a system

TABLE 35.2

Requirements to Accompany Each IMPS of the Series

1. Definition of terms
Chilled
Continuous operation
Fresh
Fresh-chilled
Frozen
Stockpile
2. Regulatory requirement
3. Ordering data to be specified by the purchaser
Meat handling
Packaging and packing
USDA certification
4. USDA certification
Certification of meat handling requirements
Purchaser specified examinations
Sealing
Destination inspection
Issuance of certificates
Waivers and amendments to specification requirements

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of meat inspection approved by FSIS. In addition, meats and meat products will be processed in establishments that meet this requirement.

Optional-ordering data include meat handling, packaging and packing, and USDA certification. All other purchaser options are contained within the individual IMPS documents. In the front of each document are the ordering data options available for the species or type of product specified within that document. Additionally, located within the individual item descriptions, are the specific options available for each item. Ordering data will be specified by the purchaser in the contract or purchase order.

The contractor will assure that products being delivered will be in compliance with all applicable contracts, purchase orders, and specification requirements. Final acceptance of all products will be by the consignee at the point of delivery. The consignee will thoroughly review each incoming delivery to insure that temperatures, box markings, appropriate stamps, delivery dates, and other obvious contractual or specification requirements have been met.

When specified by the purchaser, meat items will be subject to testing for metal contamination. Testing will be conducted after fabrication of the finished product (but may take place before or after patty formation) and before or after packaging. For bulk ground meat or for stacked patties or patties tested after packing, the vendor will install and operate equipment capable of detecting the presence of a 3.0 mm, 440 stainless-steel test standard. For patties tested in single patty layers before final packaging, the equipment used will be capable of detecting the presence of a 1.5 mm, 440 stainless-steel test standard.

The USDA website (http://www.usda.gov) presents all series for the IMPS. Sections that follow discuss some examples of IMPS for fresh beef.

35.3.2 Fresh Beef: Series 100 Product Specifications

The following discusses selected items under this series.

Item No. 100—Beef Carcass

The carcass shall consist of two matched sides each consisting of a forequarter and a hindquarter. The sides shall be produced by splitting the carcass down the back exposing the spinal groove at least 75% of

the length of either side. No more than a minor amount of major muscles shall be removed from either side. The quarters are produced by completely or partially separating the forequarters from the hindquarters by a cut following the natural curvature between the 12th and 13th ribs. The diaphragm may be removed. However, if present, it shall be firmly attached and the membranous portion shall be trimmed close to the lean. The *thymus* gland and heart fat shall be closely removed.

Item No. 103—Beef Rib, Primal

The primal rib is that portion of the forequarter remaining after removal of the cross-cut chuck and short plate and shall contain seven ribs (6th to 12th inclusive), the posterior tip of the blade bone (scapula), and the thoracic vertebra attached to the ribs. The loin end shall follow the natural curvature of the 12th rib. The chuck is removed by a straight cut between the 5th and 6th ribs. The short plate shall be removed by a straight cut which is ventral to, but not more than 6.0 in. (15.0 cm) from, the *M. longissimus dorsi* at the loin end to a point on the chuck end ventral to, but not more than 10.0 in. (25.4 cm) from, the *M. longissimus dorsi*. The diaphragm and fat on the ventral surface of the vertebrae shall be removed.

Item No. 114A—Beef Chuck, Shoulder Roast

This item is as described in Item No. 114 except that the shoulder shall be trimmed so that it is not <1.0 in. (25 mm) thick at any point, except within 3/4 in. (19 mm) of the juncture of the *M. trapezius* and *M. latissimus dorsi*. In this instance, the underlying fat must be trimmed to comply with the surface fat thickness requirements. When smaller roasts are specified, the thick (arm) end of the shoulder shall be separated from the thin (blade) end and if necessary, subsequent cuts shall be made at right angles to the blade side splitting the arm end into approximate equal portions. The blade end shall be split lengthwise into approximately equal portions, the ends shall be reversed, the boned surfaces placed together, and if necessary, a subsequent cut(s) shall be made at a right angle to the length of the blade portion into approximate equal portions. All roasts shall be netted or tied.

Item No. 123—Beef Short Ribs

This item consists of the rib section from any rib and/or plate item and shall contain at least two but not more than five ribs (ribs 6 through 10). The dorsal side shall be at an approximate right angle to the rib bones and the *M. latissimus dorsi* shall be continuous across the cut surface. The ventral side shall be a straight cut which is approximately parallel to the dorsal side and does not contain any costal cartilages. The *M. cutaneous trunci*, diaphragm, and serous membrane (peritoneum) shall be removed. The surface fat shall be trimmed NTE 1/4 in. (6 mm) at any point. The purchaser shall specify the number of ribs and the width (distance between the dorsal and ventral sides) of the rib sections.

Item No. 136—Ground Beef

Unless otherwise specified, ground beef may be derived from boneless meat which has been frozen and stockpiled. The purchaser may specify the maximum amount of frozen boneless meat that can be mixed with fresh-chilled meat prior to final grinding. All objectionable materials listed in the material requirements shall be removed.

When specified by the purchaser, "finely textured beef," low-temperature rendered beef that is processed from boneless beef trimmings, is allowed to be combined with boneless beef meeting the above material requirements provided it does not exceed 20% by weight of the combined finished product.

Grinding equipment shall have sharp knives and plates and be equipped with a bone/collector system. Objectionable material removed during final grinding may not be reintroduced into the finished product. The boneless meat shall be ground at least once through a plate having holes not larger than 1.0 in. (25 mm) in diameter, unless otherwise specified. Alternatively, boneless beef may be chopped or machine cut by any method provided the texture and appearance of the product after final grinding is typical of ground beef prepared by grinding only. Beef shall be thoroughly blended at least once prior to final grinding. However, the ground beef shall not be mixed after final grinding. Unless otherwise specified, final grinding shall be through a plate having holes 1/8 in. (3 mm) in diameter.

The purchaser may specify the use of a bone collector/extruder system on the final grind (^{3}/16 in. (5 mm) or smaller plate) to remove objectionable materials (bone, cartilage, connective tissue, etc.). For

certification purposes, the purchaser may waive examination for trimming defects provided the use of a bone collector/extruder system is specified.

When coarse-ground beef is specified, boneless meat shall be ground once through a plate having holes no larger than 1.0 in. (25 mm) and no smaller than 5/8 in. (16 mm) in diameter.

Alternatively, the boneless meat may be ground twice, with the smallest plate having holes no larger than 1.0 in. (25 mm) and not smaller than 3/4 in. (19 mm) in diameter. Coarse-ground meat may be blended after grinding or between grinds to assure uniformity of fat content. The term "coarse ground" shall appear on the product label.

Unless otherwise specified, the fat content shall be 20% fat. The purchaser may specify a different fat content provided it does not exceed 30%.

Item No. 155A—Beef Hindquarter, Boneless

This item is prepared from Item No. 155 and shall be boneless. The rib end shall be exposed by a cut that follows the natural curvature of the 12th rib mark and exposes the *M. spinalis dorsi* not extending more than half the length of the *M. longissimus dorsi*. The tenderloin shall be excluded unless otherwise specified by the purchaser. All bones, cartilages, *prefemoral* and *popliteal* lymph glands, hanging tender, *kidney*, and *kidney* knob shall be removed. The tendinous ends of the shank and round tip shall be cut to evidence no <75% lean. The purchaser shall specify if this item shall remain intact or be separated into cuts.

Item No. 167B—Beef Round, Full Sirloin Tip

This boneless item is prepared from Item No. 158A or 158B and consists of the intact sirloin tip portion of the round and the bottom sirloin (the *M. rectus femoris*, *M. vastus medialis*, *M. vastus lateralis*, *M. vastus intermedius*) and *M. tensor fasciae latae*. The sirloin tip is separated from the top (inside) round, bottom (gooseneck) round, and the top sirloin between the natural seams. All bones, cartilages, and the *M. obliquus abdominis internus* shall be removed. The tendinous end shall be removed exposing no <75% lean. When smaller roast are specified, the sirloin tip shall be split lengthwise into approximate equal portions. When the sirloin tip is removed by cutting through the natural seams and left attached to the bottom sirloin prior to separation of the round from the sirloin, then the sirloin tip may be referred to as Beef Loin, Bottom Sirloin, Sirloin Tip.

Item No. 180—Beef Loin, Strip Loin, Boneless

This item is boneless and consists of the anterior section of the loin and contains the 13th rib mark. The hanging tender and tenderloin shall be removed. The rib end shall follow the natural curvature of the 13th rib mark. The sirloin end shall be anterior to the hip cartilage, forming an approximate right angle with the length of the short loin, and exposes the *M. gluteus medius*. The flank side shall be ventral to, but not more than 3.0 in. (7.5 cm) from the *M. longissimus dorsi* at the rib end to a point on the sirloin end ventral to, but not more than 2.0 in. (5.0 cm) from the *M. longissimus dorsi*.

Item No. 189—Beef Loin, Tenderloin, Full

This item is derived from a full loin as described in either Item Nos. 172 or 172A. The boneless item shall consist of the *M. psoas major*, *M. psoas minor, M. iliacus*, and may show the presence of the *M. artorius*. The *M. obliquus abdominis internus* (flap), if present, shall be trimmed level with the fat surface. The sirloin butt end of the tenderloin shall expose the *M. psoas major*, *M. iliacus*, and *M. artorius* (when present). The surface fat shall be trimmed NTE 3/4 in. (19 mm) in depth at any point, from the posterior end to the exposed lymph gland and shall be tapered down to the lean at a point not beyond 3/4 of the length of the tenderloin. The tenderloin shall be trimmed free of ragged edges. All bones and cartilages shall be removed. A score into the tenderloin exceeding 1/2 in. (13 mm) in depth is not acceptable.

35.3.3 Fresh Beef: Series 100 Product Portion Cuts

The following describes the portion cuts for selected items under this series.

Item No. 1100—Beef-Cubed Steak

Cube steaks shall be prepared from any portion of the carcass which yields product that meets the end item requirements. However, shank and heel meat shall be excluded. Unless otherwise specified, the steaks shall be cubed twice at approximate right angles. Knitting of two or more pieces and folding the meat when cubing is permissible. After cubing, surface and seam fat shall not exceed 15% of the total area on either side of the steak. Individual steaks shall remain intact when suspended 1/2 in. (13 mm) from the outer edge. The steaks shall be free of heavy connective tissue, bones, cartilages, and lymph glands.

Item No. 1103—Beef Rib, Rib Steak

Rib steaks may be prepared from any IMPS bone-in rib item. The blade bone and related cartilage, feather bones, chine bones, backstrap, and those muscles that are immediately below (*M. subscapularis* and *M. rhomboideus*) and above (*M. latissimus dorsi*, *M. infraspinatus*, and *M. trapezius*) the blade bone and related cartilages shall be removed. The short ribs shall be removed at a point which is not more than 3.0 in. (7.5 cm) from the ventral edge of the *M. longissimus dorsi*.

Item No. 1123—Beef Short Ribs, Flanken Style

This item may be prepared from any IMPS beef chuck, rib, or plate short rib item. This item shall consist of the ribs, *M. intercostales interni* and *M. serratus ventralis* intact. The *M. serratus ventralis* shall be continuous across both the dorsal and ventral side of the specified number of ribs. The ribs shall be cut flanken style (e.g., cutting at a right angle to the rib bones) to specified thickness. The purchaser shall specify length (number of ribs).

Item No. 1138A—Beef Sandwich Steaks, Flaked, Chopped, Formed and Wafer Sliced, Frozen

The steaks shall be prepared from boneless beef that complies with the material requirements of Item No. 136. The flaking, chopping, forming, and slicing process shall be in compliance with FSIS Regulations and shall produce steaks that are moderately fine textured. Product shall comply with fat content requirements of Item No. 136. Each steak shall consist of two or more thin slices weighing approximately one ounce each. Not more than a minor amount of green/brown/gray rings shall be present. Steaks shall be packaged with paper separators between each steak. Unless otherwise specified, slices shall be ~4.75 × 7.5 in. (12.0 × 19.1 cm). The purchaser shall specify weight and/or number of slices per steak.

Item No. 1184—Beef Loin, Top Sirloin Butt Steak, Boneless

The steaks shall be prepared from Item No. 184. Prior to slicing, the heavy connective tissue closely associated with the protuberance of the femur shall be removed by a straight cut (faced) so that the appearance of the *M. gluteus medius* is oval in shape. The boneless top sirloin butt may be separated into sections reasonably parallel to the backbone line to accommodate the cutting of specified portion size steaks. The sections shall be cut into steaks reasonably parallel to the cut surface of the round end.

35.4 Commerce of Meat and Meat Products in the World

The discussion in this chapter concerns the United States. Although every Western country develops its own government standards and specifications, the discussion will help government scientists worldwide by providing a frame of reference for their deliberations. To many countries that export meat and meat products to this country, the information in this chapter plays an important role for them to have a better understanding of the rules and regulations governing meat commerce in this country.

36

Safe Practices for Sausage Production in the United States[*]

Y. H. Hui

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36.1 History of Sausages

The process of preserving meats by stuffing salted, chopped meats flavored with spices into animal casings dates back thousands of years, to the ancient Greeks and Romans, and earlier. The word "sausage" has been derived from the Latin word "salsus," which means salted, or preserved by salting. Sausages and sausage products have since evolved into a wide variety of flavors, textures, and shapes resulting from variations in ingredients and manufacturing processes.

In the United States, expansion in the meat-packing industry during the Civil War, along with development of refrigeration for use in railroad cars and slaughtering facilities, provided an incentive for meat processors to create sausage products that could utilize cheaper, perishable cuts of meat, along with scrap trimmings and offal products. In addition, persons from various nationalities and ethnic groups immigrated to the United States, bringing with them traditional recipes and manufacturing skills for creating a wide range of sausage types.

In the early 1900s, a series of scandals, including the publication of Upton Sinclair's *The Jungle*, exposed undesirable practices in the meat-processing industry, including the practice of adding variety meats and offal products to a sausage product without identifying the ingredients to the consumer. The Federal Government responded in 1906 with the Federal Meat Inspection Act (FMIA).

Today, the sausage-manufacturing industry must adhere to government standards for ingredients and processes. In addition, accurate labeling requirements ensure that the consumer is informed of the ingredients of a sausage product. The objective of these standards is to help ensure that sausage products maintain a consistent quality and are safe to consume.

This chapter discusses the manufacture of sausages and sausage products with an emphasis on the safety issues associated with the production of sausage products. Throughout, we will cover a range of issues related to sausage production, including:

- The sausage production process
- Safety and sanitation issues at each stage of the process
- Pathogens of concern to sausage makers

The objectives of this chapter include

- Describing the stages of the sausage production process, along with the production, safety, and sanitation issues as appropriate to each stage of the process.
- Identifying the range of pathogens of concern to sausage makers.

Many of the principles that apply to large food processors also apply to retail operations. This chapter reviews the sausage production process, identifies critical food safety areas within the process, and reviews the procedures that should be followed to ensure that only safe products are produced.

Increasingly stringent food safety standards are driving food industries internationally, nationally, and at the state level to adopt HACCP (Hazard Analysis Critical Control Point) system or plan. HACCP is now used to increase product safety. HACCP is also good business: it results in decreased liability potential for the establishment, and increased consistency and consumer satisfaction in the products produced.

HACCP plans are specifically required for retail facilities that use smoking, curing, acidifying, use food additives, use alternative cooking time/temperature combinations, or use reduced oxygen packaging. The FSIS technical staff has access to recognized meat safety standards that have been developed based on years of scientific study and practical experience.

Details about HACCP plans are explored in several chapters in this book.

The remaining information in this chapter has been modified from the document "Safe Practices for Sausage Production: Distance Learning Course Manual, 1999," available at the websites of:

1. United States Department of Agriculture (USDA), Food Safety and Inspection Service (FSIS), http://www.fsis.usda.gov
2. United States Food and Drug Administration (FDA), http://www.fda.gov
3. United States Association of Food and Drug Officials (AFDO), http://www.afdo.org

36.2 Examples of Sausage Products

36.2.1 Fresh Pork Sausage

Several categories of sausage products are typically consumed in the United States, each with specific production processes and storage requirements. These categories include fresh sausages, such as fresh pork sausages, cooked sausages, such as frankfurters, and dry sausages, such as meat sticks.

Fresh pork sausages are produced from selected cuts of fresh and sometimes frozen pork, pork trimmings, and water, along with seasonings. Since fresh sausages do not contain curing agents, and are neither cooked nor smoked, they require refrigeration. These types of sausages must be thoroughly cooked before serving.

36.2.2 Frankfurter

Frankfurters are examples of cooked and smoked sausages. They are produced from fresh meat that is fully cooked, and have flavors that are imparted through the addition of curing ingredients, and via various cooking and smoking processes. Although they are fully cooked they are not shelf-stable, and must be refrigerated until the time of consumption.

36.2.3 Meat Stick

Meat snack sticks are produced using controlled, bacterially induced fermentation to preserve the meat and impart a special flavor, along with a long-drying period to cure and preserve the meat. These processes produce a shelf-stable product.

This chapter, talks about the production process and production requirements for each of these categories of sausages.

36.3 Producing Sausages

This section reviews the main steps that are utilized to produce standard sausage products, including fresh sausages, cooked and smoked sausages, and dry sausages.

36.3.1 Fresh Sausages

Fresh sausages are made from selected cuts of fresh and sometimes frozen meats. Fresh sausages are not allowed to contain curing agents (i.e., sodium or potassium nitrites, nitrates, or salt in sufficient quantities to preserve the product) and are not cooked. Fresh sausages are usually seasoned, and have limited water content. These types of sausages require refrigerated storage, and must be thoroughly cooked before serving.

Fresh sausages are not as widely produced as cooked sausages, and are typically consumed as breakfast meals. Typical fresh sausages include products such as pork sausages, beef sausage, breakfast sausage, Italian sausage, and fresh chorizo sausages.

Table 36.1 provides some of the FSIS-established Standards of Identity for typical fresh sausages.

These FSIS standards are presented as guidelines for federal, state, and local inspection.

36.3.1.1 Standards of Identity for Fresh Sausages

Table 36.1 shows the standards of identity of selected fresh sausages promulgated by FSIS of the USDA. Of course, establishments may want to produce other types of sausage. Establishments have the flexibility to design their own particular sausage formulas. Many manufacturers are creating gourmet-type fresh sausage products that are acceptable as long as a truthful, descriptive product name and an accurate ingredients statement are included on the label.

36.3.1.2 Grinding Meat Ingredients

The main processes used to produce fresh sausages are

- Grinding meat ingredients
- Adding nonmeat ingredients
- Blending
- Stuffing
- Packaging

This section briefly reviews each stage of the production process for creating most types of fresh sausages. These steps are also used to create other types of sausages.

The first step in sausage production is grinding the ingredients. The grinding stage reduces the meat ingredients into small, uniformly sized particles. Ground meat is the primary ingredient in a sausage formulation. The characteristics of the meat ingredients used to create the sausage define the type of sausage—the overall taste, texture, aroma, along with the protein and fat content.

A variety of raw meat ingredients are utilized in the sausage production process. Each ingredient contributes a specific property to the final sausage formulation.

TABLE 36.1

Standards of Identity of Fresh Sausages

Type	Fat Content	Characteristics
Fresh Pork Sausage	Up to 50%	No pork by-products, paprika, binders, or extenders allowed. Water/ice up to 3% permitted.
Country Style Pork Sausage	Up to 50%	All spices and flavorings must be natural.
Whole Hog Sausage	Up to 50%	Can use meat parts from entire hog, including muscle by-products like tongue and heart, in proportions consistent with the natural animal. Water/ice up to 3% permitted.
Breakfast Sausage	Up to 50%	Can contain mechanically separated product up to 20% of the meat portion, and binders and extenders up to 3.5%. Can contain meat ingredients from multiple species. Water/ice up to 3% permitted. Paprika not permitted.
Fresh Beef Sausage	Up to 30%	Can contain mechanically separated beef product up to 20%. Binders, extenders, and paprika are not permitted. Water/ice up to 3% permitted.
Bratwurst (fresh)	No fat limitation	Usually made from pork but can be made from poultry if properly identified on label. Can contain binders and extenders up to 3.5%. Water/ice up to 3% permitted.
Italian Sausage By standard is only pork, special labeling for others	Up to 35%	Must contain 85% meat/fat. Can contain mechanically separated pork product up to 20% of the meat portion, approved condiments, and additional food ingredients. Water/ice up to 3% permitted. Paprika is permitted. Must have anise or fennel as spices, which provide product identity.

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Meats must be clean, sound, and wholesome. These products should be inspected when arriving at the facility, and just prior to use, to ensure that they were not contaminated during transit or handling.

The specific meats used in a sausage formulation must be correctly identified by type and quantity.

Prior to grinding, the meat is held in cold storage. Often processors prefer to chill the meat to below 30°F to minimize the potential for fat smearing. The grinder blades must be sharp and matched with the grinding plate to ensure an efficient grind without generating extra heat during the grinding process.

Grinding processes will vary according to the manufacturer and the nature of the product. Some sausage products use coarsely ground meats, whereas others use more finely ground meat ingredients. Some manufacturers grind the lean and fat trimmings separately, grinding the lean trimmings to a finer consistency than the fat meats.

36.3.1.3 Adding Nonmeat Ingredients

There are many nonmeat ingredients that are essential to the sausage-making process. These nonmeat ingredients stabilize the mixture, and add specific characteristics and flavors to the final product. Ingredients used in fresh sausage include water, salt, and antioxidants, along with traditional spices, seasonings, and flavorings.

It is important to note that the use of some nonmeat ingredients is limited or prohibited. In fresh sausage, for example, water is limited to 3% of the total weight, and binders and extenders such as dry milk powder are limited to 3.5% of the total weight.

Paprika is a spice that is considered to be both a flavoring and a coloring agent, because of its strong red color. For this reason, paprika or oleoresin of paprika may not be used in any fresh meat product, except for products that it is traditionally expected in, such as Italian sausage and chorizo.

The amount of nonmeat ingredients, such as spices, is determined by the overall weight of the product mixture. Since the amounts of these ingredients must be carefully controlled, and measuring very small amounts of numerous-specific ingredients within a manufacturing environment is often not practical, many manufacturers use a commercially premeasured and packaged mix of these ingredients.

36.3.1.4 Blending

Manufacturers carefully control the blending of the meat and nonmeat ingredients to create the desired characteristics for a specific sausage formulation.

The meat and nonmeat ingredients are placed in a mixer and thoroughly blended.

The manufacturer must monitor and control the blending process, since excessive mixing can cause the salts in the formulation to break down excessive amounts of protein, or friction created by the blending process can increase the product temperature and cause fats to partially render. Excessive handling also cuts protein fibers too short. All these problems could result in product quality defects.

The blending process must also obtain a uniform distribution of any nonmeat ingredients within the product formulation. For example, flavorings, salts, and other ingredients must be consistently mixed throughout a sausage formulation.

36.3.1.5 Stuffing

After the blending is complete, the blended ingredients may be bulk packaged, or they may be extruded into a casing. This process is called stuffing. Fresh sausages are typically stuffed into natural animal casings. Natural casings used for fresh sausage are derived from the small intestines of sheep. To create the natural casings, the casing processor washes, scrapes, and treats the casings, grades the casings for size and condition, then salts, packages, and ships the casings in brine or propylene glycol (for preservation) to the sausage manufacturer.

Fresh sausages may also be stuffed into a small-diameter artificial casing, or may be extruded into a short, large-diameter plastic casing called a "chub" pack, usually containing 1–2 pounds of meat.

36.3.1.6 The Stuffing Process

The stuffing process can be accomplished in a number of ways. Natural casings are typically flushed with water, and the mixture is injected into the casing at a pressure that is sufficient to fill the casing without leaving any air pockets, and without tearing the casing. The stuffing process is also sometimes conducted at lower temperatures (<35–380°F) to minimize the fat smearing on the casing.

Smaller volume or specialty producers may stuff the formulation into the casing by hand or from a screw feed. These small operations may also bypass choppers, mixers, and stuffers, and stuff the output of the grinder directly into the casing. Larger manufacturers may use air or water-piston-type automatic stuffers.

The stuffed casings are then separated into uniform segments of equal length in a process called linking. These segments form the single sausage portions. The linking process is typically accomplished by twisting the casing.

36.3.1.7 Packaging

The fresh sausage product is sometimes packaged for sale to the customer. The product may be wrapped in a gas-impermeable plastic, and placed into refrigerated storage or display. The specific packaging will vary according to the needs of the end user; however, the processor must follow hygienic standards when packaging any sausage product to avoid contaminating the product. Often retail fresh sausage is tray packed.

36.3.2 Cooked and Smoked Sausages

Cooked and smoked sausages account for approximately 85% of all sausages produced today. Typical cooked sausages, like frankfurters or bologna, are made from fresh or frozen meat that is cured during processing, fully cooked, and smoked. These types of sausages are perishable and must be refrigerated until time of consumption, or they expire. These sausages have additional flavors that are imparted through the addition of nitrites and nitrates, and via various cooking and smoking processes.

This category of sausages contains many process variations that result in the wide range of cooked and smoked sausages that can be seen in the retail trade. Although most are cooked and smoked, some are cooked without smoke, and there are many other process modifications. This manual does not cover all

the possible variations. We are only going to discuss a few of the most common processes for manufacturing cooked and smoked sausages.

One type of cooked and smoked sausage is made from emulsified (finely ground) meats. This type includes frankfurters (hot dogs), bologna, knockwurst, and vienna sausages. This class of sausage is allowed to contain a combination of fat and added water of up to 40%, with no more than 30% fat in the finished product. These product standards are found in FSIS regulation §319.180.

Some cooked and smoked sausages are more coarsely ground, such as Polish sausage, cotto salami, and smoked sausage. Fat is considered visible to the consumer in these types of sausages, and so there is no fat limitation; however, water is limited to 10% of the finished product weight. These product standards are found in FSIS regulation §319.140.

Other types of cooked sausage, such as braunschweiger, liver sausage, blood sausage, and tongue items, are cooked in water inside of an impervious casing or steel mold, often inside a steam cabinet or water bath instead of a smokehouse. The impervious casing causes these types of cooked sausage to retain all moisture during cooking, and so the manufacturer must closely control the amount of water during formulation to ensure that it does not exceed the 10% limit.

In addition to the standard production steps used to produce fresh sausages, cooked and smoked sausages have additional production stages, including:

- Smoking
- Cooking
- Showering
- Chilling
- Peeling

This section briefly reviews each of these additional stages that are used to produce cooked and smoked sausages.

Cooked and smoked sausages start out very similar to the fresh sausages. Raw meat ingredients are ground and blended with nonmeat ingredients. A bowl chopper (known in the industry as a silent cutter) is sometimes used to simultaneously blend and chop the mixture. The bowl chopper passes a series of knives through the mixture, usually resulting in an increase in temperature due to friction. A combination of ice and water is often added to the mixture to control the temperature and facilitate the grinding and blending process.

Cooked and smoked sausages are typically composed of a wide variety of meat ingredients. Each ingredient contributes a specific property to the final sausage formulation. For example, each meat ingredient will vary in overall content of protein, moisture, fat, collagen, and in its ability to encapsulate fats and hold the mixture in a stable suspension, called binding ability.

36.3.2.1 Meat Ingredients' Binding Ability

Skeletal beef muscle tissue from bull, cow, and shank meat has the highest binding capabilities. Intermediate- or medium-binding meats include head meat, cheek meat, and lean pork trimmings. Low-binding meats contain higher levels of fat, and are typically nonskeletal meats, such as jowls, fat, briskets, hearts, and tongue trimmings.

Some meat by-products have minimal-binding ability, and so the use of these products in sausage is generally avoided. These products include tripe, pork stomachs, lips, snouts, and skin.

Nonmeat proteins, such as cereal flours and nonfat dry milk must also be uniformly distributed; however, these ingredients usually are added last, since they absorb water that is required for protein emulsification.

36.3.2.2 Emulsification

Some manufacturers run the mixture through an emulsifier after the blending stage to further reduce the size of the meat particles, to achieve a very fine texture. In the emulsification process, fat, protein, salt,

and water are mixed, and combined into a semifluid emulsion. The meat muscle protein, called "myosin," is solubilized or released from the muscle fibers by contact with salt. The solubilized protein and water combine and surround the fat globules, and hold the fat particles in a disbursed suspension within the mixture, along with spices and seasonings.

Careful control of the amount of each ingredient and the grinding process is essential. The manufacturer must select a mix of raw meat materials with the appropriate emulsifying and binding characteristics. Emulsifying properties are dependent on the hydrophobic (water repulsing) and hydrophilic (water attracting) characteristics of the specific protein. Myosin protein has the best emulsifying properties (possessing both hydrophobic and hydrophilic properties), while proteins such as collagen have little or no emulsifying properties, and tend to break down at higher temperatures during the cooking. Manufacturers typically limit the use of low-binding materials to <15% of the product formulation to ensure the finished product quality.

If too much fat is added, or overchopping of the ingredients exposes too much fat, there may not be sufficient protein to encapsulate the fat. This creates a condition called "shorting out," "greasing out," or "fat capping." In this case, fat droplets migrate to the product surface and form small pockets of fat called "fat caps." The emulsion can also break down when the mixture is chopped too long, causing too short protein fibers, or the product temperature is increased, causing some of the fats to partially render. In this condition, the protein is unable to hold liquid fat in suspension. In addition, emulsion breakdown can occur in products with a high acid content, or when heating coagulates protein before it has a chance to surround the fat and "set" the emulsion.

36.3.2.3 Stuffing

Cooked and smoked sausages are stuffed into casings in a process similar to fresh sausages; however, manufacturers of these products utilize a wider range of casing materials.

Natural casings used for cooked and smoked sausages are derived from the stomach, and intestines of hogs, the intestines, bung and bladder of cattle, and the intestines of sheep. They are edible, and allow smoke and moisture to permeate the sausage during processing.

Hog middles are produced from the large intestine, and are used for Italian sausages and liver sausages. The small intestine is utilized to create small hog casings that are utilized for smaller-diameter products, such as Polish sausage, smoked pork sausage, and bratwurst. And hog bladders are used as casings for minced luncheon meats.

Sheep casings are generally made from the small intestine. These small diameter casings are typically used for frankfurters and pork sausages.

Beef rounds are casings that are produced from the small intestine, and are used for products such as bologna and mettwurst. Beef middles are larger casings created from the large intestine, and are used to produce sausages such as bologna, cervelat, and salami.

Sometimes manufacturers use casings that are not derived from the same type of meat as the main meat ingredients of the sausage. For example, a manufacturer can use a pork casing on a beef and lamb combination sausage. Currently, manufacturers are not required to disclose that the natural casing is not derived from the same livestock species as the sausage itself.

36.3.2.4 Regenerated Collagen Sausage Casings

Regenerated collagen sausage casings are made from collagen extracted from cattle hides and hog skins in a process called regeneration. The extracted collagen is dissolved, and then hardened, washed, swelled with acid, and finally formed into the tubular-casing shape in an extrusion process. This final shape is then fixed in an alkali bath.

These types of lower-strength casings are typically used for smaller-diameter products.

36.3.2.5 Synthetic and Cellulose Casings

Synthetic or artificial casings are made from special papers impregnated with cellulose, saran casings made from synthetic plastics, and hydro-cellulose casings made from regenerated cellulose. Cellulose

casings are created from dissolved fibers extracted from cotton seeds or paper pulp. Each of these types of casings are available in a wide range of sizes and characteristics and are easy to handle; however, these types of casings are not edible and must be removed from the sausage prior to consumption. Artificial casings provide high strength and are available with excellent permeability to moisture and smoke, or as impermeable casings for use in producing water-cooked products such as braunschweiger.

36.3.2.6 Smoking

Smoking is used to dry and cure the meat, and impart flavors and aroma to the final product. Smoking was traditionally important because it inhibited bacterial growth on the finished product; however, in modern times we rely on refrigeration to inhibit bacteria. A smoke generator creates natural smoke via a carefully controlled burning of hardwood sawdust, wood chips, or logs. Hickory is the most popular wood used for smoking, but other hardwoods and fruitwoods are also used. Coniferous trees such as pine are unacceptable because they contain resins; smoke from that type of wood has a high tar content and imparts a bitter flavor.

The distinctive effects of smoking are imparted through absorption of the condensible phase of the smoke. This phase consists of acids, carbonyls, phenolics, and polycyclic hydrocarbons. The amount of these materials that is deposited on the product surface is controlled by varying the density of the smoke, the duration of the smoking cycle, and the air velocity within the smoking chamber.

Sausage manufacturers also control the moisture level of the product before and during the smoking process to produce a high-quality product. The surface of the product must be slightly moist for the smoke volatiles to properly adhere to the product. If the product surface is too moist, the smoke process will cause streaking. If the casing is too dry, the smoke will not properly adhere to and permeate the casing. Producers therefore ensure that the drying cycles are carefully controlled to ensure a consistent smoked product.

Artificial liquid smoke can be used to impart the smoke flavors. Liquid smoke is created by distilling and refining the condensible elements of natural wood smoke to create a liquid concentrate of the desired elements. Liquid smoke is applied by dipping or drenching the product in a bath of the liquid smoke product. It can also be applied within a smokehouse environment by atomizing the liquid smoke into a fine mist, or by superheating and evaporating the liquid smoke in a process called regeneration. It can also be in the formulation of the sausage; if so, it must be included in the ingredients statement of the product.

The length of the smoking process varies according to the type of product and desired effect of the smoking process. An effective smoke application can be accomplished in as less as 30 min. Liquid smoke can be effectively applied in <5 min. Table 36.2 illustrates the use of a smokehouse schedule.

36.3.2.7 Sample Smokehouse Schedule

Table 36.2 shows a sample smokehouse schedule.

As Table 36.2 indicates, the smokehouse may also serve as a controlled thermal processing chamber, and is used to dry, smoke, heat (cook), and add humidity to the product.

TABLE 36.2

A Sample Smokehouse Schedule

Function	Time	Smokehouse Temperature	Relative Humidity	Damper
Drying	30 min	125°F	25%	Open
Smoking	1 h	140°F	35%	Closed
Cooking	1 h	165°F	35%	Closed
Steam	Cooking 10 min	180°F	100%	Closed

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36.3.2.8 Cooking

Manufacturers cook sausage products to enhance the flavor and color, produce the desired final product, and to inhibit the bacteria responsible for spoilage. In order to produce a safe product, cooking must also destroy parasites and pathogenic bacteria. Sausages can be cooked through immersion in a heated water bath, within a smokehouse environment, or within ovens.

The cooking process is carefully controlled to ensure that the product reaches a specific internal temperature for a defined period of time. Thermocouples are used to monitor the temperature during the cooking process.

36.3.2.9 Showering/Chilling

After the smoking and cooking phase, the sausage product undergoes a cold-water shower. Showering maintains the product humidity and stops the cooking process by reducing the product temperature as quickly as possible. Showering also helps prevent shrinkage and wrinkling of the product casing.

The cooling process is also carefully monitored and controlled. The temperature of the cooked product must be lowered to a specific temperature within a desired time. The FDA Food Code specifies that a product must be lowered from 140°F to 70°F within 2 h, then from 70°F to 41°F or less within an additional 4 h. To meet the FSIS stabilization requirements of not more than 1-log growth of *Clostridium perfringens*, uncured product can be cooled from 130°F to 80°F with 1.5 h and 80–40°F in 5 h.

36.3.2.10 Peeling

Peelers are used by the sausage manufacturer to remove nonedible casings from sausage products during the later stages of the production process.

36.3.3 Dry Sausages

Producers of dry sausages and semidry sausages utilize controlled, bacterially induced fermentation to preserve the meat and impart flavor. The most common examples of dry sausages are salami and pepperoni. The dry category also includes shelf-stable nonfermented products such as beef jerky. Again, there are many variations of process steps and ingredients, resulting in the vast array of products available to the consumer. We are not going to deal with all possible products; rather, we will concentrate on the most common examples of these types of products. In addition to the standard production stages identified for fresh, cooked and smoked sausages, dry sausages have additional production steps, including:

1. Blending special curing ingredients
2. Drying process

Let us briefly review each of these additional special production steps used to produce dry and semidry sausages.

36.3.3.1 Blending Special Curing Ingredients

The meats used in dry sausages are typically ground or chopped at low meat temperatures (20–25 °F) to maintain the well-defined fat and lean particles that are desired in this type of sausage. The ground meats and spices are then mixed with curing ingredients, such as nitrates, nitrites, antioxidants, and bacterial starter cultures. Salts have traditionally been used to help preserve sausages. Eventually, producers learned that nitrates and nitrites in the salts were essential to the curing process. Manufacturers of dry and semidry sausages use a curing agent consisting of salt and nitrates and/or nitrites. Approved antioxidants may also be added to protect flavor and prevent rancidity, and are limited to 0.003% individually, or 0.006% in combination with other antioxidants. These ingredients must be uniformly distributed throughout the mixture to achieve the maximum microbiological stability.

Bacterial fermentation is then used to produce the lower pH (4.7–5.4) that results in the tangy flavor associated with this type of sausage. In earlier days of sausage making, the bacteria growth was uncontrolled, resulting from bacterial contamination of the meat or production equipment, producing unreliable results. Traditionally, sausage makers held the salted meat at low temperatures for a week or more allowing plenty of time for the lactic acid bacteria coming from the environment to reproduce in the meat mixture. Unfortunately, this traditional method was not always reliable and subject to several errors such as cutting the time short, adding too much or too little salt, or growth of the wrong lactic acid bacteria. Therefore, modern producers of dry sausage use a commercial lactic bacteria starter culture and simple sugars, such as dextrose or corn sugar, that promote lactic acid bacterial growth by serving as food to fuel the bacteria during fermentation. The bacteria starters are harmless, and are limited to 0.5% in both dry and semidry sausage formulations. Commercial bacteria starter cultures typically consist of a blend of microorganisms such as *Pediococcus, Micrococcus*, and *Lactobacillus*, using specific species such as *P. cerevisiae*, *P. acidilactici*, *M. aurantiacus*, and *L. plantarum*. The bacterial fermentation lowers the sausage pH by producing lactic acid. This lower pH also causes the proteins to give up water, resulting in a drying effect that creates an environment that is unfavorable to spoilage organisms, which helps preserve the product. However, mold may grow, and could become a problem.

Some small producers may still allow the mixture to age in a refrigerator for several days to encourage the fermentation process, even though they are using starter cultures. However, most modern processors stuff the mixture directly into casings, which then undergoes a fermentation process at about 70–110°F (depending on the starter type). This fermentation process is designed to allow the bacteria to continue to incubate. The fermentation occurs during a 1- to 3-day process that takes place in a "greening room" which provides a carefully controlled environment designed to obtain specific fermentation results. Temperatures are typically maintained at approximately 750°F, and relative humidity at 80%. Semidry sausages are usually fermented for shorter periods at slightly higher temperatures.

36.3.3.2 Cooking/Smoking

What happens after fermentation depends on the type of product being produced. Semidry sausages like summer sausage are almost always smoked and cooked before drying. Dry sausages like pepperoni are rarely smoked, and may or may not be cooked. Today, some establishments choose to heat treat these dry sausages as a critical step designed to eliminate *E. coli*. A moist heat process may be used for some products. This process utilizes a sealed oven or steam injection to raise the heat and relative humidity to a meet a specific temperature/time requirement sufficient to eliminate pathogens (e.g., 130°F minimum internal temperature for 121 min, or 141°F minimum internal temperature for 10 min). The product is then sent on to the drying stage of the process.

36.3.4 Drying Process

The drying process is a critical step in ensuring product safety. FSIS requires that these products undergo a carefully controlled and monitored air-drying process that cures the product by removing moisture from the product. Pork-containing products must be treated to destroy trichinae. Sausages not containing pork have no such requirement.

Manufacturers are required to control the ratio of moisture to protein in the final product. The Moisture/Protein Ratio (MPR) is controlled by varying the amount of added water based on the overall product formulation, and, primarily by the drying procedure. In some products, the MPR can affect the final microbiological stability of the product. In other products, the MPR is important to ensure elements of the overall product quality, such as the texture. The minimum requirement for all products produced in FSIS-inspected facilities is that they must meet the FSIS policy standards for MPR (Table 36.3). However, these prescribed treatments have proved to be insufficiently lethal for some bacterial pathogens. Thus, most of the industry has volunteered to implement a more rigorous treatment.

The drying process consists of placing the product in a drying room under a relative humidity of 55–65%, in a process that can last from 10 days to as long as 120 days, depending on the product diameter, size, and type. The drying process is designed to produce a final product with approximately

TABLE 36.3

Moisture–Protein Ratio (MPR) for Dry and Semidry Sausages

Sausage Product	Maximum Moisture to One Part of Protein
Jerky, Pemmican	0.75 : 1
Pepperoni	1.6 : 1
Dry sausage (e.g., hard salami)	1.9 : 1
(line deleted)	
Summer sausage, shelf-stable	3.1 : 1 + pH 5.0
Dry salami	1.9 : 1
Genoa salami	2.3 : 1
Sicilian salami	2.3 : 1
Italian salami	1.9 : 1
Thuringer (semidry sausage)	3.7 : 1
Ukrainian sausage	2.0 : 1
Farmer Summer sausage	1.9 : 1

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30–40% moisture, and an MPR generally of 1.9:1 or less, to ensure proper drying and a safe product. Facilities must keep accurate records of the temperature and the number of days in the drying room for each product manufacturing run to help ensure product safety and consistency. The drying environment is controlled to ensure that the drying rate is slightly higher than the rate required to remove moisture from the sausage surface as it migrates from the sausage center. Drying too quickly will produce a product with a hard and dry casing. Drying too slowly results in excessive mold and yeast growth, and excessive bacterial slime on the product surface.

The controlled drying process is designed to reduce the moisture to the point where the final product has a specific MPR. This process will vary depending on the specific facility operation and the specifications desired for the final product. For example, pepperoni is required to maintain an MPR of 1.6:1, indicating a requirement of 1.6 parts moisture to one part protein. Genoa salami should have an MPR of 2.3:1, and all other dry sausages an MPR of 1.9:1. Inspectors may sample the products to determine the compliance with the specified MPR.

Semidry sausages are prepared in a similar manner, but undergo a shorter drying period, producing a product with a moisture level of about 50%. These products are often fermented and finished by cooking in a smokehouse, at first at a temperature of approximately 1000°F and a relative humidity of 80%. The temperature is later increased to approximately 140–1550°F to ensure that microbiological activity is halted. Since the moisture level of the final product is about 50%, semidry sausages must be refrigerated to prevent spoilage. Examples of semidry sausages include summer sausage, cervelat, chorizo, Lebanon bologna, and thuringer.

Table 36.3 identifies the FSIS MPR policy standards for some dry and semidry sausage products. Although these are the FSIS standards for dry and semidry sausages, they can provide a basis for State and local decisions about whether specific processes are adequately designed to ensure the product safety.

To ensure that the fermentation and drying processes are sufficient to reduce or eliminate any pathogens present in the product, the procedures must be validated to demonstrate that they achieve a specific reduction in organisms (e.g., a 5-log reduction in *E. coli* 0157:H7).

36.4 Sausage Production Equipment

In this section, we take a brief look at some of the standard equipment and processes that are used to produce sausages, along with the safety issues that should be addressed for each item. There are many different manufactures of equipment, and you will see many variations of these basic equipment types.

36.4.1 Grinders

Grinders are used to chop the meat ingredients into small pieces. Meat is fed from a hopper, passed along a cylinder with an auger or worm, to a perforated plate where it is sliced away by a series of revolving blades. The friction produces a rise in temperature, which potentially could result in bacterial growth if not controlled.

36.4.2 Blenders

Blenders use screw-like agitators to mix the solid ground meat mixture with any additional nonmeat ingredients to create a consistent distribution of all ingredients within the formulation.

36.4.3 Bowl Choppers

Bowl choppers (silent cutters) utilize a series of knives that chop and mix the product formulation. The friction of the knives passing through the meat will raise the temperature of the meat mixture.

36.4.4 Stuffers

Stuffers are used to extrude the sausage formulation into the casing. The ground mixture is fed from a hopper into a reservoir and forced through a nozzle into the casing.

Since grinders, blenders, choppers, and stuffers come into contact with the product formulation, they must be cleaned daily, cleaned every time that a new formulation is processed, and be kept free of excess product during the process. This will ensure that any contamination is not passed on to the product formulation.

36.5 Smokehouses

Smokehouses are heated drying rooms into which the manufacturer injects smoke. The smoke adds specific flavors to the product, and helps dry and cure the product. The smokehouse may also serve as a controlled thermal-processing chamber, and is used to dry, smoke, heat, cool, and add humidity to the product.

36.5.1 Smokehouse Recorders

Smokehouse recorders are devices that monitor and track the smoking duration for each sausage production run. The smoking process must be carefully controlled to ensure a consistent level of product quality.

36.5.2 Temperature Monitoring

The temperature of drying, smoking, and cooking chambers must be carefully monitored and controlled to ensure a consistent product, and to ensure that process temperatures/times are maintained at a sufficient level to eliminate pathogens.

36.5.3 Peelers

Peelers are used by the sausage manufacturer to remove nonedible casings from sausage products during the later stages of the production process. Steam is generally used in the peeler to loosen the casings, creating a warm, and moist environment. Peelers use a blade that slices the casing, touching each sausage that goes by; this is an ideal environment for bacteria, especially *Listeria*. Peelers must be carefully monitored because of the potential for bacterial growth.

36.5.4 Greenrooms/Drying Rooms

Greenrooms and drying rooms are used to cure and dry the sausage product. In some cases, the environment is designed to support controlled, bacterially induced fermentation processes that are used to cure the product and impart special flavorings.

36.5.5 Coolers

Coolers use refrigeration to lower the temperature of the sausage product.

36.6 Packaging Equipment

The final sausage product is often packaged prior to sale. Packaging typically consists of wrapping the product in plastic film. The packaging protects the product from cross contamination, and helps retard spoilage caused by contact with the air.

36.7 Ingredients in Sausages

In this section, we will go over the types of ingredients that are used to produce standard sausage products, including fresh sausages, cooked and smoked sausages, and dry sausages. Each type of sausage uses a different mix of ingredients.

36.7.1 "Variety Meats"

Sausage products provide the manufacturer with an opportunity to utilize various types of meat by-products, also known as "variety meats," such as hearts, tongues, livers, tripe, and pork stomachs. The specific amount and type of variety meats used in a product depends on the formula designed by the processor. USDA regulations allow the use of variety meats, only in cooked sausage like hot dogs, as long as the name of the product is modified to include the words "with by-products" or "with variety meats," and the particular types of variety meats are included in the ingredients list in the order of predominance in the formulation.

Manufacturers also often use specific types of variety meat to impart specific flavors or characteristics to the final product. For example, hearts may be used to help create the dark red color desired for summer sausages.

36.7.2 Ensuring Wholesomeness

All meat and meat by-products used in sausage formulations must be clean, wholesome, and properly labeled. Receiving of raw ingredients is an important step in ensuring food safety. The manufacturing facility should inspect all incoming meat to ensure that is not contaminated. Even previously inspected meat should be reinspected to ensure that it has not become contaminated during transit.

36.7.3 Properly Identifying Meat ingredients

FSIS regulations require the manufacturer to properly identify the types and amounts of meat and meat by-products present in the final product. The actual ingredients must match the ingredients listed on the label.

However, once the meat products are chopped or ground, they lose their visual identity, making it difficult to identify specific ingredients in a formulation. Sausage manufacturers are therefore required to carefully identify and track ingredients throughout the production process, and ensure that unlisted meat items are not accidentally or purposely substituted during the production process.

36.7.4 "Rework"

Sometimes a product that has partially or fully completed the production cycle is not salable but still wholesome, and can be used for food. For example, the casing of some sausages may split during the cooking or smoking cycle. Manufacturers are allowed to reuse these edible but unsalable products by removing the casing and adding the contents to the grinder to include in another run of the same product. Manufacturers are not allowed to use this "rework" in a product with a different list of ingredients. Rework has little binding ability since the proteins are coagulated, and so the amounts added are self-limiting, in that it has a detrimental effect on product quality.

36.7.5 Nonmeat Ingredients

A number of nonmeat ingredients are essential to the sausage-making process. These nonmeat ingredients stabilize the mixture and add specific characteristics and flavors. These typically include extenders and binders, water, salt, nitrite, nitrate, ascorbates, erythorbates, sugars, antioxidants, phosphates, mold inhibitors, extenders, along with traditional spices, seasonings, and flavorings. In this section, we will look at the effect and use of each of these ingredients.

36.7.5.1 Binders and Extenders

Binders and extenders have a number of uses in a sausage formulation. Manufacturers use extenders such as dry milk powder, cereal flours, and soy protein as a lower-cost method to increase the overall yield of the formulation, to improve the binding qualities and slicing characteristics, and to add specific flavor characteristics. A sausage formulation can include up to 3.5% of these substances.

36.7.5.2 Water

While water is a naturally occurring component of meat, manufacturers also add water to the formula in specific amounts to improve the consistency of the mixture and to substitute for fats. FSIS regulations permit manufacturers of fresh sausages to add water up to 3% of the total product weight.

Cooked sausage manufacturers are allowed to vary the amount of added water according to the amount of fat. The maximum fat content is limited to 30%, and the amount of fat and water combined is limited to 40%, so the manufacturer can increase water to substitute for reduced fat. Typically, the amount of naturally occurring water is determined by computing four times the protein content. Any moisture above that amount is considered added water.

36.7.5.3 Salt

Salt is an essential ingredient of any sausage formulation. Salt is used to preserve the product, enhance the flavor, and to solubilize the meat proteins to improve the binding properties of the formulation.

Since the advent of refrigeration, the preservative properties are the least important use of salt, though dry sausages still use salt for preservation. A salt concentration of around 17% is necessary for preservation to be effective.

The most important use of salt in a sausage product is its ability to solubilize proteins. This enhances the product texture and improves water and fat binding.

Since sodium chloride (NaCl) salt has been linked to hypertension, other nonsodium salts, such as potassium and calcium chlorides, are sometimes substituted for a portion of the sodium chloride.

36.7.5.4 Curing Agents

Curing agents such as nitrite and nitrate have traditionally been used in sausage formulations, originally as a contaminant present in salts, and later added intentionally in the form of saltpeter. Nitrites provide bacteriostatic and antioxidant properties, and improve the taste and color of the sausage. Nitrites help

TABLE 36.4

Maximum Levels for Nitrate and Nitrite Permitted in Chopped Meat

Substance	Amount
Sodium or potassium nitrate	2¾ oz. to 100 lb. chopped meat
Sodium or potassium nitrite	1/4 oz. to 100 lb. chopped meat

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control the growth of bacteria in vacuum-packed products, such as frankfurters and luncheon meats. Nitrites prevent the outgrowth of bacteria, such as the lethal *Clostridium botulinum* bacterium that causes botulism. Nitrites also inhibit the oxidation of fats in meats, reducing the development of oxidative rancidity. Nitrites produce the desired reactions much faster and are much more commonly used than nitrates. The use of nitrate by large processors is rare, because the process of converting the nitrate into nitrite within the product is much slower and less reliable than the addition of nitrite directly.

Since nitrites and nitrates can be toxic to humans, the use of these ingredients in sausage formulations is carefully controlled. They are sometimes referred to as "restricted ingredients." Supplies of sodium nitrite and potassium nitrite and mixtures containing them must be kept securely under the care of a responsible employee of the establishment. The specific nitrite content of such supplies must be known and clearly marked accordingly. The maximum level of these additives that is acceptable is spelled out in the FSIS regulations, as indicated below.

Table 36.4 shows the maximum levels for nitrate and nitrite permitted in chopped meat. The amount of nitrite added to product must be regulated at the formulation step, based on the total amount of meat and meat by-products. Nitrites dissipate quickly in the finished product, and the parts per million in the finished product does not necessarily reflect the amount that was used in formulation. This makes sampling the finished product for nitrite an impractical control measure.

36.7.5.5 Cure Accelerators

Cure accelerators such as ascorbates and erythorbates are used to speed up the curing process (Table 36.5). They also stabilize the color of the final product.

36.7.5.6 Sugars

Sugars are used in sausage formulations to reduce the flavor intensity of the salt and flavorings, and to provide a food source to enable microbial fermentation. Sugars used in sausage products include sucrose and dextrose.

TABLE 36.5

Maximum Levels for Cure Accelerators Permitted in Meat

Ingredient	Maximum Amount
Ascorbic acid	3/4 oz. per 100 pounds of meat
Erythorbic acid	3/4 oz. per 100 pounds of meat
Sodium erythorbate	7/8 oz. per 100 pounds of meat
Citric acid	May replace up to 50% of the above-listed ingredients
Sodium citrate	May replace up to 50% of the above-listed ingredients
Sodium acid pyrophosphate	Alone or in combination with others may not exceed 8 oz. (0.5%)
Glucono delta lactone (GDL)	8 oz. per 100 pounds of meat

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36.7.5.7 Antioxidants

Antioxidants are approved for use in fresh sausages to retard oxidative rancidity and protect flavor. Approved antioxidants include butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propyl gallate, tertiary butylhydroquinone (TBHQ), and tocopherols. These compounds are added to the spice mixtures, based on the actual percentage of fat in the fresh product formulations (typically 0.01% separately, 0.02% in combination), or the total meat block weight for dry sausage formulations (typically 0.003%).

36.7.5.8 Phosphate

Phosphates are used to improve the water-binding capacity of the meat, solubilize proteins, act as antioxidants, and stabilize the flavor and color of the product. Their maximum benefit to the processor is to reduce purge, or water that is cooked out of product. Phosphates also help increase shelf life of a product. The maximum amount of phosphates approved for sausage products is limited to 0.5% of the finished product weight. If used, they must be food grade.

36.7.5.9 Mold Inhibitors

Mold is a commonly encountered problem in the production of dry sausages. The common technique for inhibiting the growth of mold is to dip the sausage in a mold inhibitor solution, typically 2.5% solution of potassium sorbate or a 3.5% solution of propylparaben.

36.7.5.10 Glucono Delta Lactone

Glucono Delta Lactone (GDL) is a cure accelerator that produces an acid tangy flavor similar to flavor resulting from natural fermentation. It is allowed at 8 oz. to each 100 lb. of meat.

36.7.5.11 Spices, Seasonings, and Flavorings

Spices, seasonings, and flavorings are used to add flavor to the sausage, and also affect the consistency of the ground mixture. The wide range of available spices, seasonings, and flavorings is a primary reason for the variety available in sausages.

Spices are defined as any aromatic vegetable substance that is intended to function as contributing flavoring in food instead of contributing to the nutritional substance of the food. The active aromatic or pungent properties of spices that contribute the most to the flavoring effect are mostly present in the volatile oils, resins, or oleoresins of the spice. These properties are present in the whole spice, or in extracts of the active components. The use of spice extracts has some advantages over using whole spices, including providing more control over the intensity of the flavor, less opportunity for microbial contamination, easier storage, and a less conspicuous visual appearance compared with spice particles.

Spice extracts must be labeled as "*Flavorings*" in the product ingredients list. Flavorings are substances that are extracted from a food (such as fruits, herbs, roots, or meats, seafood, etc.) that are also intended to contribute flavoring instead of nutritional substance.

"*Seasonings*" is another general term that refers to any substances that are used to impart flavor to the food product. Some examples of common spices and seasonings include allspice, pepper, cardamom, caraway, coriander, cumin, garlic, sage, mustard, nutmeg, paprika, pepper, rosemary, sage, thyme, and turmeric.

36.8 Performing Sausage Formulation Calculations

One of the most important tasks for the inspector in the sausage production facility is to check the formulation of the product. Inspection program personnel should select a batch of sausage product, check the sample batch for accurate identification of the types and weights of the component ingredients, and

calculate the maximum and minimum amounts of restricted ingredients. Establishments which produce sausage product are encouraged to use as a reference a procedure chart that identifies the production process and ingredients for the product.

The chart should include

- The product type and name
- The type and amounts of meats and meat by-products in the product
- The type and amounts of cure agents
- The method of formulation, such as chopping time and emulsifying time and method
- The type of casing and method of stuffing
- The smoking/cooking time and temperature, along with expected shrinks
- And the chilling time and expected chill shrinks

36.8.1 Calculating Added Water and Antioxidant for Fresh Pork Sausage Formulation

This sausage contains added BHA to protect flavor. The formulation is as follows:

Ingredients: Pork, water, salt, sugar, spices, BHA (formulated to yield a finished product fat limit of 40%), as shown in Table 36.6.

FSIS has issued specific limitation in fresh pork sausages:

Limited to 50% fat or less
Added water/ice up to 3%
Antioxidant amount based on fat limited to 0.01

The inspector reviews the sausage formulation and evaluates a number of issues. For example, the inspector would determine whether the added water content in the above formulation complies with the limit for this type of product, or whether the type and amount of antioxidant is allowed in this type of product. Let us quickly go over the process to answer each of these questions.

Is added water content within limits?

The amount of added water and/or ice in fresh sausage is limited to 3% of the product weight. The inspector will use these steps to determine the amount of water permitted in this formulation. First, subtract the water from the total formula weight to determine the total weight minus the water as shown below.

TABLE 36.6

Fresh Pork Sausage: Batch Formula and Seasonal Formulation

Batch Formula	
Regular pork trimmings (60% fat)	280.00 lb.
Special pork trimmings (30% fat)	156.00 lb.
Water	15.00 lb.
Fresh sausage seasoning	9.00 lb.
Total	460.00 lb.
Seasoning Formulation	
Salt	5 lb.
Sugar	2 lb.
Black pepper	15 oz
Sage	11 oz
Nutmeg	5.64 oz
BHA (antioxidant)	0.36 oz
Total	9.0 lb.

Fresh Pork Sausage Limitations

- Limited to 50% fat or less
- Added water/ice up to 3%
- Antioxidant amount based on fat – limited to 0.01%

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460 lb.	(100%)
⊠15 lb.	(3%)
445 lb.	(97%)

Then divide the formula weight minus the water by the total weight without water (0.97) to determine the total formulation weight with the allowed amount of water added.

$$445/0.97 = 458.76$$

Then multiply the total formulation weight with the allowed amount of water added by 3% (allowed percentage of water). This results in a total allowed water amount of 13.76 lb. in this formulation. In this case, the added water exceeds the allowable limits for this product.

$$458.76 \text{ lb} \times 03 = 13.76 \text{ lb}$$

Is the type and amount of antioxidant within limits?

Antioxidants in fresh sausage are limited to 0.01% of the total amount of fat in the product. In this example, we will assume that the target fat amount of 40% in this formulation has been confirmed.

The first step is to determine the weight of the fat. Multiply the batch weight by the fat content (40%)

460
×0.40
184 lb.

Then multiply the fat content by the permitted amount of antioxidant (0.01%) to determine the maximum allowable amount of BHA in this formulation.

$$184 \times 0.0001 = 0.0184 \text{ lb.}$$

Convert pounds to ounces (16 oz/lb.)

$$16 \text{ oz} \times 0.0184 = 0.294 \text{ oz}$$

In this case, the maximum allowed amount of the antioxidant BHA in this formulation is 0.294 oz. The Fresh Sausage Seasoning formulation specifies 0.36 oz. The BHA in this formulation exceeds the regulatory limit.

Inspectors can use similar calculations to determine a range of issues, such as whether the fat percentage or binder and extender amount targets will be achieved.

36.8.2 Calculating Curing Agents for Frankfurter Formulation

The ingredients are

Beef and pork
Water
Nonfat dry milk
Mustard
Spices
Erythorbic acid
Sodium nitrite

Table 36.7 presents the batch formula.

Inspectors analyze sausage formulations to determine whether the amount of nitrites is within acceptable limits. Since it may be difficult to accurately measure small amounts in a commercial production environment, manufacturers often use a commercially premixed cure mix, containing the proper amount of nitrites and nitrates along with a salt carrier. To minimize the risk of using too much of these ingredients, cure mix is often marketed in small packets that are preweighed for a certain size batch of product.

TABLE 36.7

Frankfurter Batch Formula

Beef	185 lb.
Pork	225 lb.
Bacon ends and pieces	50 lb.
Water and ice	80 lb.
Rework (like product)	50 lb.
Nonfat dry milk	20 lb.
Salt	9 lb.
Mustard (29% protein)	5 lb.
Spice mix (12% protein)	4 lb.
Erythorbic acid	4 oz.
Cure mix (8% nitrite)	12 oz.

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This eliminates the need to weigh out the cure mix at the facility. It is still important to ensure that the cure mix packet is being added to the proper size batch of product.

For example, to review the amount of nitrites in the above formulation, first determine the total amount of meat and meat by-products. In this case, add the beef and pork. Note that the bacon ends and pieces and rework are not factored in, since they are already assumed to have cure added to their formulation.

$$\begin{array}{r} 185 \\ +225 \\ \hline 410 \end{array}$$

The total allowed amount of nitrites for chopped meat sausage is 0.25 ounces per 100 pounds of meat and meat by-products, and so the next step is to divide the total amount of meat by 100 to determine how many "units" of 100 pounds are in the formulation.

$$410/100 = 4.1$$

Then multiply the units of 100 pounds of meat by 0.25 to determine how many ounces of nitrite are allowed in the formulation.

$$4.1 \times 25 = 1.025 \text{ oz nitrite allowed}$$

Since the amount of nitrite in the cure is 8%, divide the amount of allowed nitrite by 0.08 to determine the total amount of cure mix that is allowed in this mixture. In this case, the cure mix amount specified for this formulation is within acceptable limits.

$$1.025 + 0.08 = 12.81 \text{ oz cure mix allowed}$$

36.9 Sanitation Issues

As with any food product, proper worker hygiene, raw ingredient handling and storage procedures, and the final product handling and storage procedures are essential to control the product contamination by organisms that are harmful to humans. Requirements governing the safe production of sausages have two prong approaches:

- Developing and implement sanitation standards
- Developing and implement Hazards Analysis Critical Control Points program

Details on these programs are presented in several chapters. In this section, we will look at some of the sanitation issues. The next section uses the production of pepperoni and salami to provide an outline of developing and implementing HACCP.

Raw ingredients are typically inspected prior to entering the sausage production facility/area. However, the manufacturer should not assume that these ingredients are free of pathogenic organisms. The manufacturer must ensure that raw ingredients are properly stored in refrigerated areas to minimize the opportunities for growth of pathogens. Workers handling these raw materials must not also handle completed sausage products, since cross-contamination can occur. Equipment and facilities should be designed to prevent cross-contamination between raw and cooked products. And, of chapter, sausage production workers should follow the standard hygienic procedures that are required for all food production workers.

Sausage production equipment must be maintained in a clean and sanitary condition, under conditions that minimize the potential for growth of pathogens. Grinding, blending, and stuffing equipment must be completely disassembled and cleaned after each use or at least daily. And when changing to a batch of another species of meat, the entire grinding, blending, and stuffing assembly must be disassembled and cleaned.

Grinders of fresh sausage products should develop and implement rework, carry-over, and many designation procedures that reflect an acceptable degree of product exposure (i.e., economic risk) in the event that a health risk is identified that results in recalling product that is suspected of presenting a potential hazard to the public. This may include developing a rework-tracking system.

And finally, the final products must be handled and stored according to acceptable standards to minimize the opportunities for cross-contamination and spoilage.

Safety of water: Water must be potable. Private wells or sources must be certified.

Condition and cleanliness of food contact surfaces: The portions of this area that should be monitored daily are cleaning and sanitizing of equipment, utensils, gloves, and outer garments that come into contact with food, and the condition of gloves and outer garments.

Prevention of cross-contamination: The issues in the area of cross-contamination that should be monitored are employee practices and physical separation of raw and cooked products.

Maintenance of hand-washing, hand-sanitizing, and toilet facilities: The issues that should be monitored are the concentration of hand-sanitizing solutions and that the toilet facilities are in good repair.

Protection from adulterants and toxic compounds: Food must be protected from contaminants such as condensation, floor splash, glass, and toxic chemicals.

Employee health conditions: Employee health conditions must be monitored daily.

Pest control: Pests must be excluded from food-handling areas.

36.10 Pepperoni and Salami Production: HACCP

The complete process of producing pepperoni and salami is governed by

- Technical know-how
- Legal requirements for safety

This chapter has discussed the technical know how on how to produce sausages in the United States. Professional reference sources will be needed to manufacture specifically pepperoni and salami.

An outline of the legal requirements to produce these sausages safely is provided in Figures 36.1 through 36.4.

When studying the figures, note the basic premise. It is assumed that the user has a minimal knowledge of the requirements of FSIS in meat and meat processing, especially in terms of terminology and abbreviations.

1. Common name?	Pepperoni Salami
2. How is it to be used?	Consumed as purchased (ready to eat)
3. Type of package?	Bulk-packed (e.g., plastic bag, vacuum packed)
4. Length of shelf life, at what temperature?	Varies with packaging and storage temperature: may last 3 months nonrefrigerated and 12 months under refrigeration
5. Where will it be sold? Consumers? Intended use?	Wholesale to distributors only
6. Labeling instructions?	Keep refrigerated
7. Is special distribution control needed?	Keep refrigerated

FIGURE 36.1 Pepperoni and salami: Product description. (Modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. Used with permission.)

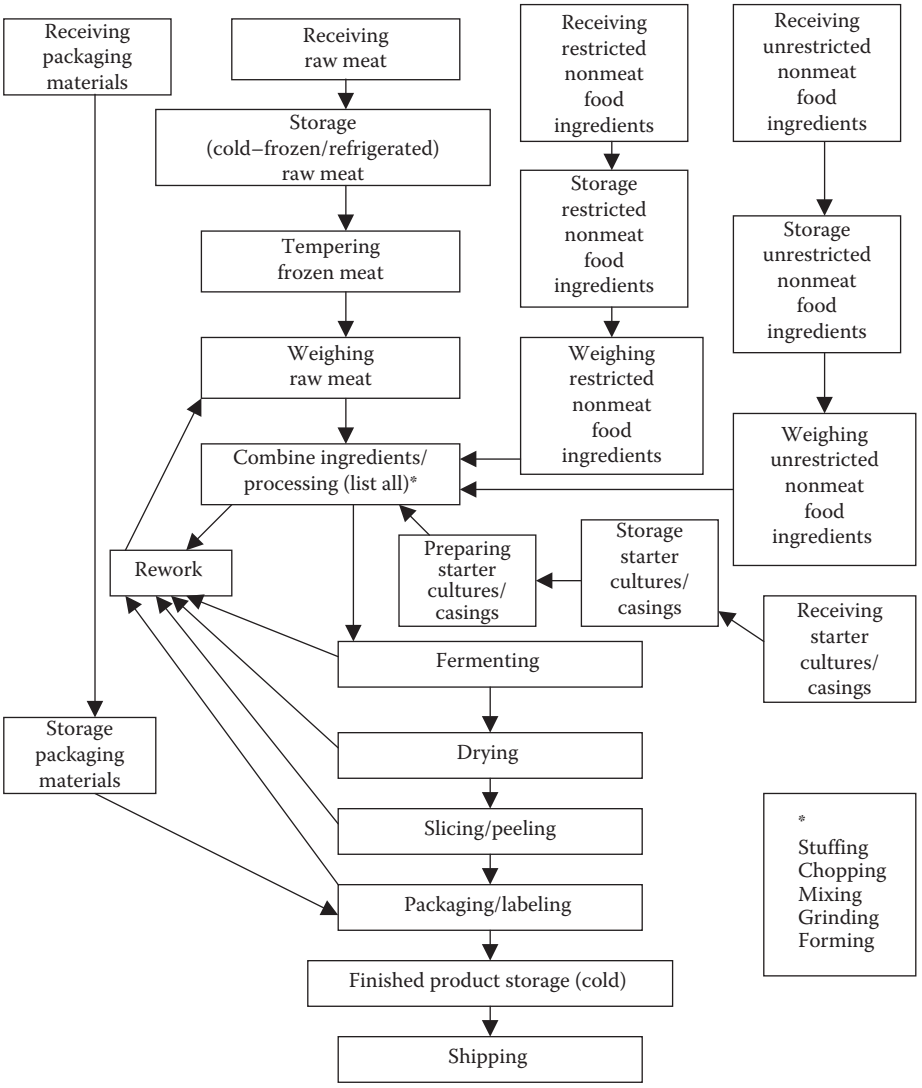

FIGURE 36.2 Pepperoni and salami: Process flow diagram. (Modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. Used with permission.)

Process Step	Food Safety Hazard	Reasonably Likely to Occur?	Basis	If Yes in Column 3, What Measures Could Be Applied to Prevent, Eliminate, or Reduce the Hazard to an Acceptable Level?	Critical Control Point
Receiving—Raw Meat	Biological: Pathogens Salmonella *E. coli O157:H7 Listeria monocytogenes*	Yes	Salmonella and *E. coli* may be present on incoming raw product. Incoming presence of Lm may impact process control and growth.	Certification from suppliers that product has been sampled for Salmonella and *E. coli O157:H7* meets FSIS performance standards. Fermentation and drying or use of postprocessing kill step could effectively control level.	1B
	Chemical—None				
	Physical—Foreign materials such as broken needles	No	Plant records show that there has been no incidence of foreign materials in products received into the plant.		
Receiving—Restricted and Unrestricted Nonmeat Food Ingredients; Starter Cultures/Casings; Packaging Materials	Biological—None				
	Chemical—Not acceptable for intended use	No	Letters of guaranty are received from all suppliers of starter cultures, casings, and packaging materials.		
	Physical—Foreign materials (metal, glass, wood, etc.)	No	Plant records demonstrate that foreign material contamination has not occurred during the past several years.		
Storage—Restricted and Unrestricted Nonmeat Food Ingredients; Starter Cultures asings; Packaging Materials	Biological—None				
	Chemical—None				
	Physical—None				
Storage (Cold—Frozen/ Refrigerated)—Raw Meat	Biological *Salmonella E.coli O157:H7*	Yes	*Salmonella and E. coli O157:H7* are reasonably likely to grow in this product if temperature is not maintained at or below a level sufficient to preclude their growth.	Maintain product temperature at or below a level sufficient to preclude pathogen growth.	2B
	Chemical—None				
	Physical—None				

FIGURE 36.3 Pepperoni and salami: Hazard. analysis (Modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. Used with permission.)

Process Step	Food Safety Hazard	Reasonably Likely to Occur?	Basis	If Yes in Column 3, What Measures Could Be Applied to Prevent, Eliminate, or Reduce the Hazard to an Acceptable Level?	Critical Control Point
Tempering Frozen Meat	Biological—Pathogens	Yes	Pathogenic microorganisms present are likely to grow if time/temperature is not maintained at or below a level sufficient to preclude growth.	Control of time/temperature during thawing process. No water tempering.	
	Chemical—None				
	Physical—None				
Weighing Raw Meat	Biological—None				
	Chemical—None				
	Physical—None				
Weighing Restricted & Unrestricted Nonmeat Food Ingredients; Preparing Starter Cultures/Casings	Biological—None				
	Chemical—None				
	Physical—None				
Combine Ingredients/Processing (Includes one or more of the following: grinding, chopping, mixing, stuffing, forming, and slicing)	Biological—None				
	Chemical—None				
	Physical—Metal Contamination	Yes	Plant records show that during mechanical processing metal contamination is likely to occur.	Visual inspection prior to stuffing and/or metal detectors are installed prior to packaging.	3P
Rework	Biological—Pathogens	No	Rework at the end of the day is condemned.		
	Chemical—None				
	Physical—None				
Fermenting (Used for flavor development and pH reduction)	Biological—Pathogens (*Staphylococcus aureus*)	Yes	Potential growth and subsequent toxigenesis of pathogens with the failure of the fermentation process.	pH sufficient to ensure correct pH reached after fermentation.	4B
	Chemical—None				
	Physical—None				

Drying	Biological—Pathogens *Salmonella*, *Staphylococcus aureus*, *Trichina*	Yes	Potential growth and subsequent toxigenesis of pathogens with the failure of the drying process.	Room temperature can be controlled to assure the drying process is achieving the desired goal of moisture protein ratio.	5B
	Chemical—None				
	Physical—None				
Slicing/Peeling	Biological—*Pathogens* (*Listeria monocytogenes*)	Yes	Potential contamination from environmental sources and/or employee handling.	Sanitizer known to be effective against *Listeria monocytogenes* will be applied every 4 h on product contact equipment.	6B
	Chemical—None				
	Physical—None				
Packaging/Labeling	Biological—None				
	Chemical—None				
	Physical—None				
Finished Product Storage (Cold)	Biological—None				
	Chemical—None				
	Physical—None				
Shipping	Biological—None				
	Chemical—None				
	Physical—None				

FIGURE 36.3 Continued.

CCP# and Location	Critical Limits	Monitoring Procedures and Frequency	HACCP Records	Verification Procedures and Frequency	Corrective Actions
1B Receiving—Raw Meat	Supplier certification that product has been sampled for *Salmonella* must accompany shipment.	Receiving personnel will check each shipment for *Salmonella* certification.	Receiving Log Corrective Actions Log	Every 2 months QA will request *Salmonella* data results from company for at least 2 suppliers.	Product without certification will not be accepted if a supplier fails to meet performance standards for 2 sample set. Supplier will not be used until a full sample set meets performance standards.
2B Storage (Cold–Frozen/ Refrigerated—Raw Meat/Poultry	Raw product storage areas will not exceed 40°F in refrigerated rooms or exceed 30°F in freezer rooms.	Maintenance personnel will record raw product storage area temperature every 2 h.	Room Temperature Log Thermometer Calibration Log Corrective Actions Log	Maintenance supervisor will verify accuracy of the Room Temperature Log once per shift. QA will check all thermometers used for monitoring and verification for accuracy daily and calibrate to within 2°F accuracy as necessary. QA will observe maintenance taking & recording temperatures weekly.	QA will reject or hold product dependent on time and temperature deviation. Product disposition will be determined by effects of deviation. Process Authority will be consulted or cooling curves will be used to make a determination. QA will identify the cause of the deviation and prevent reoccurrence by adjusting maintenance schedule & repairing equipment as required.
3P Combine Ingredients/ Processing	No metal particles to exceed 1/32 in. All kick out be reworked to meet critical limit.	Maintenance personnel will check the metal detector every 2 h to assure the kick out mechanism is working properly. All kick out product will be visually examined at the end of the shift or product line and results recorded.	Metal Detection Log Corrective Actions Log	Maintenance supervisor will verify metal detectors are functioning. QA will verify that the metal detectors are functioning as intended by running a seeded sample through the detector prior to start of each shift. QA will observe examination & rework of kick out product once per week. Kick out device will be tested each shift to determine it is functioning as intended.	Mechanical separation line supervisor will control and segregate affected product. Maintenance personnel will identify and eliminate the problem with the metal detector. Preventive maintenance program will be implemented. QA will run seeded sample through metal detector after repair. All potentially contaminated product will be run through metal detector, X-ray, or visually examined prior to further processing. No adulterated product will be shipped.

4B Fermenting	pH … 5.3 within 6 h.	QA technician will test pH of 10 sticks from each lot by probe during the fermentation process every 2 h and at completion.	Fermentation Log pH Log Corrective Actions Log	QA supervisor will observe QA technician perform pH test once per shift. QA will check all pH meters used for monitoring and verification for accuracy daily and calibrate for accuracy daily.	QA will segregate and hold all affected product until correct pH is achieved or other appropriate disposition is determined based on the nature of the deviation, time at pH of product and food safety parameters. Starter culture will be checked for appropriate amount used, dispersion, and storage parameters. HACCP plan and process controls will be changed as required. QA will identify the cause of the deviation and prevent reoccurrence.
5B Drying	Reach established Moisture: Protein Ratio (MPR) Pepperoni 1.6:1, Salami 1.9:1.	MPR checks will be done on each lot by production employee. Drying time/temperature will be monitored using room recorder charts.	Drying Room Recorder Charts Thermometer Calibration Log Corrective Actions Log MPR Log	QA supervisor will review MPR log and drying room recorder charts once per shift and observe MPR check once per week. Maintenance supervisor will verify the accuracy of the drying room recorder once per shift. QA will check all thermometers used for monitoring and verification activities for accuracy daily and calibrate to within 2°F accuracy as necessary.	If a deviation from a critical limit occurs, the following corrective actions will be taken: 1. The cause of the deviation will be identified and eliminated. 2. The CCP will be monitored hourly after the corrective action is taken to ensure that it is under control. 3. When the cause of the deviation is identified, measures will be taken to prevent it from recurring e.g., if the cause is equipment failure, preventive maintenance program will be reviewed and revised, if necessary. 4. QA will reject or hold product dependent on time/temperature deviation.

FIGURE 36.4 Pepperoni and salami: HACCP plan. (Modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. Used with permission.)

CCP# and Location	Critical Limits	Monitoring Procedures and Frequency	HACCP Records	Verification Procedures and Frequency	Corrective Actions
6B Slicing/Peeling	Application every 2 h of a sanitizer known to be effective against *Listeria monocytogenes* on product contact equipment.	QA will monitor the application and record the time of sanitizer application.	Sanitizer Usage Log Corrective Actions Log	QA will conduct a *Listeria* sampling program (both environmental and end product) as detailed in the FSIS issurance "*Listeria* Guidelines for Industry" to verify effectiveness of the sanitizer and its method of usage.	QA will address positive *Listeria* samples as detailed in the FSIS issuance "*Listeria* Guidelines for Industry." QA will stop slicing/peeling operations if time since last application of sanitizer exceeds 2 h. All end product produced after the 2 h limit is exceeded will be held until *Listeria monocytogenes* test results are final. If positive, product will be condemned and additional environmental and end product testing will be done until the source is determined. Further corrective actions will be done as detailed in FSIS regulations §417.3.

FIGURE 36.4 Continued.

37

Mold-Ripened Sausages

Kálmán Incze

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37.1 Introduction

When following collection of plants and fruits, food of animal origin also became available to humankind, in the form of predators' leftover and later by fishing and hunting, they must have realized that meat cannot be left "as is," because in some hours or some days, depending on the actual ambient temperature, it spoils or becomes poisonous, and consequently cannot be eaten. Since meat as animal protein is not only a food that tastes good but is also an excellent source of essential nutrients and some vitamins, it has always been the target of pathogenic and spoilage microorganisms that break down and transform these substances for the sake of their own growth. Keeping in mind this effect and its consequencies, humans felt forced to find ways in which to keep these hard-to-get meats available to them for a longer time.

It is probably a fairly acceptable guess that our ancestors arrived at the idea that there are also preparation methods by the help of which meats can preserve their advantageous properties for a longer period of time. We may assume that drying, cooking, frying, cooling, and freezing are the most ancient ways of preservation, yet while cooling and freezing could take place only temporarily and not under warm

climate, drying was an applicable method everywhere. In early times, meat drying took place in caves over fires and open fires; this also allowed for the advantageous effects of smoke (Incze 2007).

It seems reasonable to presume on the basis of written relicts (Rixson 2000) that our ancestors not only dried, but also salted and then dried the meat. Since salt possibly contained nitrate as contaminant, some meat pieces might have been cured to some extent by chance. Cured and dried meat was known around 1000 BC (Rixson 2000), while fish was salted and dried in ancient Egypt.

Salted and dried meat played an important role in Roman Empire as high-energy food for legions in battles. Soldiers received a salt portion on a regular basis (called "salarium," where the word salary possibly originates from) as an important material for preservation and making the food tasty. Also Hungarian warriors used salted and dried meat more than 1000 years ago but this type of preservation still exists in many parts of the world (Biltong in South Africa, Pemmican of Indians in North America or Tasajo in South America, as also Lawrie (1998) mentions).

We know also that Romans already prepared "botulus" (sausage, hence the name botulism, sausage poisoning) from smaller pieces of meat, fat mixed with salt and spices and stuffed into intestines of killed animals. According to Pederson (1979), Homer mentions a dried sausage in the ninth century BC, while Leistner (1986, 2005) suggests that sausage and salami manufacture may be considered to be 250–300 years old.

The preparation of dried sausages step by step has developed over the last century through the experience of our ancestors, and this experience has been increasingly supported by research activities and scientific evidences; however, research activities still continue to yield further information.

In the earlier stage, dry sausages were produced only from meat, fat (curing), salt, and spices. Although scientists were aware of the existence of microorganisms in the nineteenth century, and their possible role in fermentation was known, thanks to Pasteur's work, this referred to dairy and wine and no relation between microbial fermentation and dry sausage manufacture was thought of before the 1950s when Niinivaara and Niven did the excellent work in this field. (Niinivaara wrote the history of starter cultures in 1993.)

As a result of their initiating research, a determinant change came through in the dry sausage technology: with their contribution, we learned about the important role of microorganisms, and we are able to exploit their ablities in producing raw sausages, thus ensuring consistency in quality and food safety.

Interestingly enough, the conquest of useful microbes, starter cultures as they are called, was overwhelmingly successful in sausage technology, nevertheless not so successful in the production of traditional raw ham (Iberian and Parma ham). Two reasons for this difference include: large intact instead of chopped muscle; very long ripening–drying.

Mold-ripened sausages, that is, salamis, are raw-fermented products with usually longer ripening and drying time. In the traditional technology, no starter cultures and/or chemical acidulants were applied, but the old technology has been changed considerably to the starter technology all over the world applying lactic acid bacteria, staphylococci, yeasts, and molds.

Because of chemical changes, final pH is usually higher (pH > 5.5) due to the longer ripening–drying time even if the pH-value after incubation with starter culture is lower. For this reason, it is of vital importance, from the food safety point of view, that final a_w-value be sufficiently low, a requirement that is fulfilled by a long drying period.

37.2 Characterization of Mold-Ripened Sausages

Manufacturing (selection of raw material, chopping of meat and fat, blending with additives, and ripening and drying) of mold-ripened sausages is rather similar to that of sausages without mold with the basic difference that a short period of ripening–drying of mold-ripened sausages needs higher temperature and relative humidity in the ripening room to support the mold growth by ensuring optimum conditions.

Under wet conditions, any kind of molds can grow, but for technological, organoleptic, and safety reasons, growth of selected molds is desired that can be ensured either by a house mycoflora being developed during decades or by inoculation with a mold starter.

TABLE 37.1

Characterization of Mold-Ripened Sausages (Diameter: 65 mm)

Data Measured	Traditional Technology	Starter Culture in Meat Batter
Drying time[a]	10–12 weeks	5–6 weeks
pH[b]	5.6–6.2 or above	≤5.3 after incubation 5.5–5.6 or above in the final product
a_w^c	<0.90–0.91	<0.93

[a] Data given can vary depending on composition of raw material (e.g., beef dries faster), drying room parameters, and so on. Evidently, the tenures given can be extended depending on the actual sensory properties to be attained. Data given in the table can be considered as average figures.

[b] pH of sausages with starter technology is lower than that of traditional ones, yet because of longer drying, it is usually higher than 5.3.

[c] While an a_w-value below 0.95 in combination with low pH is sufficient with starter sausage without mold for the prevention of spoilage and pathogenic growth, a lower a_w-value serves for a better safety preventing lactic acid breakdown in the case of mold-ripened sausages.

Traditionally manufactured mold-ripened sausages are dried for a longer period of time to reduce the original a_w-value of about 0.97 to below 0.90–0.91, than in the case of acidified (by starter) sausages where reduced pH (<5.3) ensures safety in combination with lowered a_w. Nevertheless, it has to be mentioned that even in the case of mold-ripened starter sausages, drying time is long enough to promote biochemical reactions responsible for raising the pH-value unlike in short-ripened–dried-sausages without mold.

Some characteristic properties of mold-ripened sausages are shown in Table 37.1.

In certain cases moisture: protein ratios are given for specifying dry and semidry sausages, but a_w-values can be considered less equivocal (Incze 2007).

37.3 Technology of Mold-Ripened Sausages

37.3.1 Raw Material

High-quality products can be manufactured only from high-quality ingredients, that is, from hygienically processed raw material with good technological and sensoric attributes produced under good manufacturing practice (GMP). This high hygienic level is even more important in case of dry sausage manufacture, since there is no way of pasteurization for reducing undesired microbial load in raw materials as it is in dairy industry. Structure and texture, as well as the sensory value, are influenced (Incze 2000) by the following factors:

- Type of meat (pork, beef, poultry, game, young, old animal, etc.)
- Type of fat (saturated-to-unsaturated fatty acid ratio)
- Type of fat tissue (marbling and back fat)
- Meat-to-fat ratio in sausage batter (higher fat: protein ratio = softer texture)
- Particle size
- Temperature of chopping, grinding, and stuffing
- Type and concentration of seasonings
- Application of carbohydrate (type and concentration)
- Casing diameter
- Smoking
- Pattern of pH-changes
- Salt concentration

- Microflora (starters or spontaneous)
- Drying parameters
- Final pH and water activity

As for the technological quality of meat, the "drier" muscle of sows—with higher intramuscular fat content—is more suitable than the lean muscle of young animals if low-water-activity dry salami is the aim, achieved through a long ripening. Meat with higher fat and lower moisture content allows for better control of drying; also, less shrinking of sausage diameter occurs, which gives a more attractive product.

The presence of pale, soft, exudative (PSE) and dark, firm, dry (DFD) meat is less of a problem because of the low incidence of such types; their presence is compensated for by comminution and mixing with normal meat. Formation of texture greatly depends on the pH-value: when lactic acid is produced by starter cultures, gelifying a protein solution between meat and fat particles, a firm texture and sliceability is attained more rapidly than when the sausage pH is far from the isoelectric point of muscle. This means that with a higher sausage pH (low acidity), a significant rise in salt concentration (through long drying) is the only factor responsible for gelifying the protein solution and for sliceability, which is the result of the gel structure of the protein matrix. Further details on influencing factors are presented in the following chapters. Figure 37.1 shows the process flow of dry sausage manufacture.

37.3.2 Prefreezing and Comminution Techniques

Depending on the comminution technique (chopping or grinding), prefeezing is either a must—or can be omitted. If raw material is ground (e.g., in Italy), no prefreezing is needed; chilling is adequate. But if chopping (bowl chopper) is applied (e.g., in Germany, Hungary, the Netherlands, etc.), prefreezing of fat (–5–7°C) and meat cannot be avoided; otherwise, smearing of fat particles during chopping and stuffing causes drying failure, loss of sensory quality and leads to hygienic risk too.

During comminution and blending by bowl chopper meat, fat, and other ingredients, curing salt, seasonings, carbohydrates, and starter cultures (if applicable) are added and thoroughly mixed. Care should be taken with the order of addition: simultaneous addition of curing salt and starter culture should be avoided for the sake of protection of starter culture against initial high-salt concentration. If grinding technology is applied, in addition to mixing the ingredients before grinding another mixing after grinding may be necessary.

Even if comminution is done properly, adequate stuffing is needed to avoid smearing of sausage batter; in addition to the appropriate temperature, the right choice of a vacuum stuffing machine is of crucial importance, because air bubbles in the sausage could cause spoilage in the form of rancidity, discoloration, not to mention the visual defect of the cut surface with air holes. In order that moisture can evaporate from sausages during drying, casings with proper vapor permeability are used. Details on casings can be found in the book by Savic and Savic (2002).

After stuffing, sausages are washed and dried, incubated (if starters are used), the surface is inoculated with mold starters, and smoking is finally applied.

During ripening, the temperature, relative humidity, and air velocity are adjusted to promote mold growth. Major critical points during dry sausage manufacture are somewhat different whether traditional or starter culture technology is applied (Incze 2004a).

37.3.3 Fermentation

In original sense, in the case of fermentation, we think of the breakdown of added and tissue-carbohydrate, but with raw sausage fermentation, it can be used in broader sense, consisting of other metabolic activities as well. We may make a distinction betwen the first days' fermentation processes and the ripening that follows, but these activities of tissue and microbial enzymes are continuous with descending intensity as a_w decreases causing partial or total inactivation at a later stage. Meat fermentation principles and application are discussed in detail by Demeyer (2004) and Petäjä-Kanninen and Puolanne (2007).

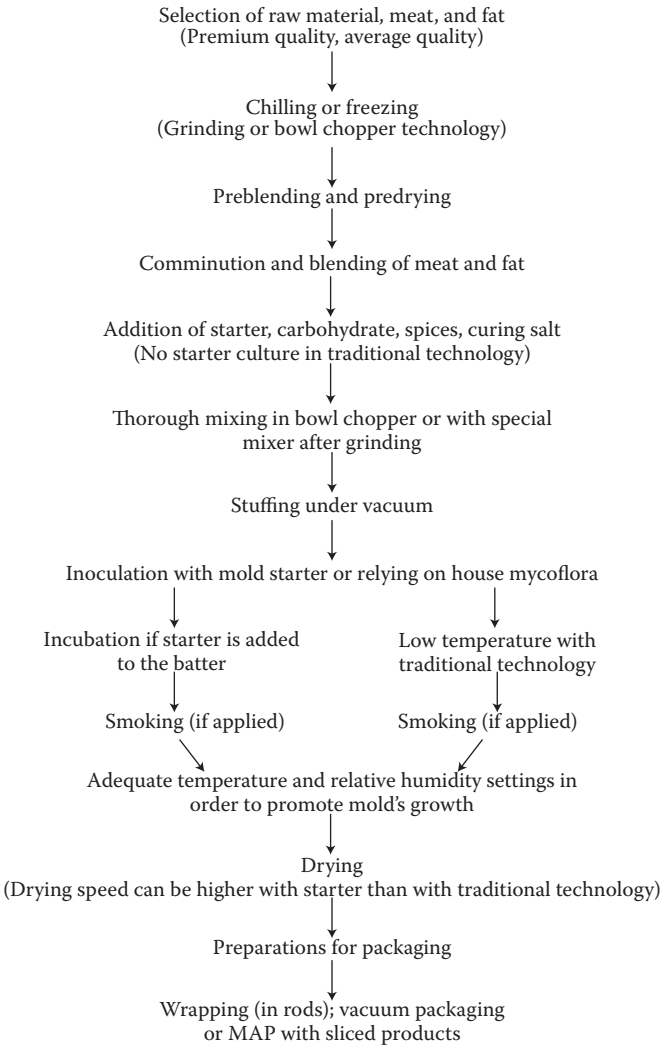

FIGURE 37.1 Process flow of dry sausage manufacture.

37.3.4 Use of Starter Culture

In long-ripened sausages, just as in short-ripened ones, lactic starters reduce pH and contribute to aroma; apathogenic staphylococci play a role mainly in color and aroma formation. Since staphylococci require higher pH, the pH-drop caused by lactobacilli may retard their growth. Preripening (keeping the sausage at 5°C for some days) can give staphylococci a chance to grow before pH-drop (Sanz and others 1997).

Depending on whether starter cultures (lactic starters alone or in combination with staphylococci and yeasts) are used or not, initial temperature (incubation) is basically different. In the presence of starter culture, the temperature has to be adjusted to the microorganisms' need, usually below 30°C (18–24°C) in Europe and above 30°C (27–32°C) in the United States.

The presence and metabolic activity of the starter culture contribute to product safety through its inhibitory effect against undesired microbes. But if no starter culture is added to the sausage batter, another inhibiting factor, low temperature, has to be relied on. This low temperature (usually 10–12°C), together with common and curing salt, ensures sufficient protection against growth of pathogenic and

spoilage microorganisms until adequately reduced water activity ensures inhibition also at higher temperatures.

Types and application of starter cultures are discussed in several publications; some of them are mentioned here (Leistner 1990, Knauf 1995, Cocconcelli and Fontana 2010).

Earlier starter cultures were applied mainly for controlling pH-changes or nitrate reduction and color stability, respectively, but nowadays other tasks are also accomplished by microbial cultures: they increase safety by inhibiting growth of undesired, that is, pathogenic and spoilage microorganisms. This is specially the case with starters and protective cultures that in addition to lactic acid produce also specific antibacterial substances, bacteriocins as they are called.

These protective or bioprotective cultures can be effective against bacteria such as *Listeria monocytogenes*, *Clostridium botulinum*, and *Staphylococcus aureus*.

Some more details concerning mold-ripened sausages can be found with Incze (2004b, 2010).

37.3.5 Color Formation

Raw-fermented sausages are usually manufactured with curing salt, in earlier times, and currently, in some cases, with KNO_3, but mostly with nitrite ($NaNO_2$), or a mixture is applied. Since sodium nitrite ($NaNO_2$) is poisonous in small concentrations, for the sake of prevention of eventual poisoning, a curing salt, that is, mixture of 99.5% NaCl and 0.5% $NaNO_2$, is used which excludes the possibility of overdosage of nitrite. (In Europe, 0.4% $NaNO_2$ content in curing salt is also applied; in the United States a much higher $NaNO_2$:NaCl ratio is applied, and the mixture is diluted by common salt during technology.) At the end of the 1970s, apprehending the risk of nitrosamine poisoning, use of nitrite and nitrate was reduced, even prohibited in some countries, but a few years later application of curing salts is further on allowed with preference of nitrite. In this change of situation, basically two facts played a decisive role: the very intensive investigations did not find the danger unequivocally as real if there was no abuse in nitrite concentration, temperature, and so on and a reducing agent was applied on the one hand, and never since has been found any other compound that could substitute the favorable effects of nitrite with emphasis on its *C. botulinum* inhibitive capability on the other.

If nitrate is used, it has to be reduced to nitrite by microorganisms, which then reacts with myoglobin to give nitrosomyoglobin. The more stable pigment is formed most likely by denaturation through increasing salt concentration during drying. Dry sausage can also be manufactured with common salt only, yet the microbiologcal risk is higher if the antibacterial effect of nitrite cannot be relied on. This is even more pronounced if for health purposes sodium content is reduced. In this case, safety requirements can be met only if other a_w-reducing substances are applied. In the absence of curing salt, color stability of sausage is reduced, but intensive smoking improves it to some extent because NO-compounds from smoke deposit and diffuse inside (Incze 1987). Details on color formation are discussed thoroughly by Møller and Skibsted (2007) and Honikel (2010).

37.3.6 Smoking and Mold Growth

Most mold-ripened sausages are not smoked; this is explained by the long traditions that offer some advantages, mainly in recent decades since artificial inoculation of the surface by starter molds like *Penicillium nalgiovense, Penicillium chrysogenum*, and *Penicillium camembertii* (Knauf 1995) has been applied. Starter molds are sensitive to smoke constituents in general, and this retards mold growth, resulting in scarce and uneven growth. There are, nevertheless, mold-ripened sausage technologies in which after intensive smoking, surface inoculation with mold starters is applied, or spontaneous mold growth covers the surface (e.g., Hungarian salami). For even growth on the whole surface of this latter type, sporadic colonies are spread by brushing. Smoking has manifold advantages:

- Inhibition of growth of yeasts (slime-producing), *Kocuria* strains and undesired
- Toxigenic molds

- Strong antioxidative effect retarding rancidity very efficiently
- Significant positive influence on sensory characteristics

As a result, smoked products are significantly different in taste and aroma. The mold layer on sausages has several advantages:

- Drying is more balanced, thus reducing frequency of drying failure.
- It slows down moisture loss in the final product.
- Because of its light protection, discoloration and rancidity is retarded.
- Molds metabolize peroxides, thus protecting against rancidity (Spotti and Berni 2007).

The source of molds is either the air in old ripening rooms with selected mold strains (it is actually a kind of "autoselection" controlled by temperature, relative humidity, and eventually by smoke and certainly by manufacturing for decades) that deposit on the surface of sausages, or mold starter culture is used for artifical inoculation. Inoculation may take place in the form of dipping the sausages in starter culture suspension after stuffing or in the form of atomizing of the suspension. This spraying can be applied either before or after smoking if the product is smoked. If applied after smoking, molds sensitive to smoke may have better chance to grow.

Growth of molds, be it inoculated or spontaneous, has to be supported by suitable temperature and relative humidity parameters. This is a process involving risk, because with too low a temperature and relative humidity, mold growth is retarded and undesired microbes might grow instead; on the other hand, with too high a temperature and relative humidity, pathogenic and/or spoilage microorganisms are able to grow inside the sausage, mainly if a_w is not low enough, and molds metabolize lactate, which allows growth and toxin production of staphylococci (Rödel and others 1993). Consequently, strict control of these parameters in accordance with the requirement of mold applied is of vital importance.

If mold growth is well supported, an even mold cover is formed on the surface that sticks well to the product and remains intact during handling, unless abuse occurs during storage: either too high a relative humidity or an impermeable packaging film.

A well-developed mold layer plays an important role during further technological steps and also during storage in retail and in households, as mentioned earlier. After the mold layer is completed, relative humidity, and in some cases also temperature, is decreased and drying is continued. Thanks to this mold layer that the risk of too-rapid drying causing case hardening is significantly less.

37.3.7 Ripening–Drying

Ripening is a most complicated process controlled only to some extent by technologists and completed mostly by tissue enzymes and by metabolic activity of inoculated and/or spontaneous microorganisms.

Intentional means for control are application of starter culture, type and concentration of additives (intrinsic factors), and mainly adjustment of temperature, relative humidity, and air velocity in function of time (extrinsic factors), controlling in this way the drying rate (Leistner 1990). Uniform air distribution is a precondition for uniform and consistent quality. In contrast to short-fermented sausages, in which, to some extent, also tissue enzymes and definitely bacterial enzymes are active in the final product too, this process can slow down or even stop after an extremely long-drying period of several months, resulting in a_w-values as low as 0.85.

Since there is a difference between the pH-value before drying of the traditional and starter sausage, the latter can be dried faster without the risk of case hardening. The well-known reason is that at a pH close to the isoelectric point of muscle protein (pH 5.3) the less strongly bound moisture can be given off easier than in traditional sausage with a typical initial pH-value of 5.6–5.8. This also means that greater care has to be taken in the latter case with drying to avoid case hardening. This explains why differences between drying times of traditional and starter sausages are significant as shown in Table 37.1.

For details on theory and practice of sausage drying, see Zukál and Incze (2010).

37.4 Changes during Ripening–Drying: Aroma Formation

Controlling temperature and drying rate plays a decisive role in food safety by inhibiting the growth of undesired microbes, but this control is needed also for a consistent sensory quality. As a result of drying (i.e., a_w-reduction), less resistant microorganisms gradually disappear (members of Enterobacteriaceae, pseudomonads, bacilli, etc.); less sensitive ones (*Kocuria*, staphylococci, enterococci, listeriae, etc.) may survive for a longer period of time. In the final product of long-ripened dried sausages, spoilage microflora has practically no chance to grow and cause deterioration. The situation is somewhat similar with pathogenic microorganisms, yet their very presence is not accepted by food authorities requiring sometimes zero tolerance—a standard that is hard to support scientifically with all types of microorganisms.

In order to meet safety requirements, a complex strategy has to be followed by adding and/or supporting growth of useful microorganisms in the hygienically produced sausage and by controlling ripening–drying parameters, thus favoring the growth of useful microorganisms and inhibiting growth of undesired ones, as discussed above. While doing so, not only are safety requirements met but aroma formation is also contributed to.

Starter cultures and bacteriocin-producing microorganisms can help in inhibiting pathogenic microbes (Demeyer and Vandekerckhove 1979, Rödel and others 1993, Aymerich and others 1998, Hugas and others 1998, Leroy and others 2001), where inhibition of staphylococci and listeriae is desired mostly because these bacteria are difficult to combat, as a result of their resistance to low a_w, the main controlling factor in long-dried sausages.

It should be kept in mind that bacteriocin producers are in general more effective under laboratory conditions on media than in a food matrix (Leroy and others 2001).

Unlike in dried ham, tissue endoenzymes in long-dried sausages play a less important role in aroma formation, and these are active mainly in the first part of ripening (Harnie and others 2000). In long-dried sausages, the metabolic activity of microorganisms contributes more to aroma but other factors — such as spices, lipolysis, and so on—may rather be involved in this complex process too.

As mentioned already, smoking and pH-changes (low- and high-acid sausages) do influence aroma characteristics, but the types and concentrations of spices used determine aroma even more (Chizzolini and others 1998). In this respect, spices that can be evenly distributed in sausages batter and react immediately with meat and fat matrix have a much more pronounced impact on aroma characteristics than occurs in long-ripened hams, where spices are generally not applied or they are rubbed on the muscle surface only. Natural spices in sausages influence aroma as well as safety indirectly too by enhancing metabolic activity of starter cultures (Nes and Skjelkvale 1982, Hugas and others 1998).

As for taste and aroma formation by microbial metabolism, the breakdown of proteins, peptides, lipids, and carbohydrates is discussed next.

Depending on application of lactic starters and addition of carbohydrate, lactic acid is produced through homofermentation, reducing the pH-level (Demeyer and Vandekerckhove 1979, Roca and Incze 1990). Concentration of D-lactate may be five times higher in high-acid sausages than in low-acid products (Montel and others 1998). At a later stage, pH also increases in sausages with lactic starters; this is related to the formation of ammonia and some amino acids (Demeyer and Vandekerckhove 1979). Nevertheless, this occurs mainly in products in which apathogenic staphylococci are used without lactobacilli (Marchesini and others 1992). In traditionally manufactured molded dry sausage (e.g., genuine Hungarian salami), no starter culture and very small amounts of carbohydrate are added; as a result, pH in the final product is around 6.0 (Incze 1987).

As for the breakdown of muscle proteins, endoenzymes (cathepsin D) are active at the beginning of ripening (Harnie and others 2000) and are enhanced by lower pH (Montel and others 1998). Lower pH, on the other hand, does not favor endogenic aminopeptidases. Microbial aminopeptidases that break down peptides into amino acids also contribute to aroma development (Flores and others 1998). Their activity is influenced by a_w, pH, and temperature too. Bacterial enzymes play a role in proteolysis at a later period of ripening (Verplaetse and others 1992).

Of microorganisms, the proteolytic activity of lactobacilli, micrococci, and molds has been more thoroughly investigated, and it has been found that some lactobacilli (*L. casei* and *L. plantarum*) can break down sarcoplasmic and myofibrillar protein (Fadda and others 2000).

Intensity of protein metabolism is higher by micrococci (staphylococci) that are also responsible for formation of volatile and nonvolatile aroma compounds (Stahnke and others 2000, Sunesen and others 2000).

Nielsen and Coban (2001) found that *P. nalgiovense* has proteolytic activity, breaking down peptides in addition to muscle protein stimulated by salt. Ordónez and others (2000) found strong proteolytic (and lipolytic) activity with *Pencillium aurantiogriseum*.

In lipolysis, endo- and exoenzymes participate, yet microbial enzymes also play a more intensive role here (Talon and others 1992). Typical flavors and aromas are related to the hydrolytic and oxidative changes occurring in the lipid fraction during ripening. Lipid oxidation may cause off-flavor but may contribute to the development of desirable flavor too (Talon and others 2000). Lipid oxidation is mostly associated with unsaturated fatty acids and is often autocatalytic. For this reason, risk is higher if the raw material contains a higher ratio of unsaturated fatty acid (Rödel and others 1993, Bloukas and others 1997) either by partially replacing fat with vegetable oil or by using the fat of animals that consumed elevated levels of unsaturated fatty acid. Risk of oxidation, on the other hand, can be reduced by the antioxidative effect of smoking and by mold ripening because of the light protection and direct oxygen consumption provided by mold, as pointed out by Ordónez and others (2000).

It is interesting to note that as a result of microbial lipolysis in mold-ripened Hungarian salami, a significant increase in the acid number of the fat fraction takes place, reaching a value of 15 without any sign of rancidity as judged by peroxide or thiobarbituric acid (TBA) number or organoleptically (Nagy and others 1988). Such a high acid number can otherwise be detected only in strongly rancid lard with a high peroxide number, where because of high-temperature rendering, no microbial activity takes place and moisture is also very low.

Because physical, chemical, biochemical, and microbiological changes in mold-fermented sausages are rather similar to that of dry sausages without mold, the reader is referred to publications on these topics (Navarro and others 1997, Chizzolini and others 1998, Flores and others 1998, Incze 1998, 2000, Paramithiotis and others 2010, Stahnke and others 2000, Sunesen and others 2000, Talon and others 2000, 2004, Zanardi and others 2000); mainly those features that are characteristic to mold-ripened sausages have been discussed. It has to be mentioned that sensory characteristics may significanty differ depending on the diameter for two reasons: (1) the metabolic pathway is different in the case of anaerobiosis (large-diameter salamis) compared to small-diameter sausages, in which anaerobiosis occurs to lesser extent; and (2) a thinner sausage has much less time for aroma formation because of the significantly faster drying.

37.5 Safety of Mold-Ripened Sausages

37.5.1 Bacterial Risk

The problem of possible growth, survival, and/or toxin production of salmonellae, toxinogenic clostridia, staphylococci, listeriae, and mycotoxic molds has to be dealt with, as well as the potential risk of biogenic amines.

Safety requirements can only be met if hygienically produced raw materials, ingredients, and additives are used during GMP, applying a well-based hazards analysis critical control points (HACCP) system. For traditional technologies, long-existing safety records prove the right way of manufacturing (Incze 1998). Should a new manufacturing technology be started, challenge tests with pathogens must be used. (With enterohemorrhagic *Escherichia coli*, the food safety and inspection service (FSIS) requires the following challenge test with raw-fermented sausages: it has to be proved that a five-logarithmic reduction is caused by the given technology.)

It is relatively easy to inhibit the growth of salmonellae and clostridia because both are sensitive to low pH and to low a_w, and clostridia are inhibited by nitrite too. Staphylococci are inhibited by low temperature

and low pH, and although they are rather resistant to low a_w, no enterotoxin can be produced at an a_w-value that is common with long-ripened sausages ($a_w < 0.90$).

Listeriae are rather resistant to the pH and a_w-values of fermented sausages, but if the initial count is low and GMP is applied, listeriae are affected by low pH, low a_w, growth, or the presence of starter cultures causing reduction in their number, mainly if bacteriocinogenic microbes are applied (Aymerich and others 1998, Hugas and others 1998). A new, possibly efficient way of inactivating listeriae in foods of animal origin may be the application of bacteriophages specific for destroying *L. monocytogenes*. Soni and Nannapaneni (2010) found that LISTEX P 100 phage (qualified as GRAS) caused significant reduction on raw salmon tissue. Further research needs to clarify whether this phage works under the conditions of dry sausage or not.

Enterohemorrhagic *E. coli* are also rather resistant to low pH and low water activity, but they are reduced in number if a low a_w (≤0.91) condition exists for longer time at elevated ambient temperature (Nissen and Holck 1998). This phenomenon, also called metabolic exhaustion, is a reliable means that helps ensure a five-log reduction in case of long-ripened dry sausages.

Further details on spoilage and pathogenic risk and control have been discussed by Skandamis and Nychas (2004) and by Labadie (2007).

37.5.2 Mycotoxic Molds

Since the discovery of aflatoxin, numerous other mycotoxins have been detected and identified on agricultural products, in foods, and in nature. Ever since we became aware of the potential risk of mycotoxins, foods with molds growing inside or outside, spontaneously or intentionally, are considered "suspicious." With this in mind, mold-ripened sausages (and hams) have been investigated thoroughly, and the isolated molds were tested for toxin production (Bullerman and others 1969, Ciegler and others 1972, Alperden and others 1973). It was possible to isolate mycotoxic mold from mold-fermented meat products, and mycotoxins were also found in products artificially inoculated with pure culture of toxic molds, but in general no mycotoxins were detected in commercially manufactured meat products. In further experiments, the effect of extrinsic and intrinsic factors on toxin production potential were investigated (Incze and Frank 1976a,b). In these latter experiments, mixed culture ("house flora") of apathogenic molds, low temperature (13°C), and low a_w-value (0.94) as well as intensive smoking have been shown to be growth inhibitors of mycotoxic molds (eight aflatoxin and 11 sterigmatocystin producers were inoculated). This finding has been supported by investigation of 800 salami samples taken from retail shops: testing analytically as well as biologically for mycotoxins gave proof of the absence of mycotoxins (Incze and others 1976). On the basis of these results, "house mycoflora" of ripening rooms can be considered harmless if traditional technologies with reliable hurdles are applied during ripening–drying; nevertheless, technologies have been changed in general for the application of mold starters.

Summing up, we can conclude that fermented, dried meat products are stable and safe without refrigeration because of low a_w-value (<0.90) or because of a combination of low a_w and low pH-value.

37.5.3 Biogenic Amines

These compounds are mainly formed by decarboxylation of amino acids or by amination and transamination of aldehydes and ketons (Eerola and others 1992). Occurrence and concentration of biogenic amines (tyramine, putrescine, cadaverine, etc.) in fermented foods have been intensively investigated (Pipek and others 1992), and it has been found that starter cultures may play a role in the extent of biogenic amine formation but freshness of raw material can be considered a major factor in keeping occurrence and concentration of biogenic amines low (Hernández-Jover and others 1997, González-Fernández and others 2000). Consequently, dry sausages properly manufactured from fresh raw materials can be considered safe also from this point of view. For further details on biogenic amines, see Vidal-Carou and others (2007).

37.6 Shelf Life

From microbiological point of view, long-ripened sausages are stable because of their low-water activity or because of the combination of low-water activity and low pH. This refers to sausages stored under adequate conditions, otherwise at high relative humidity, the mold cover may loosen and get off or secondary mold growth may occur.

Too low a relative humidity and too high a temperature during storage shortens shelf life but only because it lowers sensory quality and has nothing to do with microbiological activity, that is, with spoilage or with safety risk. While vacuum and modified atmosphere packaging (MAP) is an excellent method for extending the shelf life of sausages without mold, it is no real alternative for mold-ripened sausages, the reason for which is manifold.

The problem with both types of packaging is that moisture migrating from the core to the surface cannot evaporate making it wet; thus, the mold layer loosens and comes off giving the product a bad appearance, and wild strains of yeasts and some bacteria eventually present under the mold layer start grow causing bad odor too. For shorter storage of several weeks, either wrapping in cellophane or perforated polypropylene can be used to let moisture evaporate, with awareness that weight loss occurs even if at lower speed. Evidently, if mold-ripened dry sausage is sliced after peeling of the casing and packaged under vacuum or in MAP, a much longer shelf life, lasting for several months, can be guaranteed. Zanardi and others (2002) claim that because of less residual oxigen in MAP, less oxidation effect was detected than in vacuum packaging, which is not always the case and depends very much on how precisely the packaging technology is carried out. On the other hand, it is true that MAP is favored because slices do not stick together as strongly as in vacuum packages and can be separated easier.

As mentioned, long-ripened dry sausages are stable and safe also at ambient temperature, and cold storage in refrigerator is rather disadvantageous because phosphate crystals can appear on the product. This can occur even if no phosphate was added: muscle tissue′s own phosphate compounds are sufficient for causing this phenomenon, supported by decreasing the solubility of phosphates at low a_w and higher pH.

37.7 Quality Defects

Reasons for quality defects can be as follows: use of poor-quality raw materials, technological failures, and abuses during storage. Some of the more common faults and causes are discussed below.

37.7.1 Defects in Appearance

A properly manufactured mold-ripened dry sausage has a uniform, even mold layer that sticks to the surface. If the mold layer comes off, possibly yeasts and/or microbes grow first on the surface of sausage thus leading to adhesion of molds to the surface eventually, and/or wet conditions are responsible as mentioned. If pieces of the mold layer comes off, quite often with the cellulose casing itself, followed by greasing through, it can be explained by presence and activity of cellulase-producing molds. As for adequate prevention, use of a proper mold starter in necessary concentration and activity and/or use of casings that cannot be attacked by cellulase enzyme (e.g., collagene casing) can be considered. Another reason for an uneven mold layer may be technological in nature (temperature and relative humidity unfavorable for mold growth and inefficient mold starter) or storage abuse (high relative humidity). Another type of defect occurs if yellow, green, or black molds grow on the surface distorting the required grayish-white mold layer.

Deformation of sausages is caused by improper drying (too rapid, mainly in the first period), resulting in case hardening that does not allow evaporation of moisture from the core. As a consequence of case hardening, spoilage and pathogenic microbes may grow because a_w-reduction is slowed down significantly. Another reason for case hardening may be the wrong comminution temperature and wrong selection of stuffing machine causing fat smearing that inhibits proper drying of the inner part of sausages.

In some cases, there are consumers who are accustomed with excessively dried, distorted, deformed susages with dents (e.g., in France, Spain, Switzerland, China etc.), be it with or without mold, having the impression of a very low-moisture product suggesting both good safety record, long shelf life, and higher nutritive value (high muscle protein content). This actual demand is met quite often by local smaller manufacturers. These sometimes intentionally pressed, oval-cut surface sausages can be of excellent quality, but deformed by chance and rancid products occur too.

37.7.2 Color Defects

Color defects can be caused by insufficient amount or absence of curing salt by mistake and by a wrong way of smoking the sausages. If sausages with a wet surface are smoked at high relative humidity in the smoking chamber, brownish–grayish discoloration occurs, not only on the surface but also in a thicker layer, a fault that is most of the times irreversible. Color defects can occur also if air holes are numerous and extended caused by failure during stuffing or by faulty drying causing case hardening with longer holes. In both cases, discoloration and rancidity effect of oxygen are the causative agents.

37.7.3 Odor Defects

Odor defects can be caused if the surface is wet for long, and yeasts as well as micrococci grow, making the surface slimy and malodorous. This slimy layer inhibits good adhesion of molds that can slip off easily. If raw material is not of good hygienic quality or ripening–drying is not done properly (too high temperature, too slow drying, inefficient starter culture, etc.), spoilage can occur with unpleasant odor and taste; health risk cannot be excluded either.

As mentioned earlier, it is very important that initial microbial load of the raw material has to be low because the means are not plenty for inhibiting spoilage and pathogenic microorganisms in case of raw sausage manufacture: only low temperature, and low pH (if a starter is applied), can combat their undesired growth.

To this inhibitive effect, common and curing salt contribute as well, as a result of which either reduction of common salt and/or omission of nitrite increases health and spoilage risk.

37.7.4 Flavor Defects

Flavor defects can be caused if the product is too acidic (higher carbohydrate concentration, higher temperature for long) or rancid. The latter case can occur if the raw material was not fresh, if fat ingredients contain too high a concentration of unsaturated fatty acids, drying and storage are done at too high temperatures and for too long, nitrite is not used, and sausages are not smoked.

An intensive smoking treatment, evidently at low temperature (<15°C) because the sausage is raw dried, has a very efficient antioxidative effect preventing rancidity thus extending shelf life. If the sausage is manufactured with fresh meat and fat with proper redox state and the product is intensively smoked, practically no rancidity occurs even in spite of slight abuse during storage. On the other hand, the technology of preparing sausages without smoking is typical in the Mediterranean area; these sausages are not only significantly different in flavor from smoked products, but also have more chances to become rancid to some extent by mold cover.

More details on quality defects, as well as their reasons and possible prevention, can be found in Incze 2010.

37.8 Conclusion

Mold-ripened sausages are commodities that have been known for thousands of years; nevertheless, somewhat detailed knowledge of them in Europe has a history of some hundred years only. Controlled fermentation is even younger and application of known microorganisms aiming at influencing the organoleptic

properties as well as the safety of this type of products for ensuring a reproducible consistency in quality has a history of 50 odd years.

Mold-ripened sausages are air dried or smoked and dried products with a special appearance due to the mold layer on the surface contributing to a balanced drying process and extended shelf life as well as a unique sensory value highly esteemed by conscious consumers. Because of the low water activity (traditional products) or combination of low pH and low water activity (starter culture technology) raw dried mold-ripened sausages manufactured under hygienic conditions with properly controlled technology have an excellent safety record and can be considered as most valuable meat products beloved not only by conoisseurs.

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38

Chinese Sausages

Zhang Xue Gang and Tan Sze Sze

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38.1 History

Traditional Chinese meat products are famous for their attractive color, strong aroma, and unique taste. They are part of the precious cultural heritage of China as they symbolize ancient Chinese imagination and creativity in meat processing spanned over a long period of time and space. Records have shown that animal domestication and meat processing started in China since time immemorial. Isotope dating on unearthed historical relics in recent years has confirmed that wild boar taming in China has a history of more than 5600 years. In addition, archaeological studies on Qujialing ruins in Hubei Province have indicated that poultry farming in China began as early as 4000 years ago.

The processing techniques of Chinese meat products have been well depicted in numerous ancient manuscripts and books. These written records show that meat processing may have begun in China since 3000 years ago. For instance, the ritual of Zhou have recorded that meats from six domesticated animals,

namely horses, oxon, goats, pigs, dogs, and fowls were served as meal during the Western Zhou Dynasty. Salted eggs and sausages have been "fast food" snacks and delicacies for centuries among the Chinese.

Having an extended culinary history of thousands of years, China is renowned as "the Kingdom of fine food and delicacy" in which traditional Chinese sausages are one of the most important delicacies. Due to the lack of written records, it is difficult to trace the origin of Chinese sausages. However, it is generally accepted that sausage making in China began at least 2000 years ago. Archaeological studies have shown that during the Northern and Southern Dynasties (317 AD–589 AD), sausages were already a popular food item among the people, especially during Spring Festival. The ancient Chinese started to record the techniques of sausage making since the fifth or sixth century and the techniques developed rapidly during the Tang, Song, and Ming Dynasties. Some of these processing techniques are in fact still being used by the present sausage manufacturers in China.

38.2 Types of Traditional Chinese Sausages

China is the most populated country in the world with an enormously diverse distribution of people and food preferences. As a result, there are many sausages produced in different regions as shown in Table 38.1.

Typically, traditional Chinese sausages are produced by filling raw minced meat/offal into casings followed by drying and/or cooking. Fermentation may occur in certain types of Chinese traditional meat products. They are mainly made of pork but sometimes mutton, beef, chicken, duck, and/or offal are also added. Besides animal intestines, animal bladders are also used as casings in the processing of traditional Chinese sausages. All these products are characterized by a strong flavor generated by unique spices and maturation.

Among all these products, *la* sausages (*la chang*, *lap cheong*, or *Cantonese sausages)* are the most important variety of traditional Chinese sausages available in China. This product makes up the largest proportion of China annual sausage production. It is consumed widely all over the world especially in south-east Asia.

38.3 *La* Sausage (*Lap Cheong*)

The *la* sausages are the most common Chinese sausages currently available in the markets worldwide. Some scholars refer to them as Chinese sausages while others refer to them as sausages made in China. Most of the *la* sausages are produced in south China. Guangdong, being the largest producer of *la* sausages, accounts for 60% of the entire *la* sausage yield in China. Similar *la* products from other regions of China include Sichuan sausages, *Rugao* sausages, and big sausage of Hunan.

In Chinese language, the word "*la*" as in *la* sausages carries two meanings. First, due to the lack of food preservation techniques in ancient time, the sausages were originally processed during the coldest

TABLE 38.1

Traditional Chinese Sausages Available in Different Regions of China

Region	Typical Sausage
Guangdong Province	Dried pork sausage (*la* chang, *Lap Cheong*, *Cantonese sausage*)
Nanjing Province	Spiced pork in pork bladders, jujube-shape sausages
Hei long-jiang Province	Harbin Red sausages (a famous delicacy of Zhengyanglou Eateries)
Sichuan	Sichuan spicy sausage
Jiang Su Province	Rugao sausages
Beijing	Fragrance sausage in pork bladder
Tianjin	Rose sausages

period of the year, which is known as the "*la*" month, to avoid spoilage. Hence, "*la*" indicates how the sausages were processed during ancient times. Second, since the final dried sausages possess a shiny appearance resembling wax, it is given a description of *la*, the Chinese pronunciation for wax. Today, *la* meats indicate specific traditional Chinese meat products with lustrous surfaces, produced by preserving and drying, for example, *la* meat or *la* sausage.

38.3.1 Ingredients and Materials Used in *La* Sausage Processing

The "secret" to ensure the excellent microbiological safety of Chinese *la* sausages lies in their formulations and processing techniques. Ingredients used in the processing of *la* sausages include high amounts of sugar, salt, and liquor (referring to alcohol). Sugar and salt reduce water activity (A_w) of meat and thus prevent the meat products from deterioration. Furthermore, Chinese liquors are capable of inhibiting growth of many microorganisms. Ancient Chinese meat manufacturers may not know why and how these materials can preserve food, that is, by inhibiting the growth of microorganisms, but they were ingenious enough to make use of these ingredients. However, it is fair to say that the safety assurance of traditional recipes (not formulas) for any food in any country is most likely the result of trial and error. Many of such recipes have been handed down over generations; they have become commercial formulas in the modern world with details specifications as a result of development in science and technology over the years.

Such Chinese technology in sausage making does not only occupy an important place in the world of meat processing, but also promises inspiration to the development of food science and food technology globally. The use of salt and sugar in food processing and preservation is simple requiring no complicated equipments. Salt, sugar, and white liquor are inexpensive additives and available almost everywhere. The products can be preserved without packaging for several months at room temperatures. Such processing parameters is useful for developing countries to improve meat processing and developed countries to research new products.

38.3.1.1 Formulation of La Sausages

A typical formulation for the production of 100 kg of first-grade Chinese *la* sausage is shown in Table 38.2.

38.3.1.2 Raw Meat

In China, it is recognized that the higher the lean meat in the *la* sausage formulation, the better is the quality of the sausage. However, without pork fat, the sensory attributes especially flavor of the sausage will suffer. For *la* sausage processing, the best sources of lean meat are the back legs followed by the fore legs of pigs. On the other hand, pork fat should ideally be collected from the back of pigs; a good-quality pork fat should be hard in texture and white in color. The different pork fat:lean meat ratios in formulations led to the four grades of sausages, as shown in Table 38.3.

TABLE 38.2

A Typical Formulation of *La* Sausages

Ingredient	Weight (kg)
Lean meat	70
Fat	30
Refined salt	2.5–2.9
Sugar	7
Light soy sauce	3–4
Fen distilled spirit	3.0–3.5
Sodium nitrate	25 g

TABLE 38.3

Grading of *La* Sausages based on Port Fat: Lean Meat Ratios

Pork Fat:Lean Meat Ratio	Grade of *La* Sausage
2:8	Top grade
3:7	Grade 1
4:6	Grade 2
5:5	Grade 3

33.3.1.3 Salt

Salt is indispensable in meat processing. Besides its antimicrobial effect, salt improves the flavor *la* sausages. Refined salt is commonly used in the processing of most of the Chinese sausages. Locally produced salt is preferred for some sausage products. The salt content in sausage varies with regional conditions and preferences. In the southern part, about 1.5% of salt is used for *la* sausage processing, while ~3.0% of it is used in the northern part of China.

38.3.1.4 Refined White Sugar

Besides improving flavor, refined white sugar speeds up the pickling process of *la* sausages. To some extent, it prevents oxidation of nitrous acid and hence preserves the desirable bright red color of the *la* sausages. However, it encourages bacterial growth if not properly used. In different regions of China, the amount of sugar used in *la* sausage processing varies and the maximum level used is 12–20%. But, generally it is added at ~6% at southern China, while ≤2% at the north China.

38.3.1.5 Sodium Nitrate and Sodium Nitrite

The detailed biochemistry of nitrate and nitrite as a food additive in meat preservation is discussed in another chapter. Depending on the type of *la* sausages, they are commonly dried by air or mild roasting at temperatures between 25°C and 45°C, a temperature range best suited for microorganism reproduction. Hence, besides enhancing the color development of *la* sausages, addition of sodium nitrate and sodium nitrite is necessary to inhibit the growth of numerous pathogenic microorganisms in the *la* sausages. Although many options have been explored, sodium nitrate and sodium nitrite remain as the most effective antimicrobial agents in the manufacturing of traditional Chinese sausages. The *la* sausages manufacturers can opt to add either sodium nitrate alone or combination of both sodium nitrate and sodium nitrite into the formulation.

38.3.1.6 Soy Sauce

Soy sauce is a common flavoring agent used in making Chinese sausages. High-quality soy sauces contain ≤18% salt and >4% protein. Most soy sauces are in the form of brown or black liquids; however, colorless soy sauces and solid soy paste are also available in the market. Soy sauces help maintain the natural color of the *la* meats and are normally added at level of 0.8–1.5%. To use soy sauces in the processing of *la* sausages, the level of salt in the formulation must be carefully calculated. In recent years, some *la* sausages in China have been found to contain the prohibited additive, namely sodium benzoate; the reason for the existence of sodium benzoate could be due to the addition of soy sauces, in which sodium benzoate is used as a permitted antimicrobial agent for soy sauces in China.

38.3.1.7 Liquors

Liquors are used to reduce unpleasant smell and enhance aroma and mellowness of processed meat. The most common liquors used in making Chinese meat products are rice wine, white spirits, and Chinese

rose liquor. To manufacture Cantonese *la* sausage with strong fragrance, liquors of high alcohol content (ideally, alcohol level of the liquors should be approx. 54%) is added during the initial curing stage to marinate the sliced or diced meat and fat for about 24 h. The aromatic *Fen* distilled spirit produced in Shanxi province is the most popular liquor used in the processing of *la* sausages.

38.3.1.8 Food Coloring

In general, the main ingredients used as coloring in *la* sausages are sugar and sodium nitrate/nitrite instead of synthetic coloring. In some places, *hong qu* (Monascus red) made from red kojic rice is added to enhance the color of the sausages.

38.3.1.9 Casing

The natural casings made from animal intestines are normally preserved or dried before used. Chinese manufacturers often use thin casings (ø2.2–2.5 cm) derived from the small intestines of pigs. For some sausages produced locally, casings derived from sheep, horse, cattle, and rabbit intestines are also used. In modern factories, synthetic casings made of protein and fiber are increasingly popular. The collagen casings are the most important substitute for natural casing and are produced by extracting collagen from the skin of cattle.

38.3.2 Processing Steps

The processing of *la* sausages is similar to those of Western sausages; they are mixtures of high-quality pork, pork fat, soy sauce, salt, nitrite, and sugar stuffed in casings made from animal intestines. The raw *la* sausages are sun dried, air dried, or mildly roasted. The use of additive commonly found in Western style sausages (such as starch, blood meal, coloring, and nonmeat protein) is not allowed in the production of *la* sausages.

Lean meat is minced using mincers with orifice diameter of 6–8 mm. Solid pork fat is diced into 5–8 mm cubes and rinsed in water for several minutes to remove impurity and melted fat. Salt, water, diced fat, and other seasoning are then mixed with the minced meat using a mixer. The mixture is fully agitated for about 15 min at temperature below 18°C. The resulted meat paste is kept at 4°C overnight to allow thorough penetration of additives into the meat and fat, thus allowing desirable physical changes (such as increased water-holding capacity of the meat) and biochemical reactions (such as color formation) to take place in the meat.

The meat paste is stuffed into sausage casings at the end of the curing process and links are formed at every 15 cm. Special needles are used to pierce numerous holes on the sausage casings to ease the release of air and moisture from the meat paste during drying. The raw sausages are then rinsed with potable water and hung to dry.

As smoking is not used in the processing of *la* sausages, sufficient drying is crucial to preserve this semidried meat product. To ensure their microbiological safety, the raw *la* sausages must be dried until a moisture level of 18–35%. Traditionally, natural wind drying has been the most common method used to dehydrate the raw *la* sausages. This method, however, is unable to effectively control the moisture content of the end products due to fluctuations of temperature and humidity caused by changes in seasons and weather. Most manufacturers today use hot-air drying system to produce *la* sausages. The raw *la* sausages are hung on a rack trolleys such that the space between sausages is 5–6 cm apart; this arrangement permits proper ventilation so that sausages at the upper level of the rack trolleys to absorb heat. The rack trolley is then pushed into drying chamber. There are 3–4 levels in drying chambers with the newly stuffed sausages at the bottom level and they are progressively raised to the next level each day. The drying time of *la* sausages varies with the ingredients used, size of the *la* sausages, and the drying parameters. The *la* sausages are normally dried in a drying chamber of ~50°C for 3–4 days. During drying, the internal temperature of the sausages is controlled to be <50°C. At the end of drying, the dried *la* sausages are cut, graded, and packaged.

38.3.3 Microbiological Safety of *La* Sausages

A group of German scientists studied the microbiological safety of various intermediate moisture foods (IMF) (Leistner 1992). The study included Chinese-cured sausages collected from 24 countries including China, Singapore, the United State, Canada, Britain, France, Belgium, and so on. The result shows that the presence of miscellaneous bacteria in Chinese *la* sausages is very low. Most of the bacteria found are relatively harmless, for example, *leptosphaeria* and *lactobacillus*. Leistner (1992) found that heating at 50°C for several hours was sufficient to inactivate most pathogenic bacteria, including *Staphylococci*, in *la* sausages. The result indicates that Chinese *la* sausages are relatively safe for consumption.

38.3.4 Flavor Formation of the *La* Sausages

Hierarchical cluster analysis indicates that ripening and storage play a significant role on the formation of volatile compounds of *la* sausage (Sun and others 2010a). Various biochemical processes, including lipid degradation, protein decomposition, as well as Maillard reactions, are important in the flavor formation of *la* sausages.

The enzymatic hydrolysis of lipid is one of the most important biochemical changes in the *la* meats for flavor generation. The pH of *la* sausages at 5.5–6.2 activates neutral and acidic lipases to hydrolyze lipids into free fatty acids, monoglycerides, and diglycerides. Compounds derived from lipid oxidation, namely alcohols, ketones, and linear chain aldehydes account for >60% of the volatile components of *la* sausages. In addition, the presence of carboxylic acids and ester indicates that lipid oxidation plays a key role in flavor formation of Cantonese *la* sausage (Sun and others 2010b). Excessive oxidation is undesirable as it leads to an unpleasant rancid flavor of *la* sausages.

Protein decomposition is an important biochemical reaction during *la* sausage processing and is influenced by salt content, water activity, temperature, and humidity. Results from protein solubility analysis show that hydrophobic interactions, hydrogen, ionic, and disulfide bonds played important roles in determining the sensory properties of Cantonese *la* sausage (Sun and others 2009). Peptides and free amino acids derived from protein hydrolysis continue to participate in biochemical reactions and influence the aroma and taste of *la* sausages. The best flavor profile of *la* sausages can be achieved at a protein decomposition index (IP) of 23–28%. If IP is lower than 22%, the unique *la* flavor is not obvious. In contrast, excessive protein hydrolysis (IP > 29–30%) is detrimental to the flavor and results in loose texture of *la* sausages. Excessive protein hydrolysis produces high concentration of low-molecular-weight nitrogen-containing chemical compounds (peptides and free amino acids), which results in unpleasant bitter and metallic flavors.

Sugars, in the forms of glucose and ribose, react with amino acids, peptides, and protein to form the characteristic cooked meat flavor through Maillard reaction. As *la* sausages contain a high amount of sugar, the influence of Maillard reaction on the flavor is highly significant. The chemical components of meat flavor derived from Maillard reaction are mainly heterocyclic compounds containing nitrogen, sulfur, oxygen, as well as sulfurous compounds. In addition, the intermediate Maillard reaction products can further react with lipid and molecules decomposed from thiamin to generate meat flavor compounds. Moreover, studies have shown that compounds derived from the hydrolysis of Maillard reaction products possess antioxidative activities as they are able to improve the sensory and textural properties of *la* sausages (Sun and others 2010c).

38.3.5 Grading and Standards

Here are the current laws and regulations, as issued by the National Department of China Health, the govern the production of Chinese *la* sausage:

- 1989. Basic requirements of the Chinese *la* sausage are regulated by the Hygienic Standard of Chinese sausage (*la* sausage) and supplemented by standards related to the Fragrant bladder.
- 2005. The *la s*ausage must comply with the requirements of Cured and *la* Meat Hygienic Standard.

- 2009. New standards on raw materials and grades of the products. These new requirements have replaced some of those established in 1989.

The current law on Chinese sausage, implemented since October 1, 2009, strictly regulates the *la* sausage and wind-dried sausage in all aspects, quoting many other relevant standards and technical requirements on raw material, factory lay-out, standard for additives detection method, sampling procedures, and so on.

Provincial governments and some big factories also have their own standard to certify the local sausage specialties. These standards regulate the quality and hygiene requirement of

- Chinese sausage (*La* sausage)
- Wind-dried sausage without fermentation
- Fragrant bladder for sale in the market

The standards are applicable to products:

1. Using chopped or minced fresh (frozen) meat
2. Using additives such as salt, liquor sugar, and so on
3. Stuffing into casing or bladder
4. Final processing by drying or roasting

The next section provides some details on various legal requirements for processing Chinese sausages.

38.3.6 Sensory Quality of *La* Sausages

Table 38.4 shows some sensory attributes for grading purposes.

38.3.7 Physical, Chemical, and Microbiological Indices of *La* Sausages

Table 38.5 shows the levels of acceptance for physical and chemical index for major ingredients.

TABLE 38.4

Grading of *La* Sausages Based on Sensory Attributes

	1st Grade Freshness	2nd Grade Freshness
Appearance	Casing (or bladder casing) is dry and adhere closely to the meat	The casing (or bladder casing) is slightly wet or sticky, but it is intact and can be easily separated from the meat
	No slime or mould spots on the surface	Mould spots are apparent on the surface and it can be removed by brushing
	The sausages are firm and elastic	The sausages are soft and lack of elasticity
Structure of sausages	The cross section of sausages is compact	The cross section of sausages is even but cracks are readily observable
		The outer edges of cut sausages are soft
Color and luster	The cross section of sausages is lustrous	Only part of the meat is lustrous
	The meat is from reddish brown to rosy red	Meat is dark brown in color
	Fat is white or in faint red in color	Pork fat appears yellow
Aroma	Natural *la* sausage flavor	Rancid
		Sour

Source: Adapted from General Administraton of Quality Supervision, Inspection and Quarantine of P.R. China, Standardization Administration of China, State Standard of P.R. China, GB/T23493–2009 Chinese Sausage.

TABLE 38.5

Physical and Chemical Index for Major Ingredients

	Index		
Ingredients	**Excellent**	**Good**	**Regular**
Water (%)	≥25	≥30	≥38
Salt (%, count in NaCl)		≤8	
Protein (g/100 g)	≥22	≥18	≥14
Fat (g/100 g)	35	45	55
Total sugar (count in glucose, g/100 g)		≤22	
Peroxide value (count as fat, in KOH, g/100 g)		≤4	
Nitrite content (mg/kg, count in $NaNO_2$)		≤20	

Source: Adapted from Ministry of Health P.R. China GB10147–88 Hygienic Standard of Chinese Sausages.

There is a maximum limit for total bacteria count for *la* sausages. Moreover, common pathogenic microorganism, namely *Staphylococcus aureus* and *Salmonellae*, should not be detected in the products.

38.4 Other Important Chinese Specialty Sausages

38.4.1 *Hong Chang* (Red Sausage)

In the northeast China, a popular regional specialty is smoked savory *Hong chang* (Red sausage) similar to Polish sausages. It was introduced to Harbin by German sausage makers in 1931. This is a sweeter and dried version of the southern Chinese sausages. It is also known as Shanghai Red sausage in mid-south China. It is also produced regionally and nationally. One of the most famous brands is *Zheng Yanglou* restaurant in Ha'erbin province.

38.4.2 Fragrance Bladder

The shape of fragrance bladders resembles apples with a compact content inside. It possesses a strong aroma and can be stored for a long time. Fragrance bladders are made by stuffing meat paste into pig bladders. During drying, fermentation of meat also takes place inside the bladders. Because of the thickness of pig bladders, fermentation lasts for more than 40 days. At the end of drying and fermentation, green and white mold grow on the surface of the bladders. The pine-nut-flavored fragrance bladder is typically consumed in Northern China. The dried fragrance bladders are cooked steaming and smoking.

38.4.3 Sausages Made from Animal By-Products

Animal blood, tongues, tendons, and offals (livers, lungs, hearts, kidneys, etc.) are used as ingredients in certain types of sausages made in China. For instances, the hog liver sausage contains liver stuffed in dried cattle esophagus. *Runchang* made of chicken, beef, and mutton are available in Malaysia and Myanmar (known as kyet u gyaung).

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39

Dry-Cured Ham

Pere Gou, Jacint Arnau, Núria Garcia-Gil, Elena Fulladosa, and Xavier Serra

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39.1 Introduction

Dry-cured ham is generally considered to be a typical product of the Mediterranean area. Spain and Italy are the main producers, although it is also manufactured outside Southern Europe. The Mediterranean types of dry-cured ham (e.g., Spanish "Jamón Serrano", Italian "Prosciutto crudo", or French "Jambon de Bayonne") are characterized by dry salting and a long maturation period (from 7 to over 24 months). The Northern Europe types of dry-cured ham are characterized by smoking followed by a short maturation period (e.g., Westphalia hams in Germany).

Various technologies for the production of pork dry-cured hams exist, but basically all of them intend, on the one hand, to obtain a product that can be kept at room temperature without either jeopardizing health or involving any risk of alteration, and on the other, to facilitate the development of the desired sensory characteristics (texture, odor, and flavor). Despite there being important differences in the processing and sensory characteristics of dry-cured hams between countries, all the processes have two

operations in common: salting and drying. The main processing differences are due to specific raw material (specific breeds, weight, or trimming), specific treatments (e.g., different salting systems, smoking, etc.), and specific air conditions and duration of the drying process.

Innovations introduced into processing during the last century, such as the use of chilling chambers or artificial dryers, have improved the process standardization and, consequently, the control of the safety and quality of the final product.

In the last decades, research into this product has demonstrated the importance of raw material quality and of some processing parameters on the final quality. The current implementation of new methods for the selection of raw material or the control of the key-processing parameters are enhancing the safety and quality standardization of dry-cured hams.

The aim of this chapter is to describe the different steps of the elaboration process, from the selection of raw material to the commercialization of dry-cured hams, reviewing those aspects which have been demonstrated to have a major impact on the safety and sensory quality of dry-cured hams and how they can be accounted.

39.2 Raw Material Selection and Handling

Dry-cured hams are elaborated from the rear legs of pork carcasses. Therefore, the characteristics of the raw ham are partly linked to the carcass history: genetics, pig production (feeding, age, season, etc.), preslaughter treatments, slaughter conditions, and ham handling.

39.2.1 Fat Content and Weight

The quantity of fat and the weight of the ham dictate the main criteria currently used in the selection of raw material, and determine the processing time of the ham. The weight of green hams ranges from 9 to 14 kg. While in Northern European countries consumers prefer lean hams, many consumers in some countries in the Mediterranean area accept a certain amount of infiltrated fat in this product. This fat prolongs the maturation time (Figures 39.1a and b), resulting in the particular sensory characteristics

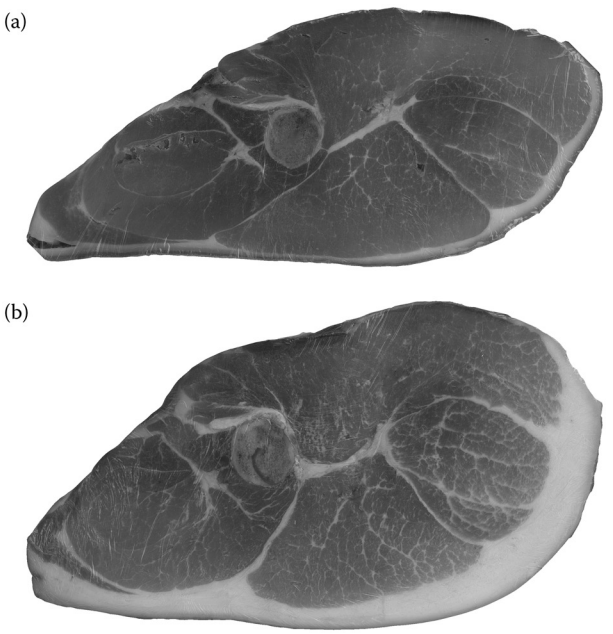

FIGURE 39.1 Slices of dry-cured ham showing different fat content levels. (a) Lean dry-cured ham. (b) Fatty dry-cured ham.

FIGURE 39.2 Ham categorizator (JMP Ingenieros, Sotés, Spain) at IRTA-CENTA facilities to classify raw hams according to fat content.

for which the product is highly valued. In dry-cured hams which have undergone a lengthy aging process, the presence of infiltrated fat and a certain amount of superficial fat slow down the drying process and impregnate the musculature. The latter enhances the mastication process during consumption, providing an oily sensation in the mouth and an aged flavor that is highly appreciated (a pleasant flavor associated with the oxidation of the fat (Antequera and others 1992). Hams that contain almost no subcutaneous fat should be avoided, as in these hams the salting and drying processes through the rind area are faster than in other hams. These hams have a saltier taste, a higher water loss and a low quality, and so are rejected by the majority of consumers (Cilla and others 2006; Morales and others 2008; Resano and others 2010).

Online systems based on electromagnetic scanning have been proposed for evaluating the fat content of fresh hams (Meseck and others 1997). Currently, fat content of raw hams can be assessed online by using the Ham Categorizator (JMP Ingenieros, Sotés, Spain; Figure 39.2). This equipment measures the disturbance that the ham produces when passing through an electromagnetic field, which has been related with the fat content (Figure 39.3).

39.2.2 Proteolytic Potential

It has been demonstrated that hams which have suffered an excessive proteolysis during processing have a disagreeable soft texture (Ruiz-Ramírez and others 2006) (Figure 39.4). Therefore, meat with a high proteolytic potential is less suitable for the elaboration of dry-cured hams, especially if a low salt content is desired (Virgili and others 1995).

The selection of raw material could be improved by the evaluation of the cathepsin B activity (Parolari and others 1994), which is one of the proteolytic enzymes that seem to be responsible for the development of soft texture. However, the current methodology has not yet been adapted for online measurements.

The selection of animals less prone to high proteolysis activity could be carried out at animal breeding or production steps. There are new technologies in the field of molecular genetics such as genetic markers that show promise for predicting the proteolytic activity of raw material (Skrlep and others 2010). Another easier approach could be the selection of older animals, which show a lower activity of proteolytic enzymes (Sárraga and others 1993).

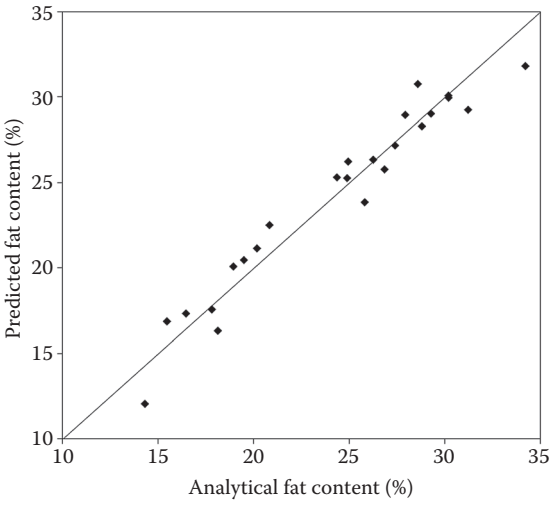

FIGURE 39.3 Analytical fat content versus predicted fat content obtained using a ham categorizator.

39.2.3 Meat pH

The pH level of the meat on arrival at the industry (time *postmortem* > 24 h, pH_u) is another important parameter that affects the maturation of ham. On the one hand, for microbiological safety reasons, the majority of authors recommend avoiding hams that have a $pH_u > 6.2$ (Leistner 1986). A pH_u higher than 6.2 also leads to an increase in the incidence of appearance problems, such as a shiny aspect of the lean meat and phosphate precipitates (Arnau and others 1998, Figure 39.5), and texture problems, such as a soft texture in the internal part of the ham (Guerrero and others 1999; Morales and others 2007) and a crust development at the lean surface (Ruiz-Ramírez and others 2006). The problem of soft texture is greater in hams of larger sizes.

On the other hand, hams with a $pH_u < 5.6$ show higher proteolysis especially at low salt content (Ruiz-Ramírez and others 2006). Therefore, they are more prone to pastiness than hams with intermediate pH.

Furthermore, pH values vary considerably between the muscles of the same ham (Arnau and others 1995). Consequently, in order to carry out an easy measurement, which should be representative of the ham as a whole, it is advisable to measure it on an external muscle of considerable size, such as the *Semimembranosus* muscle.

The selection of hams by pH can be done with a meat quality scanner (TIMPOLOT, Olot, Spain) calibrated and adapted to the industrial scale (Figure 39.6).

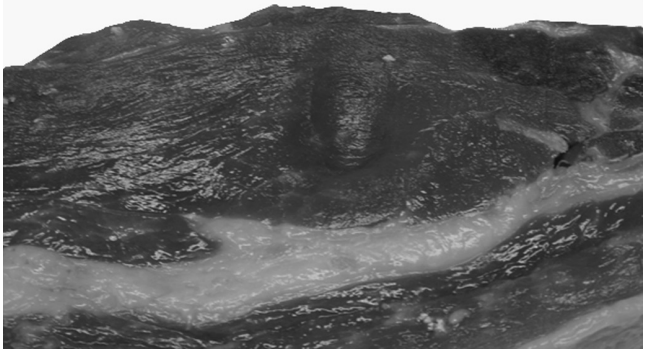

FIGURE 39.4 Slice of dry-cured ham showing soft texture.

FIGURE 39.5 Phosphate precipitates on both lean surface and edges of dry-cured ham slices.

39.2.4 Shape of Hams and Types of Cut

The elaboration of cured hams is carried out using whole pieces or parts of the leg. The whole pieces can be presented in different ways. In some traditional dry-cured hams, the presentation with hoof included is usual (e.g., in Iberian and Serrano hams in Spain, in San Danielle ham in Italy and in Jinhua ham in China). However, the majority of the hams that end up boned are elaborated without the hoof (e.g., in Parma ham in Italy and Bayonne ham in France). The presence of the hoof does not significantly affect the salting and drying processes (Boadas and others 2001) and, though it can reduce the incidence of

FIGURE 39.6 Meat quality scanner (PH120 TIMPOLOT, Olot, Spain) at IRTA-CENTA facilities to classify raw hams according to weight and pH.

FIGURE 39.7 Raw ham showing skin trimmed into a V shape.

color fading of the shank caused by the entrance of air, the main reason for the elaboration of hams with hoof seems to be mostly cultural.

In Spain, unlike other countries, it is usual to cut the rind into the shape of a V (Figure 39.7). The reason for this could be that the rind and part of the fat can then be used for other purposes. Moreover, a higher standardization of the thickness of the subcutaneous fat is achieved, and the retraction of the lean meat is facilitated during the drying process (Gou and others 2000) as well as during the shaping before slicing the ham. In addition, taking off part of the rind reduces the problem of whitish appearance observed at the rind surface zones when hams are dried at low air relative humidity (Arnau and Gou 2001).

In Spanish hams, the aitch bone remains, which means the morphology of the muscles remains intact, while in French (Jambon de Bayonne) or Italian hams (Parma and San Danielle), this bone is eliminated, leaving only a small part of it ("anchetta") in order to avoid cavities which hinder the drying process. The elimination of the aitch bone allows a more rapid salt absorption and water losses, especially in the *Biceps femoris* muscle.

Pieces of ham are used for the production of Jambon d'Ardennes in Belgium, culatello and fiocchetto in Italy, or baiona in Spain.

In all cases, it is important to avoid damaging the structure, as this can facilitate the entrance of microorganisms thus causing deterioration in the product.

39.2.5 Refrigerated Versus Frozen–Thawed Raw Hams

While the use of refrigerated raw material is usual in areas where there are abattoirs and cutting rooms in abundance, frozen hams are more frequently used when long transportation is involved, as it ensures no break in the cold chain during transport.

The use of frozen–thawed hams makes the dissolution of salt on the surface, the migration to the inner part (Sorheim and Gumpen 1986; Poma 1989) and probably the diffusion of water toward the outside easier. Therefore, salting and drying processes have to be adapted to frozen–thawed raw hams to enable the production of dry-cured hams without affecting the sensory quality of the ham (Kemp and others 1982; Motilva and others 1994; Bañon and others 1999).

However, the use of frozen–thawed meat impedes the direct confirmation of the quality of the raw material, facilitates crystallization of the tyrosine formed during the maturation process, giving way to a higher number of white crystals (Arnau and others 1994), and produces some cracks/cuts in the product. If there

are many oscillations in temperature during the storage process, or the storage period is prolonged, superficial dehydration can take place in the rind and the lean meat of the ham, which causes freezing burns. This can hinder the salting process and can damage the final appearance of the product. To prevent this, it is advisable to cover the ham with a close fitting plastic protection. To avoid problems of growth of undesirable microorganisms, it is desirable to ensure that at no point of the surface the temperature rises above 5°C during the thawing stage. On the other hand, if various days are necessary to complete a batch, which can be frequent in small cutting rooms of low production, it is preferable to freeze the hams than refrigerate them for an excessive period of time.

39.2.6 Elimination of Residual Blood

The elimination of residual blood from veins and arteries is advisable as it reduces possible microbiological problems and improves the appearance of the cut surface. The best time to carry out this operation is during the cutting process, as the blood exits more easily. In the case of no reassurance that this operation has been carried out, it should be done when the raw material is received.

39.3 Ingredients and Additives

Salt is essential for the elaboration of cured ham and it is the only substance that is permitted for the elaboration of some hams, for example, the Parma ham. It contributes to the reduction in water activity and to the sensory characteristics of dry-cured hams (e.g., safety and sensory quality), but an excessive sodium intake can produce adverse cardiovascular effects related to hypertension (Kaplan 2000; WHO and ISH 2003; FSAI 2005). Therefore, the tendency during recent years has been to reduce salt content to the minimum level without having a negative impact on safety and sensory quality.

Hams are often cured with nitrifying agents (nitrates and nitrites). Both nitrite and nitrate are used to obtain the desired red color in dry-cured hams. Nitrate is present at very low levels in meat and has been the preferred nitrifying agent in products of lengthy aging, as it is slowly transformed into nitrite by bacterial action. Nitrite plays an important role in color development and as a preservative, exerting an anticlostridial effect in cured meat. Nitrite is reduced to nitric oxide, which is combined with the pigment myoglobin forming nitrosylmyoglobin, responsible for the stable red color of cured meat. The reduction of nitrite to nitric oxide increases as pH decreases (Wirth 1984).

Cured color is obtained faster with nitrite than with nitrate and therefore is preferable in fast elaboration processes. However, nitrite causes the appearance of concentric red rings and paler core in sliced dry-cured ham (Figure 39.8) if it is added at very low levels in hams with low pH (Arnau and others 2003b).

Ascorbate is added to accelerate the transformation of nitrite into nitric oxide. It prevents greenish colorings which are the result of the reaction of nitric oxide with oxygen (Arnau 1998) and restrains the formation of nitrosamines in lean meat (Pegg and Shahidi 2000).

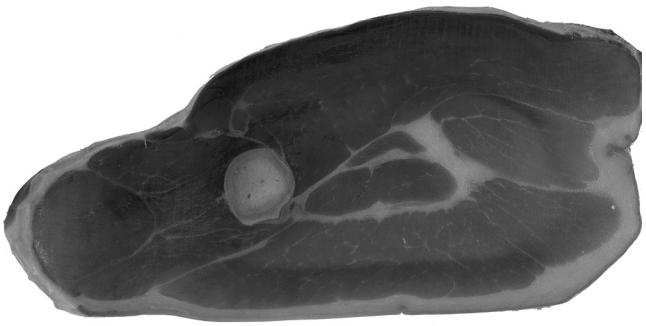

FIGURE 39.8 Concentric red ring and paler core defect in dry-cured ham with low pH (pH < 5.6).

Sugars, on the one hand, favor the implantation of surface flora and lend a light sweet flavor, but on the other hand, they can bring about acidic flavors in the case where lactic flora develops in the internal parts of the ham (Boadas and others 2000).

Parma ham usually has pepper added to the head of the femur or to the fat which is used to cover the ham. In pieces of ham that are elaborated by means of a short duration process, it is usual to add spices to improve their sensory quality.

39.4 Processing

Although salting and drying are the main processes involved in dry-cured ham manufacturing, there are many other operations, some of them specific of a certain types of hams. The different steps of the whole process, described in chronological order, can be found below.

39.4.1 Pre-Salting Operations

During the different operations before salting (trimming, shaping, pre-salting and curing), a low internal temperature ($T < 3°C$) must be assured.

Trimming is a vital operation for salt uptake; therefore, it must be performed and controlled by trained personnel, in order to achieve a similar lean surface on hams from the same class and to avoid damages to the structure. When skin is partially removed ("V" shape, only in the fattiest hams) special attention must be paid to maintaining a minimum thickness of subcutaneous fat, because it can modify the salt uptake and the drying (Garcia-Gil and others 2009a,b).

In order to facilitate the penetration of the curing salts (in hams where nitrifying agents are used), to eliminate the blood that could be present in the veins and arteries and to mould the ham, a massage of the surface is carried out using a mixture which contains salt, nitrate, nitrite, ascorbate, and sugars. Although the massage can be done manually, it is normally carried out by continuous machines or in drums. In this way, the absorption surface is increased and penetration of the salt is enhanced.

39.4.2 Salting

The aim of salting is to incorporate the correct amount of salt to the raw ham. The amount of salt used depends on both the raw ham characteristics and the salting conditions.

Salting must be carried out as soon as the temperature in the core of the ham reaches a level of between 1°C and 3°C, as in this way not only is the growth of undesirable bacteria hindered but there is also a reduction in the percentage of nonedible hams at the end of the process. Temperature should be maintained at a low value during salting.

In Mediterranean countries, the dry salting process is used, while in Northern Europe this process is also used as well as salting in brine. In the dry salting process, a higher level of osmotic dehydration is obtained, whereas with salting in brine, there is a lower consumption of salt.

The dry salting process can be carried out using two different methodologies:

1. Salting of the hams by covering them in salt. This can be done by individual salting of the hams (San Danielle) or by piling them in stainless-steel containers for a time period of approximately 1 day/kg for the refrigerated hams, and some days less for the frozen–thawed ones (typical of Serrano and Iberian hams). The salt used is normally humid to ensure correct salting, and the atmospheric humidity is high to avoid drying out and to facilitate the formation of saturated brine on the surface of the ham. For this operation, maintaining a high level of atmospheric humidity is recommended, at a level which should not be below 75%, as this would result in the dehydration of the salt. The piling up of the hams induces a pressure which is especially high in the lower stratums, facilitating the loss of water.
2. Salting by means of a quantity of salt which is in proportion to the weight of the ham. The most well-known example of this method is found in Parma ham, in which the relative humid-

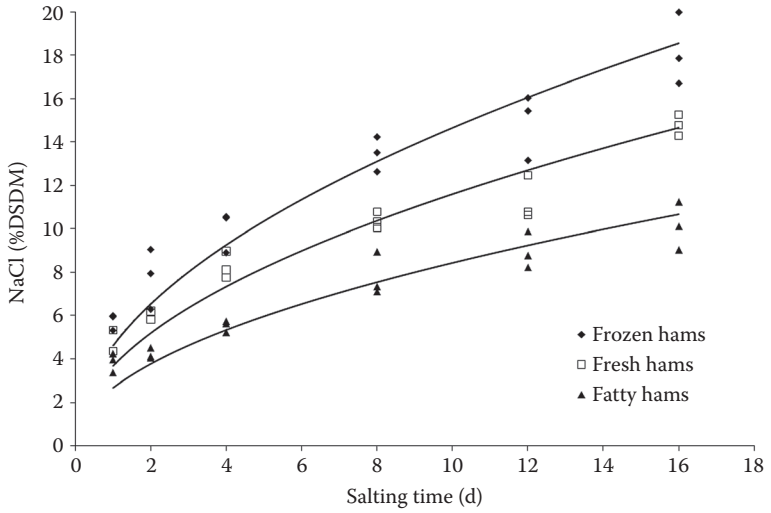

FIGURE 39.9 Salt uptake models to predict the final salt content on a desalted-dry-matter basis (%DSDM) in three classes of ham.

ity is maintained between 70–75% minimum and 85–90% maximum. Using this method, the salting process of the areas covered in salt is ensured while at the same time allowing the drying of those areas which are not covered by salt. In this case, the hams remain in a horizontal position for a period of 3–4 weeks, which results in the thickness being less than it would be if they were left hanging. With this process, the penetration of salt and the evacuation of water from inside are enhanced. In other cases, salting takes place in salting drums, with a posterior post-salting period in containers where the ham is in contact with the exuded brine. It is important to treat the ham with salt twice so that the parts of the ham which have been less salted during the first treatment are treated again a second time. In this type of process, the post-salting period should not be excessively prolonged, as the exuded water can be reabsorbed therefore encouraging the growth of undesirable microorganisms in those areas which are not covered by brine.

The amount of salt (in salting systems that use a fixed amount of salt per kg of ham) or the duration of salting (in "salt pile" systems) have to be adjusted to each class of ham (according to weight, fattiness, and fresh/frozen origin). Models of salt uptake should be constructed for each class of hams and salting conditions before selecting the correct time. For example, Figure 39.9 shows three salt uptake models corresponding to three different classes of ham at specific salting conditions (Serra and others 2010).

39.4.3 Washing

In former times, the Spanish Serrano hams were desalted by immersing them in running water to eliminate the excess salt acquired during the long salting process. However, nowadays, because a temperature of between 1°C and 5°C can be maintained during the post-salting phase, it is possible to reduce the quantity of added salt. Therefore, the main objective of washing is to remove any salt crystals that remain from the salting process.

Italian hams are usually washed after the post-salting period and therefore, during this stage, it is possible to maintain the head of the femur at a lower water activity. In this case, firstly, washing removes not only crystals of phosphate and salt, but also the slime which can form on the surface of the joint. Secondly, this method ensures that a later drying process can be achieved faster than in Serrano ham because drying is carried out at a temperature which is higher than can be used after the washing of Serrano ham.

From a technological point of view, washing is more effective using warm water than cold water. The ideal moment to carry out the washing can also be conditioned by the cost of the handling that is involved and the dehydration capacity of the post-salting or drying rooms.

At the end of the salting process, the blood vessels usually contain a certain amount of brine, so that it is advisable to perform a light pressing operation to eliminate it together with any residual blood. By pressing and forming the ham, it is also possible to improve the evenness of the cut of the product. However, the ham structure should not be damaged, because this facilitates the entrance of microorganisms that can spoil the ham. Regardless of the moment at which the washing is performed, a quick drying of the lean meat of the ham is always recommended to prevent the growth of undesirable flora. However, it must be taken into consideration that an excessively fast-drying process can produce a reduction in the surface water activity, to values lower than 0.75, which causes a local crystallization of the salt. This facilitates the drying up of the lean meat and causes white-colored stains on the rind, resulting in a deterioration of the appearance of the ham (Arnau and Gou 2001).

39.4.4 Smoking

In countries with a cold damp climate, hams have traditionally been smoked. The smoke provides a typical color and smoky flavor, an antioxidant action and also impedes the growth of surface flora (Lenges 1986).

39.4.5 Convective Drying

The aim of drying is to reduce the water content of ham to a level at which the dry-cured ham stability is assured. However, the sensory quality of the final product depends on the drying conditions (temperature, drying ratio) used in the process.

Hams are dried in "natural" or in "industrial" dryers, using dry air at different temperatures. "Natural" dryers use air from the outside, which must be dry. These dryers are placed in zones where the outside air has a low relative humidity (RH). Their main problem is a poor control of drying conditions.

Industrial dryers, however, have an air-conditioning system to control the temperature and the RH, working in discontinuous RH cycles. An interval of RH is fixed, thus when the upper limit is attained, a cooling device is activated until RH drops to the lower limit. The speed at which air RH rises depends on both the ability of the hams to transfer water into the air and on the amount of product in the dryer (load of the dryer). In order to have a regular drying process, the quantity of water which evaporates must be compensated by the diffusion of water from the inner part to the outer part of the ham. However, due to discontinuous control, the lower limit is frequently exceeded, which can produce excessive dehydration at the ham surface.

Depending on the air temperature, two drying periods can be defined (see below).

39.4.5.1 Post-Salting Period

During this period, hams are dried at a temperature lower than 5°C, until the water activity (a_w) all through the ham decreases to values which do not permit the development of pathogenic microorganisms ($a_w < 0.96$) (Leistner 1986).

The a_w at the surface mainly depends on both the drying capacity of the air and on the capacity that the ham has to release water from the internal zones to the surface. Therefore, on the external part of the ham, the flora is affected by environmental humidity conditions, and a growth of fungal flora can often be observed. At this stage, it is advisable to carry out a more energetic dehydration during the first and second weeks, as it is important to reduce the surface water activity to hinder the growth of undesirable microorganisms that give to the ham a slimy appearance.

To avoid whitening of the rind brought about by the crystallization of the salt in its interior, a relative atmospheric humidity of above 75% is recommended. Once the majority of the salt from the rind has

penetrated through the fat to the lean of the ham, the atmospheric humidity level can be reduced to below 75% to promote the sweating of the fat (Arnau and others 2003a).

In the internal part of the ham, the gram (+) catalase (+) cocci become the dominant flora. The reduction of the a_w in this zone is due, in part, to the increase of salt content due to the tendency to equilibrate the NaCl/water relationship within the whole piece (Gou and others 2004). Additional reduction is achieved by reducing the water content. This could be the reason for the higher saltiness inside the product at the end of the process. So, in order to achieve a homogeneous salty taste it is advisable to perform a homogeneous drying.

The duration of the post-salting period varies according to the size of the piece, the surface of the exterior lean part, the type of trimming, the intermuscular and intramuscular fat, etc. In small-sized hams, a minimum period of 1 month is recommended. In the case of Parma ham, it is necessary to prolong this period, sometimes for 3 months because of its low salt content and absence of nitrite. In Serrano ham, this period should be higher than 40 days.

In Iberian hams this period is 2–3 months due to the high content of fat that hinders salt and water diffusion.

If salt content is reduced, technologies such as computed tomography based on x-ray can be used to monitor the local salt content in the inner parts of the ham and to adjust the post-salting duration. This technology permits the estimation of salt and water content distribution within the dry-cured ham during the process (Fulladosa and others 2010b; Santos-Garcés and others 2010).

39.4.5.2 Drying-Maturation and Cellar Period

In the drying-maturation phase, dehydration and the phenomenon of proteolysis and lipolysis, which determine the aroma, continue. In Serrano hams, temperature is slowly increased from 10°C to 12°C until it reaches a maximum of between 28°C and 34°C and the RH is between 60% and 80%. However, in recent years, there has been a decrease in the length of time at high temperature to reduce the incidence of hams with soft texture and white film, both of which can cause a problem for the commercialization of sliced products. On increasing the temperature, the melting of the fat is produced, which then impregnates the muscle tissue and makes up one of the typical characteristics of Serrano hams.

A high-drying rate can produce crustiness, which reduces the capacity of the ham surface to retract (Olmos-Llorente 2006). This can produce cracking and hollows in the *Adductor* muscle and around the coxofemoral joint. The growth of aerobic bacteria, molds, and mites in this area may lead to the development of musty off-flavors in this region and the adjacent muscles (Fulladosa and others 2010a). This problem is more common in blocky and lean hams (Gou and others 1995; Guerrero and others 1996). The most effective measure to reduce these problems is the application of a layer of fat to this area during the drying stage (Hugas and Arnau 1987; Arnau 1998). The application of fat can be preceded by a washing and drying of the ham surface.

For lengthy processes, Serrano hams are submitted to a final phase named "cellar phase" at temperatures from 12°C to 20°C and RH from 50% to 80% to dry the ham until the desired texture is obtained. This phase begins after the drying period and continues until a minimum processing time of 7 months is reached. However, high-quality Serrano hams can attain a processing time of up to 16 months.

In Parma ham, the drying phase takes place at a temperature of about 15°C and RH around 65–80%. After 6–8 months, the drying rate is slowed down by means of several applications of a mixture of fat, flour (to make the paste more permeable), pepper, and salt. This paste is added several times as the ham becomes drier. Matured hams are fire stamped with the Parma ham brand after at least 10 months processing for pieces weighing between 7 and 9 kg, after at least 12 months for pieces over 9 kg while pieces of <7 kg cannot be branded.

With Iberian ham, after the post-salting period, hams are kept for approximately 90 days in a chamber where the temperature is slowly increased until it reaches 18–20°C. Following this, the temperature is increased slowly for a period of around 1–1.5 months until it reaches up to about 30°C and RH of 60–80%. Finally, the hams are kept in a cellar from 12 to 18 months with a temperature ranging from 10°C to 22°C and RH from 65% to 80%.

39.5 Commercial Presentation

The commercialization of dry-cured ham has traditionally been carried out in whole pieces. However, in recent years there has been an increase in the commercialization of vacuum-packed boned dry-cured hams and slices.

39.5.1 Boned Dry-Cured Hams

Hams are boned at the end of the process and then shaped and vacuum packed. Although no water losses occur during packaged storage, the diffusion characteristics are still very important for the product quality. Vacuum packaging of boned hams facilitates the slicing process, the homogenization of the ham texture, and prevents problems of mites or excessive drying. When the storage of vacuum packed ham is prolonged for a long period of time, it causes a moistening of the surface which can result in an unpleasant aroma. If the water activity is high, undesirable microorganisms can proliferate, and in this case, it is necessary to keep the product refrigerated and/or to apply additional treatments, for example, freezing or high-pressure processing (HPP).

39.5.1.1 Freezing

Although the storage of hams by freezing permits the conservation of flavor for long periods of time and slows down the formation of white film in sliced products (Arnau and Lloret 2007), it also facilitates the formation of white crystals. On the other hand, the freezing of unpacked pieces causes the loss of volatile substances, which decreases the aroma of the product.

39.5.1.2 High-Pressure Processing

This technology has also been applied to increase the microbiological stability of boned dry-cured hams vacuum packed without the modifications that a thermal treatment produces. The application of HPP (600 MPa) to boned dry-cured hams can provide additional microbiological benefits (Garriga and others 2004; Tanzi and others 2004). However, it modifies the sensory attributes of dry-cured hams. Tanzi and others (2004) and Fulladosa and others (2009) found that HPP treatment on boned dry-cured hams with high moisture content reduced softness and pastiness and increased saltiness, hardness, and fibrousness, which can be considered positive if NaCl content is reduced, but it also modified the color. However, the HPP effect on the sensory attributes is lower if the product has lower water content (Tanzi and others 2004).

39.5.2 Sliced Dry-Cured Ham

In products for slicing, hams are frozen to obtain a firm texture, thus facilitating a better cutting process and a neat cut. As the structure of the product has been broken and so the risk of microbiological contamination during slicing is higher than in non-sliced hams, slicing and packaging have to be done under especially hygienic conditions (e.g., clean room). Therefore, as in the case of boned hams with high a_w, it is necessary to keep the product refrigerated and additional treatments can be applied to increase the product microbial stability, for example, packaging in modified atmosphere or HPP treatment on vacuum-packed slices.

39.5.2.1 Packaging in a Modified Atmosphere

Packaging in a modified atmosphere allows a more natural presentation of the slices, as this prevents adhesion and the waxy appearance of slices typically found in vacuum-packed hams. In order to prevent the deterioration of color and aroma, the gas mixture must not contain oxygen. The gas most frequently used is nitrogen, although CO_2 can be useful to improve the conservation of hams with high water activity

values. The type of plastic used must be a high barrier to prevent the gas mixture being affected. When using this type of packaging, abrupt changes of temperature must be avoided, as this could result in condensation on the internal surface of the packaging which in turn causes a local increase in water activity and, therefore, a reduction in the safety of the product (Arnau 2006).

ACKNOWLEDGMENTS

This work was partially supported by the TRUEFOOD European Commission Integrated Project within the Sixth RTD Framework Program (Contract no. FOOD-CT-2006-016264) and by INIA (Contract PET-2007-08-C11-08). The information in this document reflects only the authors' views and the Community is not liable for any use that may be made of the information contained therein.

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Turkish Pastirma: A Dry-Cured Beef Product

Ersel Obuz, Levent Akkaya, and Veli Gök

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40.1 Introduction

Pastirma, a popular dry-cured beef product made from whole muscle, can be considered an intermediate moisture food (Gök and others 2008). Several whole meat-dried products such as ham, bacon, corned beef, Bündnerfleisch, and pastrami are produced all over the world. Pastirma is generally produced from whole muscles that are obtained from certain parts of cow and water buffalo carcasses (Aktaş and Gürses 2005). In order to produce pastirma, all the exterior fat and connective tissue are removed from the meat, and then curing, drying, pressing, and coating the resultant meat with çemen paste processes are applied (Aksu and Kaya 2002a). Although pastirma is defined as cured and dried meat product, partial fermentation takes place during the production step thanks to natural microbial flora (Katsaras and others 1996; Aksu and Kaya 2001a).

40.2 Processing Technology

Pastırma is derived from the Turkish verb "bastırma," meaning a strong pressing action (Tekinşen and Doğruer 2000). Although beef is the main meat used in pastirma production (Aksu and others 2005; Gök and others 2008) meat from camel, water-buffalo, sheep, turkey, and chicken can also be used (Doğruer 2001). Pastirma production takes place under environmental conditions in a much "master-apprentice" fashion. Therefore, skill rather than technology is in the center of a successful pastirma production. This

brings up economical, quality-centered, hygienic, and lack of standardization issues to our attention (Tekinşen and Doğruer 2000). "Pastirma summer" is the best period of time for natural pastirma production beginning in the second half of October and ending in the first half of November. The temperature difference between day and night is around 15°C; the days are sunny, and the relative humidity is the optimum during "pastirma summer" (Öztan 2005). However, a more recent trend is to produce pastirma in so-called "climatic rooms" where temperature and relative humidity can be effectively controlled.

The production scheme for pastirma is as follows:

- Slaughter of the animal
- Fabrication of meat
- Dry-curing (salt)
- First drying
- First pressing
- Second drying
- Second pressing
- Coating with çemen paste (paste seasoning)
- Final drying
- Packaging (see Figure 40.1).

40.2.1 Raw Material

Meat from 3- to 6-year-old cow and water buffalo is generally used in pastirma production (Anıl 1988). Meat from very young animals is not preferred since meat from these animals has too high a moisture content causing excessive moisture loss in drying. Meat from very old animals is also not preferred due to its coarse and tough texture. Moreover, fatty meat from very old animals results in a low-quality pastirma and is not preferred (Gökalp and others 1999).

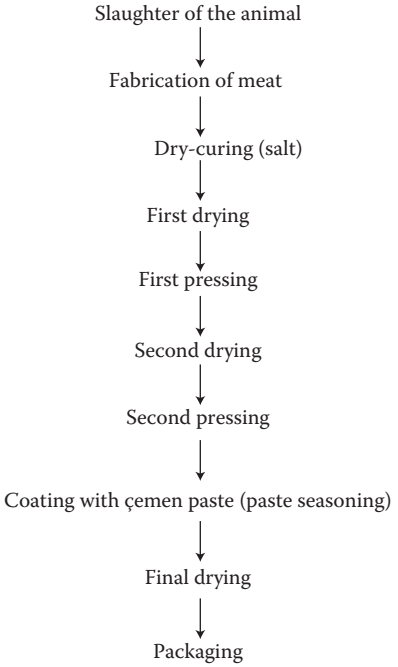

FIGURE 40.1 Pastirma production scheme. (Adapted from Gök V, Obuz E, Akkaya L. 2008. *Meat Sci* 80:335–44.)

The amount of stress before animal slaughter significantly changes the course of pH decline postmortem (Lewis and others 1989). The rate and extent of this pH decline affect several meat quality attributes including water-holding capacity and color stability (Briskey 1964). Meat having pH values ranging from 5.4 to 5.8 is optimum. After slaughtering the animal, the blood is effectively drained and rigor mortis develops. The muscles are then fabricated and they are ready to be processed for pastirma making (Öztan 2005).

40.2.2 Fabrication

Fabrication of meat is a critical step in pastirma production, which affects the final quality grade of pastirma (Gürbüz 1994). Eight pieces of meat are taken from the carcass (two pieces from loin, two from rib, two from flank, and two from round). Then, they are fabricated to be used for pastirma production. To easily remove meat pieces from the carcass, rigor must be completed and adequate cooling must be applied to the carcass (Doğruer 1992). Up to 16 different pastirmas having different names and quality attributes can be produced from a cow carcass (Institute of Turkish Standard 1991; Aksu and others 2005; Ceylan 2009). For example, pastirma from loin has a rectangular shape (the longer side measures 60–70 cm, and shorter side measures 15–20 cm) and is about 3–4 kg. It has optimum eating qualities and is the most preferred pastirma (Gökalp and others 1999, Tekinşen and Doğruer 2000). The next step is "şaklama" where deep cuts, up to half thickness of the meat, are made on the meat surface with a 45–60° angle by hand (Anıl 1986).

40.2.3 Curing

Curing agents and curing methods have a direct effect on final pastirma quality (Gökalp and others 1999). Salt along with nitrite, nitrate, and ascorbic acid is used as curing agents. Using salt as a sole-curing agents results in a tough, dry, and bland-flavored pastirma (Pearson and Tauber 1984). The curing agents are responsible for esteemed and stable red color; nitrite is a strong antioxidant and prevents or retards microbial growth and gives a pleasant flavor (Honikel 2008). Dry curing is the traditionally used curing method in pastirma production. Salt is a major ingredient in cured meat products and has a bacteriostatic effect; inhibits the growth of undesirable microorganisms; contributes to salty taste; and increases myofibrillar protein solubility (Toldra 2002). Salt used in pastirma production has medium size and is called "ant head" (Tekinşen and Doğruer 2000). Coarse salt cannot be adequately absorbed by meat and can negatively affect histology of meat resulting in salt burn. Very fine salt is readily absorbed by meat and causes several quality defects such as oxidation, black color, and excessive salty taste (Gökalp and others 1999). Curing mixture for pastirma production usually contains 94.5% NaCl, 1.5% KNO_3, 2% glucose, and 2% sucrose (Aksu and others 2005). Salt is applied to deeply cut meat surfaces by hand. The number of cuts and the distance between them are very critical. Cuts too close to each other may result in excessive salt absorption leading to a tough and bland product. On the other hand, too few cuts are responsible for less salt absorption and decreased product shelf life. Salted meat pieces are piled up and measure about a meter in height. Then, the piled meat pieces are turned upside down (Gökalp and others 1999). Pieces, which could not absorb enough salt, are resalted. Salted meat pieces are called "salty."

40.2.4 Drying

Dried meat products achieve shelf stability after drying and curing (sometimes salting) processes because these processes decrease water activity (Comaposada and others 2000). After the curing process, excess salt is drained away by washing the cured meat for at least 60 s (Özeren 1980; Gökalp and others 1999). After the washing step, drying by hanging meat pieces is carried out. The time frame of drying period depends on the environmental conditions. Drying lasts about 3–5 days in pastirma summer and it lasts about 15 days in winter months. However, recently, drying is finished much quickly in controlled climatic rooms, where parameters such as temperature, relative humidity, and speed of air can be controlled. The adequacy of drying is subjectively judged by smashing the processed meat between two fingers and by smelling it (Anıl 1986).

40.2.5 Pressing

The first pressing (cold pressing) step follows the drying process (Gürbüz 1994). This process is accomplished in a fairly large area made from çemen or mosaic in small plants, which traditionally produce pastirma (Tekinşen and Doğruer 2000). Cured and dried meat pieces are piled up on each other and a weight of 250 kg is put onto this pile (Gökalp and others 1999). The pressure of 0.9–1.0 kg is applied to per centimeter square area (Özeren 1980). The time of first pressing ranges from 6 to 16 h (Gökalp and others 1999). Pressed meats start losing water immediately due to the effect of curing and applied pressure. The pressing process also modifies the shape of meat pieces (Öztan 2005) and deep cuts on the meat surface vanish (Tekinşen and Doğruer 2000).

40.2.6 Second Drying and Hot Pressing

Second drying follows the cold pressing and is accomplished under minimal air speed. Second drying takes about 3 days at 20°C (Aksu 2002a). Fats on meat surface melt and form a white layer. Pastirma manufactures name this process "sweating or bleaching" (Gökalp and others 1999). After the second drying, second pressing or hot pressing starts. Since meat pieces have high amount of fat, they are soft and fairly warm. Therefore, they will change their shape readily and will lose water. Fats on the surfaces migrate to the interior of meat modifying meat texture significantly (Tekinşen and Doğruer 2000). Meat becomes very tender during this stage (Öztan 2005).

40.2.7 Çemenleme (Coating with Çemen Paste)

Çemenleme is to coat pastirma with cemen paste, which gives pastirma its characteristic taste, color, aroma, and flavor (Anıl 1988). A typical Cemen mixture has 500 g flour of *Trigonella foenum-graecum* seed, 350 g smashed fresh garlic and 150 g paprika, and 1200 mL water (Institute of Turkish Standard 1983, 1991). Çemen significantly affects the appearance, color, texture, taste, and flavor of pastırma and helps inhibit microbial contamination and excess drying of pastirma (Isıklı and Karababa 2005). Çemen (paste seasoning) is reported to have a protective effect on some pathogens (Yetim and others 2006) and mold growth (Kaya and others 1996; Berkmen 1960). Cemen paste is very sticky and encloses meat much like a casing (Öztan 2005). Çemenleme lasts between 16 and 36 h. The thickness of the cemen paste is about 3–4 mm (Gökalp and others 1999). Coated meat pieces are dried for 1–2 days during summer, 6–7 days during winter.

40.3 Pastirma Quality

To evaluate pastirma quality, physical, chemical, microbiological, and sensorial analyses can be used. Pastirma should meet certain physical, chemical, and microbiological standards and should not cause harm to human health. Turkish standards for pastirma lists maximum fat of 40%, salt of 8.5%, pH 4.5–5.8, maximum moisture content of 50%, maximum çemen level of 10%, no rancidity and zero tolerance to pathogens (TS-1071). Based on the Turkish Standards (TS 1071) (Institute of Turkish Standard 1983), pastirma is classified into one of the three quality grades. The first grade pastirma is of the highest quality. It has abundant level of marbling and the least amount of intermuscular fat. It is the most tender and has a fine structure and texture. The color of its interior is bright red and it does not have visible crust. The thickness of its çemen (paste seasoning) does not exceed 4 mm. The second-grade pastirma has less amount of marbling, but more intermuscular fat than the first-grade pastirma. Its çemen is not very homogeneous. The color of its interior is dark red and it has a visible crust. The third-grade pastirma does not have a fine structure and texture and is very dry. Its çemen is excessive. It does not have a significant amount of marbling, but has the most intermuscular fat. The color of its interior varies from dark-red to brown (Gökalp and others 1999).

Therefore, a high-quality pastirma:

- should have abundant and homogeneous amount of marbling
- should have minimum amount of intermuscular fat
- should have typical çemen color on the interior and bright-red color inside
- should have a fine texture, should be easily sliced, and should not be excessively tough nor should it be extremely tender.

40.3.1 pH

TS 1071 (Institute of Turkish Standard 1983) gives the pH of pastirma ranging from 4.5 to 5.8. However, pH values lower than 5 are very unlikely for pastirma since starter cultures are not used in pastirma production. pH values higher than 6.2 mark the beginning of pastirma spoilage. Storage period affects pH values of pastirma. Nitrogenous compounds formed by proteolysis during the extended storage period may be responsible for this increase as suggested by Lücke (1998). Aksu and Kaya (2005) and Gök and others (2008) reported increased pH values with storage time for pastirmas produced from fresh beef. As reported by Kaban (2009), pastirma does not undergo true lactic acid fermentation. The absence of true lactic fermentation has also been reported in other raw-cured meat products such as French dry-cured hams (Buscailhon and others 1994), Italian dry-cured ham (Virgili and others 2007), and dry-cured lacón (Lorenzo and others 2003; Marra and others 1999). The pH, albeit being less significant for pastirma than as in other fermented meat products, should not be <5.5 for sensorial reasons (Leistner 1988).

40.3.2 Water Activity and Moisture Content

Pastirma can be considered an intermediate moisture food and is shelf stable due to its relatively low water activity (a_w). Leistner (1988) reported that aw of pastirma must be between 0.85 and 0.90. Meat products having a_w values <0.90 do not have to be refrigerated, but can be stored at room temperature (Öztan 2005). Kaban (2009) reported that a_w values of pastirma changed from 0.98 (before curing) to 0.87 (final product). The current Turkish Food Codex on pastirma suggests the moisture content of not more than 40% for pastirma. Moisture content of less than or equal to 40% should ensure that the a_w of pastirma does not exceed 0.90. The chemical analysis of the pastirma samples sold in Turkish markets revealed that the moisture content ranged from 39% to 52% (Aksu and Kaya 2001a). This is very alarming since moisture content exceeding 40% may bring up food safety issues to our attention and need for refrigeration of the pastirma. In the related literature, moisture content as low as 32% (Berkmen 1940) and as high as 61.34% (Goma and others) have been reported. Setting the drying procedure for pastirma in a way that the final moisture content is <40% should ensure that the a_w for pastirma will fall between 0.85 and 0.90.

40.3.3 Thiobarbutiric Acid-Reactive Substance (TBARS) Content

TBARS are one of the significant lipid oxidation markers in meat and meat products (Shahidi and others 1987). TBARS are reported to change from 0.16 to 2.46 mg malonaldehyde/kg for pastirmas (Askar and others 1994; Yagli and Ertas 1998; Aksu 1999; Aksu and others 2005). TBARS are mostly affected by storage time, storage temperature or method of packaging. Aksu and others (2005) reported that TBARS value for pastirmas increased from 0.64 mg malonaldehyde/kg on day 0 of storage to 1.91 mg malonaldehyde/kg on day 150 of storage. Similarly, Gök and others (2008) suggested that TBARS value for pastirmas increased with increased storage time. Storing pastirmas at 10°C resulted in higher TBARS values than storing pastirmas at 4°C. Gök and others (2008) reported that the lowest TBARS values occurred for pastirmas packaged in modified atmosphere packaging followed by those packaged by vacuum packaging and aerobic packaging. Modified atmosphere packaging should be the right choice of packaging to retard oxidative rancidity over the pastirma storage period.

40.3.4 Color (L*, a*, and b* Values)

The attractive pink-red color of dry-cured meat products is mainly contributed to nitrite (Pearson and Gillett 1997). Instrumental redness (a*) is a reliable indicator of dry-cured meat product quality. With storage time, a* was reported to decrease indicating loss of cured color of pastirmas (Gök and others 2008; Aksu and others 2005). Packaging method has certain implications on cured-meat color. Gök and others (2008) reported that modified atmosphere packaging preserved typical cured-meat color of pastirmas much better than vacuum packaging or aerobic packaging. Storage temperature also affects a* values. Aksu and others (2005) concluded that storing pastirmas at 10°C resulted in higher a* values than storing pastirmas at 4°C. L* and b* values of pastirmas were reported decrease with increased storage time which may be attributed to myoglobin (pigment) oxidation (Gök and others 2008; Aksu and others 2005). However, L* (lightness) and b* (yellowness) are not very significant as far as pastirma quality is concerned.

40.3.5 Microbiological Properties

Results of studies on the microbial flora of pastırma in the Turkish market were as follows: The total viable mesophilic count and *Lactobacilli* count ranged from 4 to 8 log cfu/g, *Micrococci/Staphylococci* (4–7 log cfu/g), *Enterobacteriaceae* (<2 log to 4 log cfu/g), coliform bacteria (<2 log to 3 log cfu/g), *Enterococci* (<2 log to 4 log cfu/g), *C. perfringens* (<1 log cfu/g), *Pseudomonas* and yeasts (<2 log cfu/g), and molds (<2 log to 5 log cfu/g) (Kılıç 2009). However, *Salmonella*, *Clostridium botulinum*, and *L. monocytogenes* were not detected in pastırma. Özdemir and others (1999) and Soyutemiz and Özenir (1996) reported that Lactic acid bacteria, *Micrococcus* and *Staphylococcus*, were the dominant flora of pastirma. The type of packaging and storage time may have a commanding effect on the microbial stability of pastirma. Modified atmosphere packaging was, by far, the best method of packaging for pastirmas as far as overall microbiological quality is concerned (Gök and others 2008). Kaban (2009) concluded that significant increase in microbial counts occurred during the course of pastirma processing. On the other hand, Gök and others (2008) and Aksu and others (2005) reported decreased Mesophilic bacteria count with increased storage time. Microbiological quality problems should be of less concern for pastirma because it is an intermediate moisture food and has relatively low water activity. However, postprocessing contamination or postprocessing operations such as slicing may increase the microbial risk. Therefore, it is highly recommended that pastirmas be packaged appropriately (Modified atmosphere packaging) and be refrigerated (at 4°C) to optimize shelf life.

40.4 New Trends in Pastirma Processing

Pastirma is generally made from certain beef cuts; however, there has been research on the use of alternative meats such as turkey, chicken, and fish meat in the manufacture of pastirma (Arslan and others 1997a, b; Doğruer 2000; Gök and Uzun 2010). The use of turkey, chicken, or fish meat may increase market share of pastirma because these meats are much cheaper than beef.

Using brine solution instead of drying in the processing of pastirma is another new approach in pastirma manufacture. Güner and others (2008) investigated the use of tumbling and brine injection on pastirma quality. They concluded that brine injection of curing agents minimally affected pastirma quality. However, tumbling significantly decreased microbial count and improved the sensory properties of dry-cured pastirma.

Application of heat process in pastirma manufacture was also investigated. Ibrahim (2001) reported that heat treatment (71°C) decreased moisture content, pH, the residual nitrite and aerobic plate count, but increased total fat, ash, and protein contents. Heat treatment also positively affected sensory properties.

Effects of *Lactobacillus sakei* and *Staphylococcus xylosus* (starter cultures) on the inhibition of *Escherichia coli* O157: H7 in pastirma were investigated by Aksu and others (2008). They reported that the processing step of drying rather than use of starter culture was the significant factor for the inhibition

of *E. coli* O157: H7. The use of starter cultures decreased nitrite and nitrate residues in pastırma (Aksu and Kaya 2001b, 2002b) The authors further concluded the use of starter cultures (especially *S. xylosus* + *L. sakei* combination) in pastirma improved chemical quality parameters such as pH, residual nitrite and nitrate, and instrumental color.

40.5 Conclusions

Pastirma, as a traditional meat product, has not gained enough recognition and market share among traditional meat products. Its relatively high price, longer production time, and nonstandardized production procedures may be responsible for this problem. Standardizing production steps especially pressing stage, using controlled-artificial drying methods and optimizing raw meat quality are among some of the important issues requiring to be addressed. Moreover, a better production control system to satisfy consumer demands, but to avoid potential food safety hazards is promptly needed. In addition, alternative ingredient technologies (use of starter cultures) and processing steps (i.e., tumbling) may be implemented to further improve the quality of pastirma.

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Part VII

Meat Safety and Workers Safety

41

An Overview on Meat Safety in the United States[*]

Y. H. Hui

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41.1 Introduction

41.1.1 Abbreviations

Meat and poultry are the major sources of protein in the human diets for many countries, especially in the west. In the United States, the majority of the population like and prefer meat and poultry. In view of this, the federal government exercises tight control over their commerce, in terms of safety and economic fraud. After 9/11, food defense against terrorisms, including meat and poultry, became the third priority consideration in relation to the food industry. Before we discuss the safety system for meat and poultry in commerce, we will provide a list of abbreviations of U.S. government agencies that are used in this book's remaining chapters:

CFR: Code of Federal Regulations, http://www.gpoaccess.gov

DOL: Department of Labor, http://www.dol.gov

EPA: Environmental Protection Agency, http://www.epa.gov

[*] The information in this chapter has been modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. Used with permission.

FDA: Food and Drug Administration, http://www.fda.gov
FR: Federal Register, http://www.gpoaccess.gov
FSIS: Food Safety Inspection Service, http://www.fsis.usda.gov
HACCP: Hazards Analysis Critical Control Points
MPI: Meat Poultry Inspection
OSHA: Occupation Safety Health Administration, http://www.osha.gov
SPS: Sanitation (cleanliness) Performance Standards
SSOP: Sanitation (cleanliness) Standard-Operating Procedures
USDA: United States Departure of Agriculture, http://www.usda.gov
WMA: Wholesome Meat Act of 1967
WPPA: Wholesome Poultry Products Act of 1968

41.1.2 Premises

All information in this chapter has been adapted from public documents made available at the Web sites of the agencies listed under abbreviations above. From this perspective, all remaining chapters in Part VII of this book address the safety of meat processing and must be used with the following premises:

1. The information is based on the status of legal requirements, science, technology, and engineering available at the time of publication.
2. All information has been edited and modified to fit the objectives of this book. As such, the information cannot be used as *prima facie* evidence in a court of law.
3. All abbreviations named above will be used throughout the remaining chapters in this book.
4. Poultry is briefly mentioned in some discussion.
5. In the original documents, the style and format of the language used reflect the relationship between teachers and students in the government agency, for example:
   a. Teacher to students
   b. Supervisor to employees
   c. Supervisor to trainees
   d. Agency personnel to safety managers of food establishments
6. Such a format is kept wherever possible in this chapter so as to emphasize the importance of legal and scientific requirements presented in an educational format. Also, the language serves a useful purpose, when a supervisor uses it to train employees in a meat-processing company.
7. Every reasonable effort has been exercised to assure accuracy in the scientific and legal information. It is emphasized that the original documents at the Web sites should be consulted for details. Current technology permits easy identification of a document when a standard or specific key word or phase is used in the search engine.
8. Although food microbiology plays a major role in meat safety, food safety in general is a combined discipline of food science, food technology, food engineering, and food laws. In view of this, information on meat safety in the remaining chapters of Part VII of this book is presented with the understanding that users have some basic backgrounds in the discipline of food safety. This premise serves to explain why some knowledge of the whole field presented here is excluded due to the limitation of space.

With regard to the safety of meat and meat products in the country, the following discussion summarizes the contents of remaining chapters in Part VII.

41.2 Current Laws, Regulations, and Jurisdiction

The Federal Meat Inspection Act (FMIA) of 1906 mandated that the USDA inspect cattle, hogs, and sheep for animal diseases, verify that carcasses are fit for human consumption, and ensure the cleanliness of slaughter and processing plants. More recent regulations stemming from the enactment of the WMA of 1967 and the WPPA of 1968 charged the FSIS with the responsibility of implementing new regulations to assure the safety of meat and poultry in commerce.

Thus, the MPI program has a series of regulations and requirements that the establishments must comply with. Such regulations can be studied from a perspective of five core components.

1. Apply fundamental principles of good manufacturing practices in the production of all foods and beverages for human consumption.
2. Develop and implement a detailed set of SPS in meat and poultry-processing establishments.
3. Develop and implement SSOP in meat and poultry processing establishments.
4. Develop and implement HACCP plan.
5. Develop and implement procedures to work with other federal agencies in the safety of such matters as other food and beverages (FDA), drinking water (EPA), and workers (DOL, OSHA). The same cooperation is important in relation to state regulators.

A major goal, among many others, of the above safety system is to reduce the amount of pathogens potentially present in meat and poultry in an amount that will be harmful to the consumers. The MPI applies to all meat and poultry commerce that crosses state lines.

The law requires FSIS to cooperate with State agencies in developing and administering their own MPI Programs. Individual State MPI programs are required to operate in a manner and with authorities that are "at least equal to" the federal system. The concept does not require that the States operate their MPI Programs in a manner that is the same as or identical to the FSIS program, nor does it prohibit the State MPI Programs from establishing safeguards that they believe to be more effective than those employed by FSIS. Federal MPI programs will apply in those states that do not have an "equal" system or prefer the federal system.

To achieve its goals, the FSIS has established three types, among others, of mandatory requirements:

1. Basic official regulations published in the U.S. CFR Title 9.
2. Daily official announcements of rules and regulations published in the U.S. FR.
3. Official documents on regulations and requirements distributed within the FSIS. Most of them are available at its website.

The official regulations and requirements are located in 9 CFR 300-592. The regulations cover many topics. Those directly related to this book include

1. Antemortem and postmortem inspection
2. Preparation and processing operations
3. Definitions and standards of identity or composition
4. Produce categories
5. Hazards
6. Meat plants
7. Meat-processing equipment
8. Sanitation
9. Hazard Analysis and Critical Control Point (HACCP) Systems
10. Enforcements

41.3 Product Categories

In general, the FSIS's regulations are designed to government the safe production for nine product categories divided into

- Slaughter
- Raw product—not ground
- Raw product—ground
- Heat treated but not fully cooked—not shelf stable
- Fully cooked—not shelf stable
- Product with secondary inhibitors—not shelf stable
- Not heat treated—shelf stable
- Heat treated—shelf stable
- Thermally processed—commercially sterile

The safety considerations in relation to some of these products will be discussed in the remaining chapters of Part VII of this book on meat processing safety. Here, we will provide a summary of the contents of these chapters.

41.4 Hazards

When consuming a food is unsafe, it means that it contains a hazard that can cause potential harm to the consumers. These hazards can be biological, chemical, and physical. Although these hazards occur during food manufacturing, they can also occur after consumers have bought them. This happens as a result of mishandling during food preparation and storage at home. Each group is studied in detail below with an emphasis on consumer practices. The remaining chapters of Part VII provide more details on the hazards during the manufacturing of meat and meat products.

41.4.1 Biological Hazards

Biological hazards include bacterial, viral, and parasitic microorganisms. See Table 41.1 for a listing of selected biological hazards. Bacterial pathogens comprise the majority of confirmed foodborne disease outbreaks and cases. Although cooking destroys the vegetative cells of foodborne bacteria to acceptable levels, spores of spore-forming bacteria such as *Bacillus cereus*, *Clostridium botulinum*, and *Clostridium perfringens* survive cooking and may germinate and grow if food is not properly cooled or held after cooking. The toxins produced by the vegetative cells of *Bacillus cereus*, *Clostridium botulinum*, and *Staphylococcus aureus* may not be destroyed to safe levels by reheating. Postcook recontamination with vegetative cells of bacteria such as *Salmonellae* and *Campylobacter jejuni* is also a major concern for operators of retail and food service establishments.

Viruses such as norovirus, hepatitis A, and rotavirus are directly related to contamination from human feces. Recent outbreaks have also shown that these viruses may be transmitted via droplets in the air. In limited cases, foodborne viruses may occur in raw commodities contaminated by human feces (e.g., shellfish harvested from unapproved, polluted waters). In most cases, however, contamination of food by viruses is the result of cross-contamination by ill food employees or unclean equipment and utensils. Unlike bacteria, a virus cannot multiply outside of a living cell. Cooking as a control for viruses may be ineffective because many foodborne viruses seem to exhibit heat resistance exceeding cooking temperature requirements, under laboratory conditions. Obtaining food from approved sources, practicing no bare hand contact with ready-to-eat food as well as proper handwashing, and implementing an employee health policy to restrict or exclude ill employees are important control measures for viruses.

Parasites are most often animal host-specific, but can include humans in their life cycles. Parasitic infections are commonly associated with undercooking meat products or cross-contamination of

TABLE 41.1

Selected Biological Hazards, Associated Foods, and Control Measures during Food Preparation and Storage

	Hazard	Associated Foods	Control Measures
Bacteria	*Bacillus cereus* (intoxication caused by heat stable, preformed emetic toxin and infection by heat labile, diarrheal toxin)	Meat, poultry, starchy foods (rice, potatoes), puddings, soups, cooked vegetables	Cooking, cooling, cold holding, hot holding
	Campylobacter jejuni	Poultry, raw milk	Cooking, handwashing, prevention of cross-contamination
	Clostridium botulinum	Vacuum-packed foods, reduced oxygen packaged foods, under-processed canned foods, garlic-in-oil mixtures, time/temperature-abused baked potatoes/ sautéed onions	Thermal processing (time + pressure), cooling, cold holding, hot holding, acidification and drying, etc.
	Clostridium perfringens	Cooked meat and poultry, cooked meat and poultry products including casseroles, gravies	Cooling, cold holding, reheating, hot holding
	E. coli O157:H7 (other shiga toxin-producing *E. coli*)	Raw ground beef, raw seed sprouts, raw milk, unpasteurized juice, foods contaminated by infected food workers via fecal–oral route	Cooking, no bare hand contact with RTE foods, employee health policy, handwashing, prevention of cross-contamination, pasteurization, or treatment of juice
	Listeria monocytogenes	Raw meat and poultry, fresh soft cheese, paté, smoked seafood, deli meats, deli salads	Cooking, date marking, cold holding, handwashing, prevention of cross-contamination
	Salmonella spp.	Meat and poultry, seafood, eggs, raw seed sprouts, raw vegetables, raw milk, unpasteurized juice	Cooking, use of pasteurized eggs, employee health policy, no bare hand contact with RTE foods, handwashing, pasteurization or treatment of juice
	Shigella spp.	Raw vegetables and herbs, other foods contaminated by infected workers via fecal–oral route	Cooking, no bare hand contact with RTE foods, employee health policy, handwashing
	Staphylococcus aureus (preformed heat-stable toxin)	RTE PHF foods touched by bare hands after cooking and further time/temperature abused	Cooling, cold holding, hot holding, no bare hand contact with RTE food, handwashing
	Vibrio spp.	Seafood, shellfish	Cooking, approved source, prevention of cross-contamination, cold holding
Parasites	*Anisakis simplex*	Various fish (cod, haddock, fluke, pacific salmon, herring, flounder, monkfish)	Cooking, freezing
	Taenia spp.	Beef and pork	Cooking
	Trichinella spiralis	Pork, bear, and seal meat	Cooking
Viruses	Hepatitis A and E	Shellfish, any food contaminated by infected worker via fecal–oral route	Approved source, no bare hand contact with RTE food, minimizing bare hand contact with foods not RTE, employee health policy, handwashing
	Other Viruses (Rotavirus, Norovirus, Reovirus)	Any food contaminated by infected worker via fecal–oral route	No bare hand contact with RTE food, minimizing bare hand contact with foods not RTE, employee health policy, handwashing

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RTE = ready-to-eat.
PHF = potentially hazardous food (time/temperature control for safety food).

ready-to-eat food with raw animal foods, untreated water, or contaminated equipment or utensils. Like viruses, parasites do not grow in food, and so control is focused on destroying the parasites and/or preventing their introduction. Adequate cooking destroys parasites. In addition, parasites in fish to be consumed raw or undercooked can also be destroyed by effective freezing techniques. Parasitic contamination by ill employees can be prevented by proper handwashing, no bare hand contact with ready-to-eat food, and implementation of an employee health policy to restrict or exclude ill employees.

41.4.2 Chemical Hazards

Chemical hazards may be naturally occurring or may be added during the processing of food. High levels of toxic chemicals may cause acute cases of foodborne illness, while chronic illness may result from low levels.

Title 21 of CFR provides guidance on naturally occurring poisonous or deleterious substances. For example:

- 21 CFR 109: Unavoidable Contaminants in Food for Human Consumption and Food Packaging Material.
- 21 CFR 184: Direct Food Substances Affirmed as Generally Recognized as Safe.
- 1 CFR 172: Food Additives Permitted for Direct Addition to Food For Human Consumption.

FDA documents also provide information on naturally occurring chemicals. Examples include:

1. Fish, Shellfish, Crustaceans, and Other Aquatic Animals—Fresh, Frozen or Processed—Methyl Mercury.
2. Foods—Adulteration with Aflatoxin.
3. Aflatoxin in Brazil Nuts, peanuts and peanut products, and pistachio nuts.

Table 41.2 of this provides additional examples of chemical hazards, both naturally occurring and added.

41.4.2.1 Food Allergens as Food Safety Hazards

Recent studies indicate that more than 11 million Americans suffer from one or more food allergies. A food allergy is caused by a naturally occurring protein in a food or a food ingredient, which is referred to as an "allergen." For unknown reasons, certain individuals produce immunoglobulin E (IgE) antibodies specifically directed to food allergens. When these sensitive individuals ingest sufficient concentrations of foods containing these allergens, the allergenic proteins interact with IgE antibodies and elicit an abnormal immune response. A food-allergic response is commonly characterized by hives or other itchy rashes, nausea, abdominal pain, vomiting and/or diarrhea, wheezing, shortness of breath, and swelling of various parts of the body. In severe cases, anaphylactic shock and death may result.

Many foods, with or without identifiable allergens, have been reported to cause food allergies. However, FDA believes that there is scientific consensus that the following foods can cause a serious allergic reaction in sensitive individuals; these foods account for 90% or more of all food allergies:

1. Milk
2. Egg
3. Fish (such as bass, flounder, or cod)
4. Crustacean shellfish (such as crab, lobster, or shrimp)
5. Tree nuts (such as almonds, pecans, or walnuts)
6. Wheat
7. Peanuts
8. Soybeans

TABLE 41.2

Common Chemical Hazards at Retail, along with Their Associated Foods and Control Measures

Chemical Hazards	Associated Foods	Control Measures
Naturally Occurring		
Scombrotoxin	Primarily associated with tuna fish, mahi-mahi, blue fish, anchovies bonito, mackerel; also found in cheese	Check temperatures at receiving; store at proper cold holding temperatures; buyer specifications: obtain verification from supplier that product has not been temperature abused prior to arrival in facility.
Ciguatoxin	Reef fin fish from extreme Southeastern United States, Hawaii, and tropical areas; barracuda, jacks, king mackerel, large groupers, and snappers	Ensure fin fish have not been caught: 1. Purchase fish from approved sources. 2. Fish should not be harvested from an area that is subject to an adverse advisory.
Tetrodoxin	Puffer fish (Fugu; Blowfish)	Do not consume these fish.
Mycotoxins		
Aflatoxin	Corn and corn products, peanuts and peanut products, cottonseed, milk, and tree nuts such as Brazil nuts, pecans, pistachio nuts, and walnuts. Other grains and nuts are susceptible but less prone to contamination	Check condition at receiving; do not use moldy or decomposed food.
Patulin	Apple juice products	Buyer specification: obtain verification from supplier or avoid the use of rotten apples in juice manufacturing.
Toxic mushroom species	Numerous varieties of wild mushrooms	Do not eat unknown varieties or mushrooms from unapproved source.
Shellfish toxins		
Paralytic shellfish poisoning (PSP)	Molluscan shellfish from NE and NW coastal regions; mackerel, viscera of lobsters and Dungeness, tanner, and red rock crabs	Ensure molluscan shellfish are 1. From an approved source; and 2. Properly tagged and labeled.
Diarrhetic shellfish poisoning (DSP)	Molluscan shellfish in Japan, western Europe, Chile, NZ, eastern Canada	
Neurotoxin shellfish poisoning (NSP)	Molluscan shellfish from Gulf of Mexico	
Amnesic shellfish poisoning (ASP)	Molluscan shellfish from NE and NW coasts of NA; viscera of Dungeness, tanner, red rock crabs and anchovies.	
Pyrrolizidine alkaloids	Plants food containing these alkaloids. Most commonly found in members of the Borginaceae, Asteraceae, and Fabaceae families.	Do not consume of food or medicinals contaminated with these alkaloids.

continued

TABLE 41.2 (continued)

Common Chemical Hazards at Retail, along with Their Associated Foods and Control Measures

Chemical Hazards	Associated Foods	Control Measures
Phtyohemmagglutinin	Raw red kidney beans (Undercooked beans may be more toxic than raw beans)	Soak in water for at least 5 h. Pour away the water. Boil briskly in fresh water, with occasional stirring, for at least 10 min.
Added Chemicals		
Environmental contaminants: Pesticides, fungicides, fertilizers, insecticides, antibiotics, growth hormones	Any food may become contaminated.	Follow label instructions for the use of environmental chemicals. Soil or water analysis may be used to verify safety.
PCBs	Fish	Comply with fish advisories.
Prohibited substances (21 CFR 189)	Numerous substances are prohibited from use in human food; no substance may be used in human food unless it meets all applicable requirements of the FD&C Act.	Do not use chemical substances that are not approved for use in human food.
Toxic elements/compounds Mercury	Fish exposed to organic mercury: shark, tilefish, king mackerel, and swordfish. Grains treated with mercury-based fungicides	Pregnant women/women of childbearing age/nursing mothers, and young children should not eat shark, swordfish, king mackerel or tilefish because they contain high levels of mercury. Do not use mercury-containing fungicides on grains or animals.
Copper	High-acid foods and beverages	Do not store high-acid foods in copper utensils; use backflow prevention device on beverage-vending machines.
Lead	High-acid food and beverages	Do not use vessels containing lead.
Preservatives and Food Additives: Sulfiting agents (sulfur dioxide, sodium and potassium bisulfite, sodium and potassium metabisulfite)	Fresh fruits and Vegetables Shrimp Lobster Wine	Sulfiting agents added to a product in a processing plant must be declared on labeling. Do not use on raw produce in food establishments.

Nitrites/nitrates Niacin	Cured meats, fish, any food exposed to accidental contamination, spinach. Meat and other foods to which sodium nicotinate is added	Do not use more than the prescribed amount of curing compound according to labeling instructions. Sodium nicotinate (niacin) is not currently approved for use in meat or poultry with or without nitrates or nitrates.
Flavor enhancers Monosodium glutamate (MSG)	Asian or Latin American food	Avoid using excessive amounts.
Chemicals used in retail establishments (e.g., lubricants, cleaners, sanitizers, cleaning compounds, and paints)	Any food could become contaminated	Address through SOPs for proper labeling, storage, handling, and use of chemicals; retain Material Safety Data Sheets for all chemicals.
Allergens	Foods containing or contacted by: Milk, egg, Fish, crustacean shellfish Tree nuts Wheat Peanuts Soybeans	Use a rigorous sanitation regime to prevent cross-contact between allergenic and nonallergenic ingredients.

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Meat and meat products that include any of the above items as an ingredient will pose hazards. Consumers with food allergies rely heavily on information contained on food labels to avoid food allergens. Each year, the FDA receives reports from consumers who have experienced an adverse reaction following exposure to a food allergen. Frequently, these reactions occur either because product labeling does not inform the consumer of the presence of the allergenic ingredient in the food or because of the cross-contact of a food with an allergenic substance not intended as an ingredient of the food during processing and preparation.

The legal requirements include

1. Food manufacturers must identify in plain language on the label of the food any major food allergen used as an ingredient in the food, including a coloring, flavoring, or incidental additive.
2. Both FDA and FSIS are to conduct inspections to ensure that food facilities comply with practices to reduce or eliminate cross-contact of a food with any major food allergens that are not intentional ingredients of the food.

41.4.3 Physical Hazards

Illness and injury can result from foreign objects in food. These physical hazards can result from contamination or poor procedures at many points in the food chain from harvest to consumer, including those within the food establishment. As establishments develop their food safety management systems, Table 41.3 can be used to aid in the identification of sources of potential physical hazards to the food being prepared, served, or sold. Table 41.3 provides some examples of common physical hazards.

41.4.4 Specific Risk Materials

The USDA-FSIS has a category of Specific Risk Materials (SRM) from cattle and their handling and disposition.

The following materials from cattle are SRM, except when they are from cattle from a country that can demonstrate that its bovine spongiform encephalopathy (BSE) risk status can reasonably be expected to provide the same level of protection from human exposure to the BSE agent as prohibiting SRM for use as human food does in the United States:

> The brain, skull, eyes, trigeminal ganglia, spinal cord, vertebral column (excluding the vertebrae of the tail, the transverse processes of the thoracic and lumbar vertebrae, and the wings of the sacrum), and dorsal root ganglia from cattle 30 months of age and older and the distal ileum of the small intestine and the tonsils from all cattle. (USDA-FSIS regulations)

SRM are inedible and prohibited for use as human food. SRM must be removed from the carcasses of cattle, segregated from edible materials, and disposed of in accordance with regulations. The spinal cord from cattle 30 months of age and older must be removed from the carcass at the establishment where the animal was slaughtered.

The small intestine from all cattle may be used for human food if:

1. It is derived from cattle that were inspected and passed in an official establishment in the United States or in a certified foreign establishment in a country listed as eligible to export meat and meat products to the United States.
2. The distal ileum is removed by a procedure that removes at least 80 in. of the uncoiled and trimmed small intestine as measured from the ceco–colic junction and progressing proximally toward the jejunum or by a procedure that the establishment demonstrates is effective in ensuring complete removal of the distal ileum.

3. If the conditions are not met, the entire small intestine must be removed from the carcass, segregated from edible materials, and disposed of in accordance with regulations.

The requirements do not apply to materials from cattle from countries that can demonstrate that their BSE risk status can reasonably be expected to provide the same level of protection from human exposure to the BSE agent as prohibiting SRM for use as human food does in the United States.

Procedures for the removal, segregation, and disposition of SRM are

1. Establishments that slaughter cattle and establishments that process the carcasses or parts of cattle must develop, implement, and maintain written procedures for the removal, segregation, and disposition of specified risk materials. These procedures must address potential contamination of edible materials with SRM before, during, and after entry into the establishment. Establishments must incorporate their procedures for the removal, segregation, and disposition of SRM into their HACCP plans and/or other prerequisite programs.
2. Establishments that slaughter cattle and establishments that process the carcasses or parts of cattle must take appropriate corrective action when either the establishment or FSIS determines that the establishment's procedures for the removal, segregation, and disposition of SRM, or the implementation or maintenance of these procedures, have failed to ensure that SRM are adequately and effectively removed from the carcasses of cattle, segregated from edible materials, and disposed of in accordance with regulations.
3. Establishments that slaughter cattle and establishments that process the carcasses or parts of cattle must routinely evaluate the effectiveness of their procedures for the removal, segregation,

TABLE 41.3

Potential Physical Hazards during Food Manufacturing

Physical Hazards	Sources	Causes	Adverse Effects
Animal parts	Limbs, bones, etc.	Failure to exclude animals in processing areas	Potential choking, mouth damage, cuts, infection
Bone fragments	Muscle foods	Improper slaughter and cutting, procedures	Trauma, potential choking, mouth injury
Debris	Structure such as walls, floors, insulations	Repairs not done, foods not covered	Potential choking, intestinal tract injury
Glass fragments	Glasses, containers (bottles), light bulbs, glass tops	Breakage, absence of protective covers, accidental introduction	Cuts, bleeding, mouth and intestinal injury, infection
Human fingers	Worker's hands	Machinery mishandling	Alarm and distress
Metal fragment	Processing equipment, any external sources (jewelry, glass frames)	Chipping from metallic matters due to metal aging or accidental fallouts	Swallowing/mouth organs damage, cuts, infection, intestinal injury
Plastic fragments	Utensils, packaging, pens, toys	Breakage, improper processing, packaging, and handling	Damage to oral organs, choking, intestinal injury
Stones, pebbles	Yard tools, shoes, pockets, buckets with sand, etc.	Noncompliance with good manufacturing practices	Damage to oral organs, choking, intestinal injury
Wood chips	Splinters from work bench, boxes	Improper processing and handling	Choking, mouth damage, cuts, intestinal injury
Workers accessories and belongings	Jeweler, hair, eye glasses, pocket items (pen, coins, keys)	Noncompliance with good manufacturing practices	Swallowing and mouth damage. Chipped teeth, cuts, intestinal injury

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and disposition of SRM in preventing the use of these materials for human food and must revise the procedures as necessary whenever any changes occur that could affect the removal, segregation, and disposition of specified risk materials.

4. Record-keeping requirements are
   a. Establishments that slaughter cattle and establishments that process the carcasses or parts of cattle must maintain daily records sufficient to document the implementation and monitoring of the procedures for the removal, segregation, and disposition of the materials and any corrective actions taken.
   b. Records may be maintained on computers provided that the establishment implements appropriate controls to ensure the integrity of the electronic data.
   c. Records must be retained for at least 1 year and must be accessible to FSIS. All such records must be maintained at the official establishment for 48 h following completion, after which they may be maintained off-site provided such records can be made available to FSIS within 24 h of request.

There is mandatory sanitation requirements of equipment used to cut through specified risk materials. If an establishment that slaughters cattle, or that processes the carcasses or parts from cattle, does not segregate the carcasses and parts from cattle 30 months of age and older from the carcasses and parts from cattle younger than 30 months during processing operations, it must:

1. Use dedicated equipment to cut through specified risk materials; or
2. Clean and sanitize equipment used to cut through SRM before the equipment is used on carcasses or parts from cattle younger than 30 months of age.

If an establishment that slaughters cattle, or that processes the carcasses or parts from cattle, segregates the carcasses and parts of cattle 30 months of age and older from cattle younger than 30 months of age during processing operations, and processes the carcasses or parts from the cattle younger than 30 months first, it may use routine operational sanitation procedures on equipment used to cut through SRM.

Another requirement concerns shipping SRM. Slaughter establishments may ship beef carcasses or parts that contain vertebral columns from cattle 30 months of age and older to another federally inspected establishment for further processing if the establishment shipping these materials:

1. Maintains control of the carcasses or parts while they are in transit or ensures that the carcasses or parts move under FSIS control.
2. Ensures that the carcasses or parts are accompanied by documentation that clearly states that the carcasses or parts contain vertebral columns from cattle that were 30 months of age and older at the time of slaughter.
3. Maintains records that identify the official establishment that received the carcasses or parts.
4. Maintains records that verify that the official establishment that received the carcasses or parts removed the portions of the vertebral column designated as SRM and disposed of them in accordance with regulations.

41.5 Meat Plants

The FSIS has both mandatory and voluntary requirements for a meat-processing facility or plant. The specifications cover the following:

Location

Layout

Welfare facilities for establishment employees
Construction
Lighting, ventilation, refrigeration, and equipment
General-plumbing facilities
Establishment sewage treatment
Meat Slaughter: General Facilities Guidelines

1. For location, details are provided for site and the separation of official and nonofficial establishments.
2. For layout, recommendations are made for flow of operations, people traffic flow, separation of raw and ready-to-eat product, and so on.
3. To look after the welfare facilities for establishment employees, the management must provide dressing (locker) rooms, lockers, and so on.
4. *Construction will cover details for building construction materials for rooms (finished surfaces), floors, coving/curbs, stairs, and so on.*
5. Lighting, ventilation, refrigeration, and equipment must comply with strict specifications.
6. Water supply includes requirements for potable water, backflow.
7. General plumbing facilities will be concerned with hose connections and hoses, establishment drainage system, floor drains, and so on.
8. Establishment sewage treatment must comply with federal regulations.

41.6 Meat-Processing Equipment

Chapter 49 on sanitation and meat-processing equipment will cover a variety of topics such as materials for construction and repair, design and construction, cleanability, water usage, specialized equipment, equipment location and installation, maintenance and operation, cleaning of equipment and utensils, sanitizing of equipment and utensils, and protection of clean items.

41.7 Sanitation Standards

Proper and effective sanitation is vital to every step of a food-manufacturing process. The wholesomeness of product is directly dependent on the sanitary practices conducted in the food production operation. Insanitary facilities and equipment, poor food handling, improper personal hygiene, and similar insanitary practices create an environment conducive to contamination of products. There are direct links between inadequate sanitation and the contamination of meat and poultry products with pathogenic bacteria.

Sanitation is broadly defined as the formulation and application of procedures that establish an environmental state that promotes cleanliness and protects public health. It has many implications when applied to the food-processing environment. A safe water supply and distribution system, unpolluted air, sound construction of facilities; effective vermin control; and proper waste handling, disposal, and treatment are critical elements of environmental sanitation. Effective environmental sanitation or cleanliness coupled with sanitary maintenance of equipment and utensils, good personal hygiene, and proper food-handling practices substantially reduce the risk of direct product contamination and adulteration and are essential to the implementation of HACCP systems.

41.8 HACCP Systems

For more than two decades, the FSIS has mandated the use of HACCP system or plan in an establishment that processes meat and poultry. Under this system, the establishment identifies in the entire process

potential hazards, critical points where such hazards can be controlled, and preventive measures. Then, the establishment will monitor the production process and make corrective actions where necessary. Record keeping and verification completes the system. Obviously compliance with these requirements can only be accomplished through rigorous enforcement. This system or strategy is based on seven principles that will be explored in remaining chapters in Part VII.

41.9 Enforcement

The FSIS has many legal tools at its disposal to enforce laws and regulations protecting the American public from consuming food that may be injurious to their health. They include

- Establishment inspection
- Noncompliance records and appeals
- Port-of-entry reinspection
- Product control actions
- Food recalls
- Notices of prohibited activity
- Notices of warning
- Administrative actions
- Criminal actions
- Civil actions

Details on these topics will be provided in Chapter 50 on enforcement.

41.10 Workers' Safety

The meat-packing industry, which employs 1–2 million workers, is considered to be one of the most hazardous industries in the United States. According to the Bureau of Labor Statistics (BLS), this industry has experienced one of the highest injury rate of any industry in the country with a rate two to three times that of many other manufacturing industries.

The injuries include: cut by knives or machinery, sprains, and strains. Younger new workers are at the highest occupational risk and suffer a significant proportion of all injuries.

Workers can be seriously injured by moving animals prior to stunning, and by stunning guns that may prematurely or inadvertently discharge while they try to still the animal. During the hoisting operation, it is possible for a 2000-pound carcass to fall on workers and injure them if faulty chains break or slip off the carcass' hind leg. Workers can suffer from crippling arm, hand, and wrist injuries. For example, carpal tunnel syndrome, caused by repetitive motion, can literally wear out the nerves running through one section of the wrist. Workers can be cut by their own knives and by other workers' knives during the butchering process. Back injuries can result from loading and unloading meat from trucks and from moving meat, meat racks, or meat trees along overhead rails. Workers can be severely burned by cleaning solvents and burned by heat sealant machines when they wrap meat. It is not uncommon for workers to sever fingers or hands on machines that are improperly locked-out or inadequately guarded, or even amputations. Many workers can also injure themselves by falling on treacherously slippery floors and can be exposed to extremes of heat and cold.

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*Sanitation Performance Standards**

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42.1 Introduction

Proper and effective sanitation is vital to every step of a food-manufacturing process. The wholesomeness of product is directly dependent on the sanitary practices conducted in the food production operation. Insanitary facilities and equipment, poor food handling, improper personal hygiene, and similar insanitary practices create an environment conducive to contamination of products. There are direct links between inadequate sanitation and the contamination of meat and poultry products with pathogenic bacteria.

Sanitation is broadly defined as the formulation and application of procedures that establish an environmental state that promotes cleanliness and protects public health. It has many implications when applied to the food-processing environment. A safe water supply and distribution system, unpolluted air, sound construction of facilities; effective vermin control; and proper waste handling, disposal, and treatment are critical elements of environmental sanitation. Effective environmental sanitation or cleanliness coupled with sanitary maintenance of equipment and utensils, good personal hygiene, and proper food handling practices substantially reduce the risk of direct product contamination and adulteration and are essential to the implementation of Hazard Analysis and Critical Control Point (HACCP) systems.

In the United States, the fundamental principles of sanitation for all foods (raw, fresh, and processed), including meat and poultry, were originally those developed by the FDA. They are called Good Manufacturing Practice (GMP) regulations. Almost all of them are generic descriptions and they do not tell a food establishment what to do. Rather, they are end results that must be achieved by any food establishment. Thus, the FSIS has established sanitation performance standards (SPS) regulations. They are described below and the format used is similar to teacher to students, supervisor to employees, and so on.

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42.2 Sanitation Performance Standards Regulations

42.2.1 Basic Requirements

FSIS-inspected establishments must meet two sets of regulations concerning sanitation: The Sanitation Performance Standards (SPS) and the Sanitation Standard Operating Procedures (SSOP). Compliance with both is necessary if an establishment is to prevent the creation of insanitary conditions that can cause the adulteration of product. SPS means that an establishment must develop a frame of reference for various types of sanitation standards. Once SPS are established, the establishment must develop SSOP which describes the procedures to properly implement the SPS. In this chapter, details are only provided for SPS and interested parties should consult FSIS website for more details on SSOP. However, before we study details for SPS, a brief summary is provided for SSOP in the following paragraph.

Sanitation Standard Operating Procedures (SSOPs) are written procedures that an establishment develops and implements to prevent direct contamination or adulteration of product. The establishment must also maintain daily records sufficient to document the implementation and monitoring of the SSOPs and any corrective action taken. The establishment is required to maintain these written procedures on a file, and they must be available to FSIS on request. It is the establishment's responsibility to implement the procedures as they are written in the SSOPs. If the establishment or FSIS determines that the SSOPs fail to prevent direct contamination or adulteration of product, the establishment must implement corrective actions that include the appropriate disposition of product, restoration of sanitary conditions, and measures to prevent recurrence.

Most of the SPS address conditions within and around the establishment (e.g., ventilation, lighting, facility and equipment construction, and maintenance of the grounds). SPS are an integral part of the overall public health picture of a facility or a plant. They are used in conjunction with SSOP requirements to ensure that wholesome products are produced in a sanitary environment. In this chapter, we will provide a detailed discussion on SPS. A user can refer to documents on SSOP at the website of FSIS.

Performance standards set the results to be achieved, but they do not prescribe the step-by-step procedures to produce safe meat and poultry products. Simply put, the expected result is defined in the regulation, but the methods to achieve that result are not specified. The performance standards allow establishments the flexibility to develop and employ innovative and unique sanitation procedures to achieve the desired results. Although plants can use varying means to meet the performance standards, the required results are always the same. Plants must:

1. Operate under sanitary conditions.
2. Ensure that the product is not adulterated.

The Federal Meat Inspection Act (FMIA) and Poultry Products Inspection Act (PPIA) provide authority, requirements, policies, and standards related to sanitation. The law is quite clear:

> Meat and poultry products produced, packed, or held under insanitary conditions where they may have become contaminated with filth or may have been rendered injurious to health are deemed adulterated, without any further showing required by FSIS.

FSIS requirements for sanitation are found in Title 9, United States Code of Federal Regulations. An example of a standard citation is 9 CFR 416.1, which means: United States Code of Federal Regulations (CFR), Title 9 Section 416.1 or §416.1. Throughout this chapter, the citation of a section will be §416.1.

The sanitation performance standards identified in §416.1–416.5 focus on specific areas or conditions in and around the establishment that may result in insanitary conditions that could lead to the adulteration of product. These regulations provide the sanitation standards the establishment must meet for the mark of inspection to be applied to its products. Insanitary means "a state, condition, or occurrence which may lead to the contamination or adulteration of edible meat or poultry product when it is exposed, processed, handled, stored, or packaged." Noncompliance involves "insanitary conditions" that do not result in direct contamination or adulteration of product.

This chapter provides details on selected topics under §416.1. Users should refer to the Code of Federal Regulations for complete information for all topics.

Each official establishment must be operated and maintained in a manner sufficient to prevent the creation of insanitary conditions and to ensure that product is not adulterated.

This regulation sets the overall requirement for all of the sanitation performance standards. Establishments must ensure that conditions in and around the plant do not lead to adulterated product.

42.2.2 Grounds, Facilities, Pest Control

This sanitation performance standard regulation covers grounds and pest control; construction; light; ventilation; plumbing; sewage disposal; water supply and water; ice and solution reuse; and dressing rooms, lavatories, and toilets.

The grounds about an establishment must be maintained to prevent conditions that could lead to insanitary conditions, adulteration of product, or interfere with inspection by FSIS program employees. Establishments must have in place a pest management program to prevent the harborage and breeding of pests on the grounds and within establishment facilities. Pest control substances used must be safe and effective under the conditions of use and not be applied or stored in a manner that will result in the adulteration of product or the creation of insanitary conditions.

Proper maintenance of the grounds about an establishment is essential for ensuring good sanitation. Establishments need to keep the outside premises of the plant clean, orderly, and free from the basic essentials of life (i.e., food, water, and a place to hide and rear young) that attract and sustain a population of insects, rodents, or birds. The objective is to keep the official premises of the establishment so well maintained that pests will seek sustenance elsewhere. The best internal pest management program cannot be effective when a ready supply of vermin is present just outside the plant doors! The establishment has the responsibility to ensure that

- The outside premises of the plant are maintained in a manner that does not lead to the creation of insanitary conditions or interfere with FSIS inspection.
- Sources of product contamination or adulteration are prevented even if the source originates from outside the plant's "official premises." For example, the plant must prevent objectionable odors, smoke, flying ash, and so on, even when it is coming from neighboring industries such oil refineries, paper pulp mills, chemical plants, and so on, from adulterating product.

  The "official premises" are the boundaries of the plant. The establishment is required to designate the official premises of the plant. The Inspector may ask for written documentation of the boundaries to facilitate inspection of the plant.
- A pest control management program is implemented and maintained to prevent and/or eliminate pest harborage and breeding on the outside grounds and within the plant.

  Pests can transmit several diseases to humans through food contamination. Therefore, their presence in or around a food establishment creates an insanitary condition!
- Meat and poultry products are not contaminated or adulterated through the misuse of pest control substances.

  Pest control substances are pesticide chemicals (insecticides, rodenticides, or avicides) used to eliminate insects, rodents, and birds, and repellents used to keep them away.
- All pest control substances are safe for their intended use and applied, handled, and stored to prevent the creation of insanitary conditions that could lead to an adulterated product. Pesticides, repellents, traps, and other devices must be used in a manner that will kill, capture, or repel pests that venture onto the premises or gain entrance to the establishment without contaminating product, product containers, or equipment.
- Documentation substantiating safe use of a pest control substance in a food-processing environment is available for FSIS review.

- The Environmental Protection Agency (EPA) requirements under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) are followed. FIFRA addresses the application of pesticides and the safety of chemicals.

All the above items must be legally documented. Outside premises and pest control program can be verified as follows. Examine and assess the outside premises of the plant and the plant's pest control system in one or more places within the plant. Verify that the outside grounds are maintained in a sanitary manner, there are no areas where pest can breed, live, or hide outside or inside the plant, and there is no evidence of pest infestation outside or inside the plant. Answers to the following questions will help

1. Are all outside areas on the official premises maintained in a manner to prevent harborage and breeding of pests?
2. Are all areas within the establishment maintained in a manner to prevent harborage and breeding of pests?
3. Does the establishment have a pest management program?

The following list of observations may assist in answering these questions:

- Look for objectionable odors and flying dust, smoke, and ash in the air outside the plant.
  Note: Meat and poultry products may be exposed to airborne contaminants that can enter the plant through necessary openings such as loading and receiving docks, employee and visitor entrances and exits, etc.
- Look for accumulations of trash, debris, board piles, scrap metal, old equipment, or other refuse lying around outside the plant or useful materials and equipment stored in direct contact with the ground outside the plant for several days.
- Look for tall or overgrown vegetation.
  Note: Accumulations of rubbish, equipment stored on the ground, and tall weeds and dense grass are potential homes and hiding places for rodents and other vermin.
- Look for accumulations of hog hair, bones, viscera, paunch contents, feathers, manure, and other inedible materials in inedible operating and storage rooms and outside the plant on shipping docks, near dumpsters, or in live-bird areas, driveways, pens, alleys, and so on.
  Note: These organic waste products contain most of the contaminants, disease-producing, and spoilage microorganisms from plant production processes. If not handled properly, they can produce objectionable odors, serve as fly-breeding materials, and attract rodents and other vermin.
- Look for low areas with standing water outside the plant.
  Note: Surface and run-off water are usually heavily contaminated. Stagnant water in pools or puddles can emit offensive odors, serve as a breeding place for insects, and a source of water for rodents and other vermin.
- Look for conditions inside and outside that interfere with inspection, for example, inaccessible storage rooms or buildings on the official premises; no passageways or aisles between rows of supplies, boxes, and ingredients; or rows of supplies, boxes, and ingredients stored too close to the wall or stored in a manner that the entire floor-wall junction is not visible for detecting pest activity in dry storage areas, and so on.
- Look for indications that the plant has a pest management program in place, for example, an outside pest extermination firm has serviced the plant, rodent bait stations and glue boards are used in the dry storage and inedible areas, holes are sealed around pipes and electrical conduits that enter the plant, or plant employees inspect incoming supplies for the presence of insects, and so on.
- Look for evidence of pest activity "around" and "within" the plant.
  Note: Evidence of rodent activity might be visual observation, sound, droppings, runways and rub marks, nests and burrows, and gnaw marks, while evidence of insect activity might be visual observation, egg capsules, and excrement.

"Around" the plant would include outside premises and outbuildings within the official premises but not connected to the production facility. "Within" the plant would include processing areas, inedible product areas, and nonprocessing areas.

Processing areas are production rooms or production-related areas such as dry ingredient storage areas, spice rooms, coolers, packaging material and container dry storage areas, or any other areas where meat and poultry product is accessible. Nonprocessing areas are electrical rooms, boiler rooms, maintenance rooms, welfare facilities, business offices, or similar places.

Evidence of pest activity in a processing area requires further investigation to determine whether products, ingredients, or packaging materials have been adulterated or contaminated.

One must assess the information gathered above. What are some examples that indicate failure to meet the requirements of this regulation?

- There is an accumulation of pipes, scrap metal, and old equipment lying on the ground outside the plant.
- A large inedible product dumpster outside the plant is emitting an objectionable odor.
- There are several low areas with standing/stagnant water in them outside the plant.
- Several rows of palletized-packaging boxes are stored so close to the wall that there is no access to view the floor–wall juncture to detect pest activity.
- There is evidence of pest activity (e.g., rodent droppings) in a nonprocessing area of the plant (e.g., maintenance room or boiler room).

One should verify the following. One should observe and assess the use, handling, and storage of pest control substances in one or more areas of the plant and review plant documentation that supports the safety of their use in the food-processing area or outside the plant. One should *verify* that pesticide sprays, gases (fumigants), powders, pellets, or baits and repellents are handled, stored, and used in a manner that does not create insanitary conditions or adulterate product and that documentation describing the safe use of the chemical is on file in the plant. Again, answers to the following questions will help

1. Does the establishment have documentation on file about the safety of the pest control substances?
2. Does the documentation on file include how the pest control substances are to be used?
3. Are the pest control substances being applied as per the conditions and use?

The following list of observations may assist you in answering these questions:

- Look for pesticides used around the outside of the plant or in the plant that are not EPA-registered.
- Look for "restricted use" (residual) pesticides being prepared, mixed, or applied by a person who is not a certified applicator or under the supervision of certified applicator.
- Look for pesticides and repellents used in a manner that is inconsistent with their label instructions, safety precautions, or conditions of use identified in the use documentation on a file.

Some application methods do not allow operations to be conducted during treatment. Other methods allow the plant to be in operation when treated but require exposed product to be removed from the treatment area or be effectively covered. Some methods of applying pesticides require the plant to thoroughly wash and rinse food-contact surfaces of equipment and utensils before reusing them.

- Look for evidence of the use of residual insecticides in processing areas or areas such as locker rooms, lunchrooms, restrooms, halls, and the inspection office that are frequently used by employees whose primary work duties are in edible food areas.

Note: If the establishment keeps pesticide use records on file, you could compare these records to the documented application methods and precautions in the documentation that is on file at the plant.

If a residual insecticide is used in an area like a locker room, it could be transferred to employees, their work clothing, or other materials and objects that may contact product and indirectly contaminate it. Floor–wall junctions in areas with natural barriers such lockers, tables, cabinets, guard rails, and vending machines may be spot-treated with a residual pesticide if they will not be contacted by employees' shoes or other objects that could lead to secondary transfer of the insecticide to product.

False or drop ceilings in processing areas or employee welfare areas cannot be spot/void treated with a residual pesticide unless the plant can ensure that there is no opportunity for the pesticide spray or vapor to drift onto food-contact surfaces or other objects or materials below that may be contacted by employees whose primary work duties are in edible food areas.

- Look for pest control substances stored directly above or adjacent to (commingled with) food ingredients or product-packaging materials.
  Note: Pesticides are toxic chemicals and if stored in this manner they could be mistaken as food additives or soil-packaging materials if they happen to leak or are spilled.
- Look for clear identification (common name of the compound) on containers used to store amounts of pest control substances that have been removed from their original fully labeled (bulk) container.
- Look for documents, for example, Material Safety Data Sheets (MSDS), letter of guaranty, previous USDA acceptance, etc., that support the safety of pest control substances applied in the food-processing environment or around the plant and how they are to be used in the plant.

One must assess the information gathered above. What are some examples that indicate failure to meet the requirements of this regulation?

- The plant's pest extermination company is applying an insecticide in a manner that is inconsistent with the instructions on the label.
- A two-gallon container of insecticide is stored on a rack next to bags of flour in the ingredient dry storage area.

42.2.3 Construction

A plant's construction, layout (placement of rooms and equipment), and product flow can have a significant impact on daily sanitation operations and the goal of minimizing or eliminating product contamination.

The performance standards for construction provide establishments, regardless of size, flexibility to design facilities, and equipment in the manner to maintain a sanitary environment for food production.

Establishment buildings, including their structures, rooms, and compartments, must be of sound construction, be kept in good repair, and be of sufficient size to allow for processing, handling, and storage of product in a manner that does not result in product adulteration or the creation of insanitary conditions.

The establishment has the responsibility to ensure that

1. Buildings, including their rooms, compartments, coolers, and freezers are of such materials, construction, and finish (surface) that permit thorough cleaning to prevent insanitary conditions and product adulteration during processing, handling, and storage.
2. Rooms, compartments, coolers, freezers, and structures are large enough to allow orderly processing, handling, and storage of product. Inadequate room size and poor placement of equipment may result in congested operations that can lead to insanitary conditions.
3. The structure of the plant, its rooms, compartments, coolers, and freezers remains in good repair. Neglected or deteriorating facilities can defeat even the best sanitation program and lead to insanitary conditions or the adulteration of product.

Floors subject to wet cleaning operations that are not watertight, that is, have cracks, crevices, or holes, allow biological material and moisture to accumulate. Organic materials (e.g., protein and fat residue) and moisture caught or stuck in cracks or holes in the floor coupled with a prolonged drying time are all important factors in microbial growth.

Walls with rough or uneven surfaces where product is handled are difficult to clean because they may have recesses or depressions where protein and fat residue and foreign materials can collect. Likewise, junctions at floors and walls in production areas that have crevices, open cracks, or separations can allow water, dust, debris, and product residue to collect.

Although ceilings seldom contact product, they are a potential source of product contamination if foreign material or moisture drops onto equipment and exposed products. Therefore, they should be maintained in good repair and free of potential overhead contaminants such as scaling paint or plaster and leaks. If you are the responsible officer in the establishment,

1. You should examine and assess the design and construction of rooms, compartments, and structures in one or more areas of the plant where product is processed, handled, and stored.
2. You should verify that the room, compartment, or structure (door, partition, or post) is made with materials that can be cleaned, is of sufficient size, and is maintained in good repair. Answers to the following questions will help
   a. Are the rooms and compartments of sufficient size to allow for processing, handling, and storage of product?
   b. Are the structures, rooms, and compartments kept in good repair?

The following list of observations may assist you in answering these questions:

- Look for doors or doorways too small to permit product transferred on rails or in hand-trucks to pass through without contacting the door jamb.
- Look for carcass rails without enough passageway space to prevent the exposed product from contacting walls, refrigeration units, columns, or other fixed parts of the building.
- Look for inadequate space and capacity in work rooms, freezers, and coolers causing congested installation of equipment and storage of product, poor product clearance from objects, trash dumpsters stored next to exposed product or food-contact surfaces, and so on.
- Look for breaks, cracks, and holes in the floors, walls, and ceilings of production areas and employee welfare facilities.
- Look for junctions at floors, walls, and ceilings in production areas and employee welfare facilities with open crevices, cracks, or separations.
- Look for flaking or peeling paint or plaster on ceilings where product is processed, handled, or stored.
- Look for leaks in the ceilings of production areas and employee welfare facilities when it is raining.
- Look for plastic strips in poor repair on doors through which the exposed product passes.

The above information must be evaluated and examples of the failure to meet the requirements of this regulation are

- Flaking or peeling paint on the walls or ceilings of edible product areas.
- Two holes in the glass board of a wall in an edible product area permitting moisture to penetrate the wood behind it.
- Open gaps in the drop ceiling of the spice room because two fiberglass plastic ceiling panels have warped and are no longer seated correctly on metal cross-straps.
- An exposed gap/separation at the wall—floor juncture in the equipment and utensil washroom because two brick tiles that cover it are gone.

Walls, floors, and ceilings within establishments must be built of durable materials impervious to moisture and be cleaned and sanitized as necessary to prevent adulteration of product or the creation of insanitary conditions. The establishment has the responsibility to ensure that

- Floors, walls, and ceilings in areas where product is processed, handled, or stored are made of durable, easily cleanable, and moisture-resistant materials. Materials that are absorbent like wood, plaster board, and porous panels and tiles are difficult to keep clean.
- Floors, walls, and ceilings are cleaned at a frequency sufficient to prevent insanitary conditions and product adulteration.

Note: In order to be susceptible to thorough cleaning, the basic building materials used in the floors, walls, and ceilings of production and storage areas should be easy to clean, rigid, smooth, impervious to moisture, and resistant to wear and corrosion. If you are the responsible officer in the establishment,

1. You should examine and assess the materials used to build and the cleanliness of the floor, walls, and ceiling of one or more rooms, compartments, and structures in the plant.
2. You should verify that the materials used in the construction of the floor, wall, and ceiling are durable and nonabsorbent.
3. You should also verify that the floor, wall, and ceiling have been cleaned and sanitized as needed to prevent insanitary conditions.

You may gather needed information by seeking the answer to the following questions:

1. Are the walls, floors, and ceilings made of materials that are durable and impervious to moisture?
2. Are the walls, floors, and ceilings cleaned and sanitized as necessary?

The following list of observations may assist you in answering these questions:

- Look for the accumulation of dried or encrusted product residue from previous days' production, dust, or debris on floors and walls where product is processed, handled, and stored.
- Look for floors, walls, and ceilings made with materials that are absorbent in areas that are wet-cleaned.
- Look for dust, dirt, and debris on ceilings and window ledges where product is processed, handled, and stored.

You must evaluate the information you have obtained from above and examples of the failure to meet the requirements of this regulation are

- There is an accumulation of dust on the ceiling around air vents in the fresh sausage-packaging room.
- There are fat particles and hog hair from the previous days' production on the wall behind the dehairing machine.

Walls, floors, ceilings, doors, windows, and other outside openings must be constructed and maintained to prevent the entrance of vermin, such as flies, rats, and mice.

The establishment has the responsibility to ensure that

- The design and construction of the plant creates a barrier that prevents the entry of pests from the outside.

  Some places where pests can gain entry into the plant include gaps or separations in exterior wall–floor, wall–ceiling, and door–jamb junctures, gaps around service entries (e.g., pipes and electrical conduits) through exterior walls, floors, and ceilings, and so on.

- Breaks in the construction such as holes or cracks in exterior walls are promptly repaired.
- Doors that open to the outside and windows are protected against the entrance of pests or equipped with suitable devices (screens, fans, seals, etc.) to keep them out.

If you are an establishment official,

1. You should examine and assess the design, construction, and maintenance of the plant's exterior walls, floors, ceilings, outside doors, and other outside openings in one or more areas of the plant.
2. You should *verify* that the outer walls, ceilings, and floors prevent the entrance of pests.
3. You should also *verify* that outside openings such as doors and windows are equipped with suitable devices or barriers to keep insects, birds, and rodents out.

You may gather needed information by seeking the answer to the following question:

- Are the walls, floors, ceilings, doors, windows, and other outside openings constructed and maintained to prevent the entrance of vermin, such as flies, rats, and mice?

The following list of observations may assist you in answering this question:

- Look for broken areas, holes, cracks, and separations in the exterior walls of the plant.
- Look for open or broken windows without devices or barriers to prevent the entrance of pests.
- Look for outside doors with gaps around the jambs, which remain open (ajar) or do not close tightly, or are in poor repair (e.g., have holes in them).
- Look for gaps around pipes and electrical conduits entering the building.

You must evaluate the information obtained above and examples of the failure to meet the requirements of this regulation are

- There are open gaps or cracks around several outside doors and pipes entering the plant.
- A window without a screen has a broken window pane.
- There is a crack in the exterior block wall of the ingredient storage room.

Rooms or compartments in which edible product is processed, handled, or stored must be separate and distinct from rooms or compartments in which inedible product is processed, handled, or stored, to the extent necessary to prevent product adulteration and the creation of insanitary conditions.

The establishment has the responsibility to ensure that areas where edible products are processed, handled, and stored are adequately separated from those areas like inedible product departments that could cross-contaminate food products with disease-causing microorganisms, chemicals, filth, or other extraneous and deleterious materials.

Note: Establishments can process, handle, or store edible and inedible products in the same room as long as they are separated by time or space in a manner that prevents adulteration of the edible product or the creation of insanitary conditions. You should examine and assess the handling and storage of inedible products. You should verify that inedible products are adequately separated from edible products. You may gather needed information by seeking the answer to the following question:

- Are edible products and inedible products processed, handled, and stored in a manner that prevents product adulteration and the creation of insanitary conditions?

The following list of observations may assist you in answering this question:

- Look for inedible product containers commingled with edible product in coolers.
- Look for inedible product contacting edible product.

- Look for inadequate separation between the handling of inedible and condemned products and edible product.

Evaluate the information you gathered and examples of the failure to meet the requirements of this regulation are

- Inedible containers with by-products to be used as animal or pet food are commingled with containers that have edible offal in them in a cooler.
- The denaturing or decharacterizing and packing of inedible by-products and the handling and packing of edible offal are in a room too small to keep employees and products separated.

42.2.4 Ventilation

Ventilation adequate to control odors, vapors, and condensation to the extent necessary to prevent adulteration of product and the creation of insanitary conditions must be provided. A good ventilation system is important to the production of wholesome meat and poultry products. Unless the quality of air entering the plant is controlled and the quality of the air in the plant is maintained, products may become contaminated by dust, odors, smoke, fumes, and condensate. A poor ventilation system can serve as a vehicle for disseminating microorganisms. The establishment has the responsibility to ensure that the ventilation system:

- Provides enough ventilation for all areas of the plant, including processing, packaging, and welfare rooms to control condensation to the extent necessary to prevent creation of insanitary conditions and the adulteration of product.
- Promptly exhausts objectionable vapors (e.g., ammonia), fumes (e.g., from gas forklifts), and odors (e.g., from hide cellars, grease traps, toilet rooms, and inedible tank rooms) to the outside so they do not accumulate and enter edible areas where they can be absorbed by exposed product.
- Provides air from the outside that is free of odors from livestock pens and inedible areas, dust, fumes, and other airborne contaminants that can contaminate product. If air is exhausted from the plant, air from somewhere else will replace it.

FSIS does not expect the establishment to completely eliminate all odors, vapors, and condensation. However, plants must control the airborne contaminants such as odors, vapors and condensate (mists) to prevent adulteration of the environment that can lead to creation of insanitary conditions or the adulteration of product.

You should examine and assess the plant's ventilation system where products are processed, handled, or stored and in employee welfare rooms. You should verify that the plant's ventilation is adequately controlling the formation of vapors (including steam) and condensate, and promptly removing objectionable vapors and odors that could create insanitary conditions or adulterate product from the plant. You may gather needed information by seeking the answer to the following questions:

1. Is the ventilation adequate to control objectionable odors and vapors that could adulterate product or mask the odor of spoiled or otherwise adulterated product?
2. Is the ventilation adequate to control condensation?

The following list of observations may assist you in answering these questions:

- Look for a build-up of condensation in rooms where a product is processed, handled, or stored.
  Note: Condensate is free moisture formed on a surface or in the air when there is too much water vapor for the air to hold. Condensate is formed only when the temperature of the air or

surface is below the dew point of the surrounding atmosphere. When condensate forms in air, it becomes fog. When condensate forms on a surface, it wets it, and if the condition persists, the moisture accumulates to the point of forming droplets that fall from a horizontal surface or run down a vertical or inclined surface.

Some areas where condensate is likely to form include ceilings above cooking vessels and poultry chill vats, the ceiling around refrigeration units, or on the bottom of refrigeration units in processing areas.

- Look for a buildup of condensation on the ceilings of coolers and freezers or the bottom of refrigeration units in these areas.
- Look for objectionable vapors and odors, for example, ammonia and diesel fumes, in areas where product is processed, handled, or stored.
- Look for air intakes in areas where product is processed, handled, or stored, bringing in air contaminated with dust, smoke, and odors.
- Look for airflow carrying contaminants from an area in the plant being remodeled to a product area in the plant.
- Look for airflow carrying foul odors from toilet rooms entering areas where product is processed, handled, or stored.
- Look for air flowing from raw product areas to ready-to-eat product areas.

Assess the information you gathered. Two possible scenarios are

Scenario 1: You observe condensation that has formed on the underside of the stainless-steel lid covering a large kettle during cooking. The cooking kettles, including the lids, are cleaned and sanitized and monitored as part of the plant's written SPS and SSOP. In this case, the condensation has no affect on product safety, sanitary conditions, or inspection. With regard to condensation, you need to keep in mind that some forms of condensation are unavoidable and acceptable as long as the establishment controls the condensation to ensure that it does not create an insanitary condition or adulterate product.

Scenario 2: You observe beaded condensation on the ceiling directly above carcasses stored in the carcass-holding cooler. You look for evidence that the condensate has dripped from the ceiling, for example, drops on the floor between carcasses. You do not see any dripping condensate or find any evidence for you to conclude that condensate has contacted the carcasses. At this point, a potential insanitary condition exists that could lead to product contamination or adulteration. If the condensate had built up enough to be dripping onto the carcasses, contamination may be evident.

Examples of the failure to meet the requirements of this regulation are

- There is air from an area where the raw product is handled flowing into an area where ready-to-eat product is handled.
- A foul odor emanating from a restroom is entering an area where the product is processed and handled.
- Diesel fumes from parked trucks are being drawn into the receiving coolers.
- Beaded condensate is on the bottom of a refrigeration unit (not equipped with a drip pan) in an area where the product is processed, handled, or stored.

42.2.5 Plumbing

Plumbing is a particularly important consideration in food plants. Because plumbing systems transport water to and from establishments, problems with the plumbing system can easily lead to product contamination or adulteration. Plumbing impacts the plant's water supply, drainage, and sewage disposal systems.

Plumbing systems must be installed and maintained to Carry sufficient quantities of water to required locations throughout the establishment. The establishment has the responsibility to ensure that the plumbing system provides enough potable water for sufficient cleaning of equipment and utensils, facilities, hands, outside areas, and so on, to prevent product adulteration or creation of insanitary conditions.

An adequate supply of water is critical in sanitation programs and plant operations. Reduced water pressures, resulting from improperly sized and maintained pipelines, may adversely affect various washing operations and cleaning equipment that depend on specific water pressure and volume to perform their intended functions.

You may gather needed information by seeking the answer to the following question:

- Are sufficient quantities of water provided throughout the establishment?

The following list of observations may assist you in answering this question:

- Look for inadequate amounts of water in areas of the plant that require wet-cleaning operations.
- Look for inadequate amounts of water for flushing inedible materials (e.g., feathers and viscera) down the troughs in poultry slaughter operations.
- Look for inadequate amounts of water for scalding birds in poultry slaughter operations.
- Look for the absence of hot- and cold-water outlets in areas that require wet-cleaning operations, for example, near livestock pens, grease catch basins or traps, and outside inedible material removal areas.

Assess the information you gathered and examples of the failure to meet the requirements of this regulation are

- The water flow rate for removing inedible poultry viscera from the production room is inadequate, allowing the viscera to pile up in the trough.
- There are no water outlets in an area of the official premises that requires the removal of fat, grease, blood, meat juices, feathers, manure, and so on.

The establishment has the responsibility to ensure that sewage lines, drainage lines, and floor drains are of sufficient size to permit prompt removal of sewage (from toilet bowls and urinals) and liquid waste or waste water (e.g., water that contains blood, fat, animal fecal material, or product residues) from the processing environment and official premises. You may gather needed information by seeking the answer to the following question:

Does the plumbing system properly convey sewage and disposable waste from the establishment?

The following list of observations may assist you in answering this question:

- Look for blocked or clogged floor drain inlets and drain lines.

Note: Frequently blocked or clogged drains may be too small to accommodate the amount waste material being removed.

- Look for accumulations of water in the bottom of ice storage compartments.
- Look for catch basins or traps for recovering grease located near edible product departments and in areas where edible products are received and shipped.

Assess the information you gathered and examples of the failure to meet the requirements of this regulation are

- There is a clogged drain in a production area allowing waste water to backup onto the floor.
- An ice storage compartment allows the water from melted ice to collect in the bottom.
- A grease trap located on the official premises gives off a foul odor.

The establishment has the responsibility to ensure that its plumbing system distributes a potable water supply throughout the plant in quantities sufficient for all operating needs and the drainage and sewage lines permit the rapid removal of waste from the plant without adulterating product or creating insanitary conditions.

All plumbing should be sized, installed, and maintained in accordance with applicable State and local plumbing laws, ordinances, and regulations. If the plumbing system is not properly designed, installed, or maintained, contamination of the water supply, product, equipment, or utensils can occur or be created from conditions like cross-connections, backflow, flooding, drain system stoppage, and overhead leakage.

You may gather needed information by seeking the answer to the following question:

- Do the plumbing system's design, installation, and maintenance prevent adulteration of the product, contamination of water supplies, equipment and utensils, and the creation of insanitary conditions in the plant?

The following list of observations may assist you in answering this question:

- Look for dead-end pipes attached to the water supply system.

Short lengths of capped pipes are considered to be dead ends. For instance, a tee with one opening plugged is not a dead-end pipe, but any length of capped pipe extending from the tee is a dead-end pipe. A dead-end pipe may serve as a reservoir for stagnant water where microorganisms can grow.

- Look for leaking waste water drainage and sewage lines in production areas and employee welfare rooms.
- Look for potable water that has an off odor, color, or taste, or contains rust and scale.

Assess the information you gathered and examples of the failure to meet the requirements of this regulation are

- A dead-end pipe is attached to a potable water supply line.
- A waste water drainage pipe that is a few feet above the floor and runs along the wall of the cutting and boning room has a small leak at an elbow.

The establishment has the responsibility to ensure that all parts of floors where waste water is discharged onto the floor, wet-cleaning operations are conducted, or where floors are frequently hosed down are well drained to prevent accumulation of waste and the spread of contaminants during operations.

Plant waste water usually contains contaminants such as disease-producing and spoilage microorganisms. Therefore, contaminated fluids like waste water that accumulates on the floor or flows across a floor for long distances can create insanitary conditions. For instance, the accumulation of liquid and suspended solid waste can create objectionable odors in working areas, make proper cleaning impossible, and be tracked through production areas by employees, increasing the likelihood of product contamination. You may gather needed information by seeking the answer to the following question:

- Does the plumbing system provide adequate floor drainage?

The following list of observations may assist you in answering this question:

- Look for floors with areas including low spots in traffic areas where solid waste or waste water pools or collects because the floor is not sloped or pitched toward the drain.
- Look for waste water discharged from soaking and cooking vats, tripe scalders, chilling tanks, or other large vessels during operations that travels across the floor in traffic areas before entering a drain.
- Look for drainage lines that allow waste water from handwashing facilities and meat wash sinks that discharge water onto floors of product handling or storage areas.

- Look for drainage lines from drip pans of overhead refrigeration units that discharge water onto the floor.
- Look for waste water from poultry transfer belts with continuous washing systems that flows across the floor carrying organic waste to other areas of the room before entering a drain.

Assess the information you gathered and examples of the failure to meet the requirements of this regulation are

- Water from drip pan drainage lines is flowing across the floor to the floor drain located in the middle of the cooler.
- There is an accumulation of liquid and suspended solids (waste water) in a low area of the production room floor where employees walk.

The establishment has the responsibility to ensure that its water distribution system is free of possible pollution or contamination sources such as backflow and cross-connections between nonpotable water, waste water, or sewage lines, and potable water lines.

Maintaining the integrity of the water supply system within the plant is critical to product safety. A chief concern is the possibility of contamination of the water distribution system within the plant, and that the water becomes a vehicle for the transmission of disease-causing organisms.

A cross-connection is an actual or potential link between the potable water supply and a source of contamination (sewage, foreign materials, chemicals, etc.). A direct (or permanent) cross-connection is one where a potable water line has actually been plumbed (solid-pipe connection) to a nonpotable water (e.g., recirculated process water, untreated river water, or waste water) line. An indirect (or temporary) cross-connection is one in which the end of a water faucet or outflow end of the line terminates below the flood level of the sink, kettle, vat, and so on. Contamination or pollution occurs when the pressure differentials between the water supply and another system (reuse water, nonpotable water, etc.), via a connection, are sufficient to transfer contaminants or pollutants into the potable supply. Temporary reversal of pressures or momentary vacuums in the water supplies can be unpredictable.

Backflow is the flow of water or other liquids that is opposite to the expected or intended direction. Backflow is undesirable; however, a potable water system protected with backflow preventing devices can remain safe. Backpressure (a pushing force) and back-siphonage (a vacuum pulling force) are two forms of backflow.

Backpressure is a concern in plants with two or more piping systems. Backpressure occurs when both systems (potable and nonpotable water) are under pressure but the nonpotable system has greater pressure than the potable system, which pushes the contaminated water into the potable water supply. For backpressure to occur, a direct (solid pipe) connection between the two water systems has to exist.

Back-siphonage occurs when the pressure in the potable system drops below zero (or has negative pressure), which draws or pulls (siphons) the contaminated water into the potable water supply. Back-siphonage can occur with an indirect (submerged inlets) or direct (solid-pipe) cross-connection.

Once a cross-connection exists, a ruptured water main, a sudden large water withdrawal, or any situation that causes a pressure differential, with the potable system having the lower pressure or negative pressure, can result in the reverse flow of fluid (backpressure or back-siphonage) and contamination of the entire water distribution system and potable water supply. Hence, direct cross-connections between potable and nonpotable water lines must not exist and equipment installations where submerged water inlets are unavoidable must be equipped with mechanical antibackflow devices (vacuum breakers) to protect the potable water supply from contamination.

A vacuum breaker admits atmospheric pressure to a piping system between the source of the pollution and the origin of the vacuum, preventing back-siphonage. To be effective, the vacuum breaker should be installed between the last cutoff valve and the submerged water line.

You may gather needed information by seeking the answer to the following question:

- Is the plumbing installed to prevent backflow conditions and cross-connections between piping systems that discharge waste water or sewage and piping systems that carry water for product manufacturing?

The following list of observations may assist you in answering this question. You may perform additional observations:

- Look for direct cross-connections between potable water lines and nonpotable water lines, waste water lines, or sanitary (toilet and urinal) lines.

Nonpotable water (e.g., untreated water from a river or lake or water from an untested source such as a private well) may be used in plants for limited purposes. Cross-connections are hard to recognize or discover because of their location. For you to determine whether there are possible cross-connections between the potable water supply and the nonpotable water supply, you may ask the plant to assist you in distinguishing potable water lines from nonpotable lines.

- Look for direct cross-connections between lines carrying water or solutions for reuse and lines carrying potable water.
- Look for water lines with submerged inlets in processing equipment not equipped with mechanical vacuum breakers to prevent back-siphonage.

Submerged inlets are created when the outflow end of a potable water line is covered with water or other liquid. The water or liquid *may not* be potable. When you identify a submerged potable water inlet on a piece of equipment, you should ask the plant to show you the location of the vacuum breaker.

- Look for cleanup hoses with ends or nozzles submerged in sinks, defrost tanks, cleaning solution tanks, clogged drains, puddles of water on floor, and so on, not equipped with mechanical vacuum breakers.

When you identify a submerged hose end in a drain, tank, sink, and so on, you should ask the plant to show you the location of the vacuum breaker.

Assess the information you gathered and examples of the failure to meet the requirements of this regulation are

- A water hose not equipped with a vacuum breaker to prevent back-siphonage has its nozzle submerged in the evisceration trough drain.
- There is a cross-connection with a shut-off valve between the potable water supply and the nonpotable water supply.

The establishment has the responsibility to ensure that foul odors (sewer gases) from sink and floor drains do not enter the plant. You may gather needed information by seeking the answer to the following question:

- Is the plumbing installed to prevent the backup of sewer gases?

To assist you in answering this question, look for drains in the plant that are emitting sewer gases. The floor drainage system is usually sealed off so that sewer gases do not enter the plant by equipping drains, including blood drains, with a deep seal trap and a vent to the outside. The effectiveness of the trap depends on enough water remaining to constitute a seal. As water flows through the trap and down the drainpipe, a suction is created that will pull the water out of the trap and break the seal unless the suction is broken by venting the drainpipe to the outside air. The seal can also be broken by evaporation of trapped water. Therefore, drains installed in areas where there is seldom any wet cleanup are more likely to emit foul odors because the water in the trap has evaporated.

When you find a drain in the plant emitting sewer gases, it is creating an insanitary condition.

42.2.6 Equipment and Utensils

Equipment used in meat and poultry slaughter and processing establishments ranges from utensils (simple hand tools) to large, highly complex electronically operated machinery. Since product contacts a

variety of equipment surfaces, there is a potential for it to be contaminated. Equipment design and construction can have a significant impact on sanitation in the establishment and the goal of preventing product contamination.

Equipment and utensils used for processing or otherwise handling edible product or ingredients must be of such material and construction to facilitate thorough cleaning and to ensure that their use will not cause the adulteration of product during processing, handling, or storage. Equipment and utensils must be maintained in sanitary condition so as not to adulterate the product. The establishment has the responsibility to ensure that

- Equipment and utensils used in the preparation, handling, and storage of edible product and ingredients are not a source of product contamination or adulteration.
- Equipment and utensils are made of materials that are easy to clean.
- Materials used in the construction of equipment will not deteriorate under normal use conditions in the plant, allowing the harborage of microorganisms that could lead to adulteration of the product.
- Equipment is designed, installed, and maintained to prevent deteriorating surfaces that could lead to both microorganisms and chemicals being transferred to the product.
- The design, installation, and maintenance of equipment in the plant ensure that all surfaces and parts are capable of being cleaned and maintained in a sanitary manner.

Microorganisms may grow and cause objectionable odors inside equipment where meat and poultry fat and juices, cleanup water, and other debris are allowed to accumulate. Food-contact surfaces with pits, open seams, cracks, crevices, interrupted welds, etc., and external surfaces of equipment with open seams, gaps, crevices, inaccessible recesses, etc., may prevent the adequate removal of organic matter (product residues) from the surface and allow the development of biofilms. A biofilm is composed of closely packed colonies of bacteria that have attached themselves to the surface by producing a complex carbohydrate or polysaccharide substance that firmly adheres to the surface (including stainless steel) and traps nutrients, other microorganisms, fat, proteins, and other debris. Once established, these biofilms can transfer pathogens to product. Biofilms are not easily removed by ordinary cleaning measures and resist penetration of sanitizers.

- Equipment is designed and installed in such a manner that foreign materials such as lubricants, heat exchanger media, condensate, cleaning solutions, sanitizers, and other nonfood materials do not create insanitary conditions or adulterate the product.
- Cleaning and sanitizing of clean-in-place (CIP) systems are as complete and effective as the cleaning and sanitizing of disassembled equipment. The CIP design should ensure that the circulating cleaning and sanitizing solutions and rinse water contact all food-contact surfaces of the equipment. Dead spots in the system, that is, areas not contacted by the circulating cleaning and sanitizing solutions and rinse water, could cause insanitary conditions and adulteration by permitting the buildup of product residue. The CIP system should be self-draining because cleaning and sanitizing solutions remaining in the system may result in insanitary conditions and product adulteration.

CIP systems are used on equipment that is difficult or impractical to disassemble. CIP equipment is cleaned by circulating detergent solutions and rinse water at a flow velocity high enough to remove all product residues from internal surfaces. Band saws, mixers, grinders, and so on, that are subject to in-place manual cleaning are not considered CIP system equipment.

You may gather needed information by seeking the answer to the following questions:

- Are equipment and utensils used in processing and other handling of edible product or ingredients of material and construction that facilitates thorough cleaning?
- Are the ice-making equipment, rooms, and augers maintained in good repair and sanitary condition?

The following list of observations may assist you in answering these questions:

- Look for corrosion, rust, blisters, pits, cracks or other defects on equipment and utensil surfaces.
- Look for surfaces of equipment and utensils that can absorb moisture.
- Look for difficult-to-clean areas or surfaces on equipment and utensils.

There are many types of equipment used in the slaughtering and processing of meat and poultry products. It is not possible to list all of the pieces of equipment or the areas of the equipment that may be difficult to clean. Most pieces of equipment have areas that are difficult to clean or are accessible for cleaning but are not cleaned on a regular basis. For instance, grinders, conveyors, augers, blenders, emulsifiers, patty machines, linkers, stuffers, and packaging machines all have areas on them that are difficult to clean. Undersides of equipment, around motors, and behind deflectors or guards are some of the areas that might not be cleaned regularly and create insanitary conditions. You should not only learn to look, but should also be sensitive to odors that might indicate that there is an accumulation of product or product juices within a piece of equipment that has not had regular cleaning.

- Look for surfaces of equipment and utensils that could allow the migration of deleterious substances or impart colors or odors to product.

Aluminum, copper, brass, and bronze are metals that can discolor, stain, or migrate into the product. For instance, when friction occurs between aluminum and meat or fat, a black oxide is produced that discolors the meat. When copper and copper alloys (brass and bronze) contact acidic foods like meat and poultry, fats or oils, an objectionable greenish discoloration is formed in the product.

- Look for rough areas or recesses, open seams, gaps, protruding ledges, inside threads, bolts, or rivets on food-contact surfaces of equipment and utensils.
- Look for open seams, gaps, crevices, inaccessible recesses, or slot or hollow-headed fasteners, and so on, on nonfood-contact surfaces of equipment.
- Look for rough, interrupted, or uneven welds on equipment and utensil surfaces.
- Look for low areas, depressions, or sagging areas on or in equipment where cleanup water may accumulate and create an insanitary condition.
- Look for parts or components of equipment such as pulleys, bearings, or gears that could contaminate product and food-contact surfaces with foreign materials.
- Look for equipment and utensils that are not in good repair.

The equipment in large establishments handles large volumes of product. Equipment parts may become worn or damaged under normal use. Equipment that is not properly operated or maintained may have damaged or worn parts. Therefore, you should also learn to listen for noises that are not normal, such as metal rubbing on metal. Worn or damaged equipment can create insanitary conditions and adulteration of the product.

- Look for equipment, compartments, and bins used to make and store ice that could contaminate the ice.

Assess the information you gathered. Some potential scenarios are

Scenario 1: You observe a rough, uneven weld with crevices that connects one of the agitating paddles to the shaft in a vertical mixer ready for use in the curing room. You closely examine the weld and conclude that no product will be contaminated when the mixer is used because you found no accumulation of product residue stuck in the crevices or around the weld. However, if you had found an accumulation of product residue stuck in the crevices or around the weld, then the product would be contaminated when it came in contact with the weld.

Scenario 2: You observe a black substance on the surface of a piece of equipment during operations. You examine the piece of equipment and the product around it and find there is no product contamination occurring. If the black substance was contacting product, there may or may not be food safety hazards associated with the contamination. If the black substance contacting the product was coming from a heavy metal, such as lead, there may be food safety hazard associated with that contamination.

Examples of the failure to meet the requirements for this regulation are

- A food-contact surface of a piece of equipment is corroded and has several pits.
- A piece of equipment used to handle edible product has an external surface (nonfood contact) with an exposed bolt and open seam that have a build-up of encrusted product residue around and in them.
- A polyester conveyor belt that moves exposed product to the packaging machines has two deep cuts and has been gouged.
- The plastic cutting boards on the pork-boning tables have been scored by hand knives and have wizard knife gouges.
- A stainless-steel shovel used to handle edible product has a rough and curled edge.
- An accumulation of product residue is under the edges of the floor mounts for the legs of three large permanently installed cooking kettles.
- A foul odor is being emitted from a gearbox on a piece of equipment.

Equipment and utensils must not be constructed, located, or operated in a manner that prevents FSIS program employees from inspecting the equipment or utensils to determine whether they are in sanitary condition. The establishment has the responsibility to ensure that

- Equipment and utensils are designed, constructed, installed, and operated in a manner that allows inspection personnel access to inspect them. Because of the closed nature of CIP systems, there should be inspection openings throughout the system so that the Inspector can verify that all parts have been cleaned and sanitized.
- Conveyor guides, splash guards, safety guards, and so on, that are installed on equipment that cover food-contact surfaces can be removed to permit inspection.

You may gather needed information by seeking the answer to the following question:

- Are equipment or utensils constructed, located, or operated in a manner that prevents inspection program personnel from inspecting the sanitary condition of the equipment or utensils?

The following list of observations may assist you in answering this question:

- Look for equipment and utensils whose construction or installation interferes with sanitation inspection.
- Look for equipment and utensils with nonfood contact surfaces that are not accessible for inspection.
- Look for equipment and utensils with food-contact surfaces that are not visible for inspection.

Assess the information you gathered. If you cannot visually inspect the surfaces or parts of equipment, you cannot determine if an insanitary condition is being created or if product is being contaminated. One potential scenario is

Scenario 1: You find that a section of the plant's CIP-piping system that conveys edible product is not disassembled for inspection and it does not have access points for you to inspect the interior surfaces for cleanliness. At this point, you do not know if there is an insanitary condition. However,

the plant installs quick disconnects and removable elbows at each change of direction to allow inspection. You ask the plant to disconnect the pipes for inspection and a solution of water pours out of them. Now you will have to gather more information and make the determination whether or not this condition is creating an insanitary condition or contaminating the product.

Examples of the failure to meet the requirements for this regulation are

- A splash guard is permanently installed on a portable screw conveyor that carries trimmings to a meat grinder hopper that prevents the Inspector from inspecting the sanitary condition of the auger.
- A large piece of equipment is installed so close to the wall that the Inspector cannot inspect the sanitary condition of one side of it.

Receptacles used for storing inedible material must be of such material and construction that their use will not result in the adulteration of any edible product or in the creation of insanitary conditions. Such receptacles must not be used for storing any edible product and must bear conspicuous and distinctive marking to identify the permitted uses. The establishment has the responsibility to ensure that

- Containers or receptacles for handling, storing, and transporting inedible materials are made of materials that do not create an insanitary condition.
- Inedible receptacles or containers are maintained in a good state of repair and cleaned as needed to avoid creating insanitary conditions.

Containers that are not water-tight or that leak can spread contaminants into the environment, which could become a source of contamination of product, equipment, and utensils.

- The permitted use of the receptacle or container is identified through conspicuous and distinctive markings, a color-coding system, or some other means. Simply put, plant employees need to be able to recognize the inedible product containers from the edible product containers.
- Inedible receptacles or containers are not used to handle, store, and transport edible product.

You may gather needed information by seeking the answer to the following questions:

1. Are receptacles used for storing inedible products marked conspicuously and distinctively to identify permitted uses?
2. Are receptacles used for storing inedible material constructed of materials that can be maintained in a sanitary manner?

The following list of observations may assist you in answering these questions:

- Look for proper identification on the inedible material receptacles.
- Look for inedible receptacles made of materials that cannot be cleaned.
- Look for inedible receptacles that are not water-tight or are leaking.
- Look for inedible receptacles that are giving off objectionable odors.
- Look for inedible receptacles being used to handle, store, and transport edible product.

If you find inedible containers that are not clearly marked or readily distinguishable from edible product containers, it would represent noncompliance. Some potential scenarios are

You observe a plastic trash can marked “inedible” in the cooler being used to store edible bologna rework (stick ends and broken slices). You take an official control action and retain the bologna

rework. To salvage the rework, the plant must demonstrate that the rework is not contaminated. Is the rework contaminated?

Examples of the failure to meet the requirements of this regulation are

- There is a hole in the bottom of an inedible container allowing purged juices (blood, water, etc.) to leak onto the production room floor.
- Several metal inedible product barrels located in production-related areas have a large buildup of dried, encrusted product residue on the inside surface and are emitting an objectionable odor.

42.2.7 Sanitary Operations' Implementation

Frequent and effective cleaning of certain areas of the plant is necessary to:

1. Prevent the accumulation of organic wastes resulting from meat and poultry operations on equipment and utensils.
2. Prevent the development of foul odors that could attract insects, rodents, and other vermin.
3. Remove product debris or dirt on nonfood-contact surfaces that may provide an environment suitable for the growth of microorganisms that employees could indirectly transfer to the product. The method, frequency, and area to be cleaned may vary with plant operations.

All food-contact surfaces, including food-contact surfaces of utensils and equipment, must be cleaned and sanitized as frequently as necessary to prevent the creation of insanitary conditions and the adulteration of product. The establishment has the responsibility to ensure that

- Utensils and equipment surfaces that contact exposed edible product are cleaned and sanitized on a schedule that prevents the creation of insanitary conditions and the adulteration of the product.
- Food-contact surfaces of utensils and equipment are cleaned and sanitized whenever there is a change from working with raw product or raw components to working with ready-to-eat products.
- Food-contact surfaces of utensils and equipment are cleaned and sanitized whenever they become contaminated during operation.

Generally, establishments clean their operations once a day; however, some establishments conduct chemical cleanup procedures less than once a day. Such extended cleanup procedures should be incorporated into the plant's SSOP. To ensure that extended cleanup procedures prevent insanitation and the adulteration of product, establishments might conduct microbiological testing to evaluate the effectiveness of the extended cleanup. An example of failure to meet this performance standard is insufficient cleaning of a belt allowing a buildup of fat. When the fat rubs against a stainless-steel guard on the belt, a black substance is deposited on the accumulated fat.

42.2.7.1 Protecting Products from Adulteration before Distribution

Products must be protected from adulteration during processing, handling, storage, loading, and unloading at and during transportation from official establishments. The establishment has the responsibility to ensure that

- The product is not contaminated or adulterated by elements in the environment such as microorganisms, dust, wood splinters, scaling paint, flaking rust, condensate, oil, grease, metal fragments, plastic, etc.

- Products are not adulterated or misbranded because they contain undeclared ingredients that are potential sources of food sensitivities and intolerances (i.e., allergens).
- Products are protected from being contaminated by dust, dirt, or insects during loading, unloading, and transportation from the plant.
- No handling or storing of nonfood materials (barrels, boxes, pallets, trash, etc.) creates insanitary conditions in areas where products are prepared, handled, or stored.
- Supplies such as packaging materials or paper wrappings that might contact edible products are handled and stored under sanitary conditions, for example, measures are taken to prevent dust collection, footwear contamination, cleanup water splash, or contact with any insanitary surface.
- Wet-cleaning operations are conducted in a manner that minimizes the danger of contaminating product or food-contact surfaces with splashes from cleanup water. The plant should have a separate area or washroom for cleaning utensils, pans, trays, hand-trucks, demountable equipment, and so on.
- Employee traffic patterns do not create insanitary conditions or lead to the cross-contamination of product. Pathogens can be transported by employee clothing or boots. Therefore, employees not routinely assigned to specific work areas should not travel through those work areas. For example, employees working in the inedible product department should not travel through edible product areas.

Similarly, employees who work in slaughter areas or raw product areas should not travel through cooked or ready-to-eat product areas.

Depending on the situation and the circumstances, the plant must decide how to protect the product through all phases of the process. For example, the plant may decide to cover all products that are stored in the finished product freezer with plastic to prevent condensate from soiling the boxes while it is working to fix the refrigeration system in the freezer.

This performance standard allows establishments to devise their own means of limiting microbial growth in their processing operations. The plant may control microbial growth in product by using the traditional method of reducing the ambient temperature or use a different method. For instance, the plant may use heat exchangers on equipment to immediately reduce the product temperature, which more effectively limits the growth of microorganisms than maintaining a specific room temperature. You may gather needed information by seeking the answer to the following question:

- Does the establishment protect product from adulteration during processing, handling, storage, loading and unloading, and during transportation from official establishments?

The following list of observations may assist you in answering this question:

- Look for inadequate sanitation between production runs of products that have ingredients that cause food sensitivities declared on their label and products that do not have these ingredients declared on their label.
- Look for raw (not rinsed or washed) vegetables being handled in the same area as the exposed edible product.
- Look for cloth or paper containers of meat or poultry or of ingredients (sugar, salt and spices, etc.) added to equipment (mixers, blenders, grinders, etc.) that allows lint, dirt, or debris from the outer surface to fall into the product.
- Look for supplies that might contact product that are not handled and stored in a manner to preclude contamination.
- Look for food ingredients that are not handled and stored in a manner to preclude contamination, for example, spices stored in containers without lids in the spice room, open broken bags of ingredients in the dry storage area, and so on.
- Look for products (carcasses or carcass parts, trimmings in combo- bins or gondolas, etc.) received and distributed from the plant that are not protected from dust, dirt, insects, and so on.

- Look for transport vehicles that are not constructed to protect product being hauled from weather or road contamination.
- Look for containers (wooden, fiberboard, or metal) *reused* as edible product containers that are structurally unsound or not clean, for example, torn, broken or wet; soiled or stained; give off odors; or contain meat and fat particles, wood splinters, and any other foreign material.
- Look for transport vehicles that give off objectionable odors or that have accumulations of dried meat and fat, or foreign materials such as grease and trash in them.
- Look for pallets in edible product areas that have a buildup of grease, meat tissues and juices, animal excreta, feathers, or hair, or are giving off offensive odors.
- Look for accumulations of rubbish such as paper towels, empty product cartons, or labeling materials in edible product areas.
- Look for employees traveling through areas of the plant where they are not assigned.

Assess the information you gathered. A potential scenario is

> You observe an employee preparing a pickle cure solution. The pickle ingredients are staged on a wooden pallet next to the stainless-steel pickle cure vat. The employee fills the vat up with water and then begins to add 50 lb bags of salt to the water. He stands the bag on end, opens the top of the bag of salt, picks it up, places it on the rim of the vat with the bag over the edge, and pours the salt from the bag. Since the bags of salt were in direct contact with the pallet and he did not strip the outer layer of the bag before placing it over the edge of the vat, you decide to examine the condition of the solution. You find extraneous material (lint, dust, debris, etc.) floating on top of the water. As a product ingredient was contaminated, you should document this noncompliance under the SSOP procedure.

If you did not find foreign or extraneous material in the solution when you examined it, there is still noncompliance with the requirements because the method the employee used to add the salt when formulating the pickle solution does not protect the solution from contaminants that may fall off the outside surface of the bags. You should document this noncompliance. Examples of the failure to meet the requirements for this regulation are

- The establishment is loading forequarters of beef onto a truck trailer that has an accumulation of dried blood, fat, and meat particles on the floor.
- Several overhead pipes in the grinding room have an accumulation of dust on them.
- Several uncovered combo bins of pork trimmings were removed from a truck trailer and are now sitting on the receiving dock waiting to move into the cooler. The receiving dock is open to environmental elements.
- The surface of a roll of packaging material that contacts product is stored in direct contact with the surface of a pallet in the dry storage area.
- Two employees who work in the picking room are walking through the cut-up room.

42.2.8 Employee Hygiene

Employees can be disease carriers and bring contaminants from the outside such as dirt, debris, and other foreign materials that can both indirectly and directly contaminate food. Foodborne illness outbreaks have been linked to poor personal hygiene of workers involved in food processing and handling. Therefore, personal hygienic practices for employees are essential to the production of safe and wholesome meat and poultry products. The performance standard allows establishments to develop alternative or innovative means to ensure that employee practices do not result in insanitary conditions or adulteration of products.

All persons working in contact with products, food-contact surfaces, and product-packaging materials must adhere to hygienic practices while on duty to prevent adulteration of product and the creation of insanitary conditions.

The establishment has the responsibility to ensure that employees are engaging in personal hygienic practices and product-handling practices that prevent insanitary conditions and reduce the likelihood of contaminating product, food-contact surfaces, and product-packaging materials.

The hands can serve as a vehicle for transferring microorganisms to meat and poultry products, food-contact surfaces of equipment, and packaging materials. The hands can pick up microorganisms from such actions as touching utensils, equipment, raw product, product cartons, pallets, and door handles; using the toilet; eating; shaking hands; and touching the nose, mouth, or face. For example, many people have the pathogen *Staphylococcus aureus* in their nasal passages and can transfer these organisms to their hands by touching or blowing their nose.

Handwashing is a very important practice in a food-processing environment. Plant employees who handle any edible product should keep their hands clean. Insanitary personal practices like indiscriminate and uncovered coughing and sneezing over exposed product, and wiping discharges from the eyes, nose, or mouth on outer clothing could result in product contamination.

Employees using poor food handling techniques can contaminate product and food-contact surfaces with perspiration, hair, cosmetics, tobacco/snuff, chewing gum, and foreign objects like jewelry. Pieces of the jewelry such as the setting or stones or the whole item itself, like a wristwatch, might fall into the product being prepared. Some jewelry may have crevices or recesses that can collect product residue and hinder routine cleaning. As a result, the jewelry can act as a reservoir of microorganisms that can be transferred to the product. You may gather needed information by seeking the answer to the following question:

- Are the persons in contact with product, food-contact surfaces, and product-packaging materials adhering to hygienic practices?

The following list of observations may assist you in answering this question:

- Look for employees who do not wash their hands or change gloves after touching a contaminated surface (e.g., pallet; box; head, nose, mouth, or other areas of the body).
- Look for employees engaging in insanitary personal practices such as uncovered coughing over exposed product or wiping discharges from their nose or mouth on their outer clothing.
- Look for employees using their mouth to temporarily hold metal tag fasteners, shroud pins, cards, or other objects, and so on, that will be handled subsequently by the employee or contact product.
- Look for employees who have handled raw product or raw components, who do not wash and sanitize their hands prior to handling ready-to-eat products.

The plant may include hygienic handling procedures for preventing cross-contamination of ready-to-eat products in its SSOP. When the plant includes these procedures in the SSOP, management must implement and monitor such procedures and document the monitoring results, including any corrective actions taken on the SSOP records.

- Look for employees handling products or components who are wearing loose jewelry such as wristwatches and bracelets, and rings with settings or high crowns.
- Look for employees who do not wash their hands after using the toilet room, eating, or smoking prior to handling product or implements used in the preparation of product.
- Look for employees who are not preventing dislodged hair, perspiration, or cosmetics from either falling into or ending up in the product.
- Look for employees eating and using tobacco products in nondesignated areas of the plant.
- Look for employees who do not remove aprons, outer garments, scabbards, knives, steels, and so on, before using the toilet or urinal.

Access the information you gathered.

1. Observe the employee who places frozen blocks of beef trimmings into the hydroflaker pick up several empty beef trimming cartons off the floor (touching the outside surface of the carton with his hands) and put them in a trash bin.
2. Watch to see if the employee realizes he has touched an unclean surface. The employee makes no attempt to wash his hands and continues to perform the same work duties. At this point, there is noncompliance because an insanitary condition exists that could lead to direct product contamination.

However, if the employee eventually touches one of the frozen blocks of beef trimmings with his hands prior to washing them, product would be contaminated. One potential scenario is

> You see an employee leave the restroom without washing his hands. Careful consideration of the circumstances is required when it is not known for a fact that an employee's hands will serve as a vehicle for cross-contamination of product. In cases where an employee has engaged in a nonwork activity like using the toilet, smoking, or eating, further investigation or observation may be necessary before you conclude that an insanitary condition exists.

It is reasonable to conclude that the employee's hands touched an unclean surface when he used the toilet. However, if the employee does not return to the production room, then the employee's unclean hands do not create an insanitary condition that could result in contaminated product or food-contact surfaces. Likewise, if the employee washes his hands at a sink immediately upon entering the production room, then his hands were cleaned prior to handling product or touching a food-contact surface. But if the employee goes directly to his workstation and does not wash his hands, his poor personal hygienic practice creates an insanitary condition and there is a potential for contamination.

Examples of the failure to meet the requirements for this regulation are

- An employee handling exposed products with her bare hands is wearing nail polish.
- An employee wipes his runny nose on the sleeve of his smock.
- Two employees handling exposed products are not taking measures to prevent their hair from falling into the products.
- An employee adding pork trimmings into grinders is wearing a wristwatch.

42.2.9 Disease Control

Any person who has or appears to have an infectious disease, open lesion, including boils, sores, or infected wounds, or any other abnormal source of microbial contamination, must be excluded from any operations which could result in product adulteration and the creation of insanitary conditions until completely cured.

The establishment has the responsibility to ensure that no person affected with a disease in a communicable form, while a carrier of such disease, or while afflicted with boils, sores, infected wounds, or other abnormal sources of microorganism contamination, works in any area of the plant where there is a likelihood of disease transmission or meat, poultry, or food ingredients becoming contaminated, until the condition is corrected. Note the following:

1. A wide range of communicable diseases and infections may be transmitted by food handlers to other employees and consumers through contaminated meat and poultry products and careless handling practices. Foodborne pathogens may be shed from infected workers during the incubation period of a disease, before the affected person displays clinical symptoms.
2. Employees working in edible product areas exhibiting flu-like symptoms such as persistent sneezing or coughing can contaminate exposed product, food-contact surfaces (hands,

equipment, and utensils), and product packaging materials with discharges from the mouth and nose.

3. Skin infections, such as boils, open lesions, and infected cuts, frequently contain large numbers of streptococci or staphylococci organisms that can easily become food contaminants. These infections may be sources of contamination that can lead to foodborne illness.

Within reason, you should be able to recognize obvious symptoms and lesions like those mentioned above. Otherwise, seek professional help. You may gather needed information by seeking the answer to the following question.

- Are persons who appear to have an infectious disease; an open lesion, including boils, sores, or infected wounds; or any other abnormal source of microbial contamination excluded from any operations that could result in product adulteration and the creation of insanitary conditions?

The following observations may assist you in answering this question.

- Look for employees who appear to be exhibiting symptoms of an infectious disease or condition.
- Look for employees with an open lesion, including a boil, sore, infected wound, or any other abnormal source of microbial contamination on their hands or forearms.

Assess the information you gathered. Potential scenarios are as follows:

You observe an employee working in the inedible product department exhibiting flu-like symptoms such as persistent sneezing or coughing. The inedible product department is separate from areas used for edible products. The plant also has separate welfare facilities (toilet and dressing rooms, lunchrooms, etc.) for employees who work in the inedible product department. Since the employee's potential health condition does not present a risk of transmitting foodborne pathogens to edible product, edible food-contact surfaces, and edible product packaging materials, there is no insanitary condition.

You observe an employee handling raw edible product with a cut on her forefinger near the first knuckle. The cut has some redness around it but you are not sure that it is infected. The cut is not protected with an impermeable cover. Because of the potential of transferring microorganisms to product, there is noncompliance with the requirements.

If the cut was infected (pus pockets and fluid oozing), then product would have been contaminated. There is food safety hazard associated with the contamination because of the possibility of transferring the pathogen *Staphylococcus aureus* to the exposed product; therefore, it is a noncompliance.

Examples of the failure to meet the requirements for this regulation are:

- An employee who is handing exposed product has an uncovered sore on his bare forearm.
- An employee moving pallets of boxed product from the end of the production lines to the freezer appears to have a cold (exhibiting symptoms such as persistent coughing, sneezing, sniffling, etc.).

42.3 Concluding Remark

This chapter provides a brief discussion on sanitation practices and remedies as the platform on which the FSIS fulfills its regulatory obligations. The next chapter will present the ultimate regulatory goal of the FSIS to prevent hazards in meat and meat processing. This is known as Hazards Analysis and Critical Control Points (HACCP) system.

43

Hazard Analysis and Critical Control Point System[*]

Y. H. Hui

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43.1 Principles of HACCP

For more than two decades, the Food Safety Inspection Service (FSIS) has mandated the use of Hazard Analysis and Critical Control Point (HACCP) system or plan in an establishment that processes meat and poultry. Under this system, the establishment identifies in the entire process potential hazards, critical points where such hazards can be controlled, and preventive measures taken. Then, the establishment will monitor the production process and make corrective actions where necessary. Recordkeeping and verification completes the system. Obviously, compliance with these requirements can only be

[*] The information in this chapter has been modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyright 2011©. Used with permission.

accomplished through rigorous enforcement. This system or strategy is based on seven principles that are described below.

Please note that the remaining information (text, tables, and figures) in this chapter has been adapted from the legal document "U.S. Federal Register, Volume 61, Number 144, Pages 38805–38916, July 25, 1996."

43.1.1 Principle No. 1

A hazard analysis of each process must be carried out. The purpose of the analysis is to identify and list the food safety hazards reasonably likely to occur in the production process for a particular product and the preventive measures necessary to control the hazards. A food safety hazard is any biological, chemical, or physical property that may cause a food to be adulterated or otherwise unsafe for human consumption. A listed hazard must be of such a nature that its prevention, elimination, or reduction to acceptable levels is essential to the production of a safe food. This point in hazard analysis consists of asking a series of questions that are appropriate to each step in the flow diagram. The hazard analysis should question the effect of a variety of factors on the safety of the food. A checklist of questions to be used in the hazard analysis process is as follows:

Ingredients:

Does the food contain any sensitive ingredients that are likely to present microbiological hazards (e.g., *Salmonella Staphylococcus aureus*), chemical hazards (e.g., aflatoxin, antibiotic, or pesticide residues) or physical hazards (stones, glass, bone, and metal)?

Intrinsic factors of food:

1. Physical characteristics and composition (e.g., pH, type of acids, fermentable carbohydrates, water activity, and preservatives) of the food during and after preparation which can cause or prevent a hazard.
2. Which intrinsic factors of the food must be controlled in order to ensure food safety?
3. Does the food permit survival or multiplication of pathogens and/or toxin formation before or during preparation?
4. Will the food permit survival or multiplication of pathogens and/or toxin formation during subsequent steps of preparation, storage, or consumer possession?
5. Are there other similar products in the market place? What has been the safety record for these products?

Procedures used for preparation/processing:

1. Does the preparation procedure or process include a controllable step that destroys pathogens or their toxins? Consider both vegetative cells and spores.
2. Is the product subject to recontamination between the preparation step (e.g., cooking) and packaging?

Microbial content of the food:

1. Is the food commercially sterile (i.e., low-acid canned food)?
2. Is it likely that the food will contain viable sporeforming or nonsporeforming pathogens?
3. What is the normal microbial content of the food stored under proper conditions?
4. Does the microbial population change during the time the food is stored before consumption?
5. Does that change in microbial population alter the safety of the food?

Facility design:

1. Does the layout of the facility provide an adequate separation of raw materials from ready-to-eat foods?
2. Is positive air pressure maintained in product packaging areas? Is this essential for product safety?
3. Is the traffic pattern for people and moving equipment a potential source of contamination?

Equipment design:

1. Will the equipment provide the time/temperature control that is necessary to meet critical limits?
2. Is the equipment properly sized for the volume of food that will be prepared?
3. Can the equipment be controlled so that the variation in performance will be within the tolerances required to produce a safe food?
4. Is the equipment reliable or is it prone to frequent breakdowns?
5. Is the equipment designed so that it can be cleaned and sanitized?
6. Is product contamination with hazardous substances, for example, glass, likely to occur?
7. What product safety devices such as time/temperature integrators are used to enhance consumer safety?

Packaging:

1. Does the method of packaging affect the multiplication of microbial pathogens and/or the formation of toxins?
2. Is the packaging material resistant to damage, thereby preventing the entrance of microbial contamination?
3. Is the package clearly labeled "Keep Refrigerated" if this is required for safety?
4. Does the package include instructions for the safe handling and preparation of the food by the consumer?
5. Are tamper-evident-packaging features used?
6. Is each package legibly and accurately coded to indicate production lot?
7. Does each package contain the proper label?

Sanitation:

1. Can the sanitation practices that are employed impact upon the safety of the food that is being prepared?
2. Can the facility be cleaned and sanitized to permit the safe handling of foods?
3. Is it possible to provide sanitary conditions consistently and adequately to ensure safe foods?

Employee health, hygiene, and education:

1. Can employee health or personal hygiene practices impact the safety of the food being prepared?
2. Do the employees understand the food preparation process and the factors they must control to ensure safe foods?
3. Will the employees inform management of a problem, which could impact food safety?

Conditions of storage between packaging and the consumer:

1. What is the likelihood that the food will be improperly stored at the wrong temperature?
2. Would storage at improper temperature lead to a microbiologically unsafe food?

Intended use:

1. Will the food be heated by the consumer?
2. Will there likely be leftovers?

Intended consumer:

1. Is the food intended for the general public, that is, a population that does not have an increased risk of becoming ill?
2. Is the food intended for consumption by a population with increased susceptibility to illness (e.g., infants, the elderly, the infirm, and immunocompromised individuals)?

We can approach the questions of hazards from a general perspective such as:

1. What potential hazards may be present in the animals to be slaughtered or the raw materials to be processed?
2. What are the avenues that might lead to contamination of finished product with pathogenic microorganisms, hazardous chemicals, or other potentially hazardous contaminants?
3. What is the likelihood of such contamination and what are the means for preventing it?
4. Does the food contain any ingredient historically associated with a known microbiological hazard?
5. Does the food permit survival or multiplication of pathogens or toxin formation during processing?
6. Does the process include a controllable processing step that destroys pathogens?
7. Is it likely that the food will contain pathogens and are they likely to increase during the times and conditions under which the food is normally stored before being consumed?
8. What product safety devices are used to enhance consumer safety (e.g., metal detectors, filters, thermocouples)?
9. Does the method of packaging affect the multiplication of pathogenic microorganisms and/or the formation of toxins?
10. Is the product epidemiologically linked to a foodborne disease?

43.1.2 Principle No. 2

The critical control points (CCP) of each process must be identified. A CCP is a point, step, or procedure at which control can be applied and a food safety hazard can be prevented, eliminated, or reduced to an acceptable level. All hazards identified during the hazard analysis must be addressed. The information developed during the hazard analysis should enable the establishment to identify which steps in their processes are CCPs. Identification of CCPs for controlling microbial hazards throughout the production process is particularly important because these hazards are the primary cause of foodborne illness.

43.1.3 Principle No. 3

The critical limits for preventive measures associated with each identified CCP must be established. A critical limit is the maximum or minimum value to which a process parameter must be controlled at a CCP to prevent, eliminate, or reduce the identified physical, biological, or chemical food safety hazard to an acceptable level. Critical limits are most often based on process parameters such as temperature, time, physical dimensions, humidity, moisture level, water activity, pH, titratable acidity, salt concentration, available chlorine, viscosity, preservatives, or survival of target pathogens. Critical limits should be based on applicable FSIS regulations or guidelines, the Food and Drug Administration

(FDA) tolerances and action levels, scientific and technical literature, surveys, experimental studies, or the recommendations of recognized experts in the industry, academia, or trade associations.

Establishments are encouraged to establish critical limits more stringent than those now required by FSIS regulations or suggested by scientific data to ensure that regulatory requirements are routinely met, even when minor deviations occur.

43.1.4 Principle No. 4

The monitoring requirements for CCPs must be established. Monitoring is an integral part of HACCP and consists of observations or measurements taken to assess whether a CCP is within the established critical limit. Continuous monitoring is preferred, but when it is not feasible, monitoring frequencies must be sufficient to ensure that the CCP is under control. Assignment of the responsibility for monitoring is an important consideration for each CCP. Personnel assigned the monitoring activities should be properly trained to accurately record all results, including any deviations, so that immediate corrective actions may be taken.

43.1.5 Principle No. 5

The HACCP plan must include corrective action to be taken when monitoring indicates that there is a deviation from a critical limit at a CCP. Although the process of developing a HACCP plan emphasizes organized and preventive thinking about what is occurring as the meat or poultry product is being manufactured, the existence of a HACCP plan does not guarantee that problems will not arise. For this reason, the identification of a planned set of activities to address deviations is an important part of a HACCP plan. In such instances, corrective action plans must be in place to determine the disposition of the potentially unsafe or noncompliant product and to identify and correct the cause of the deviation. The HACCP plan itself might require modification, perhaps in the form of a new critical limit, or of an additional CCP.

43.1.6 Principle No. 6

Effective recordkeeping procedures that document the entire HACCP system must be developed and maintained. A HACCP system will not work unless consistent, reliable records are generated during the operation of the plan, and those records are maintained and available for review. One of the principal benefits of a HACCP process control system to both industry and regulatory officials is the availability of objective, relevant data.

43.1.7 Principle No. 7

HACCP systems must be systematically verified. After initial validation that the HACCP system can work correctly and effectively with respect to the hazards, the system must be verified periodically. Periodic verification involves the use of methods, procedures, or tests in addition to those used for monitoring, to determine whether the HACCP system is in compliance with the HACCP plan and/or whether the HACCP plan needs modification and revalidation to achieve its food safety objective. Four processes are involved in the verification of the establishment's HACCP system. The establishment is responsible for the first three; FSIS is responsible for the fourth:

1. The first is the scientific and technical process, known as "validation," for determining that the CCPs and associated critical limits are adequate and sufficient to control likely hazards.
2. The second process is to ensure, initially and on an ongoing basis, that the entire HACCP system functions properly.
3. The third consists of documented, periodic, reassessment of the HACCP plan.
4. The fourth process defines FSIS's responsibility for certain actions (Government verification) to ensure that the establishment's HACCP system is functioning adequately.

This chapter is not the proper forum to provide complete details on the implementation of the seven principles. We will confine this chapter to the following:

1. The hazards of meat and meat processing under Principle No. 1.
2. A summary of the application of all seven principles.

43.2 Hazards

Before we can implement the seven principles, the foundation of the system bases on Principle No. 1, safety hazards affecting meat and meat productions. The regulation defines a food safety hazard as: "Any biological, chemical, or physical property that may cause a food to be unsafe for human consumption." This section defines the hazards and discusses in general where they may occur in meat and poultry production. It will then talk about identifying hazards in your establishment. Finally, this section explains how you can apply preventive measures to the hazards you have identified, to ensure that the products are safe for consumers. A preventive measure is defined, in the regulation, as "Physical, chemical, or other means that can be used to control an identified food safety hazard."

To identify biological, chemical, or physical hazards that are likely to occur, you need to know about the chemical, physical, and microbiological characteristics of meat, poultry, and other ingredients, as well as how various processes affect those characteristics. You also need to understand the interactions among ingredients. You need to evaluate each step in the process flow diagram to determine whether a biological, chemical, and/or physical hazard may be introduced at that step and whether preventive measures are available (see Tables 43.1–43.7).

43.2.1 Biological Hazards

Biological hazards are living organisms including microorganisms that can put human health at risk. Biological hazards include bacteria, parasites, protozoa, viruses, and the like.

Agricultural products and food animals carry a wide range of bacteria. From a public health standpoint, most bacteria are harmless. Others—the pathogenic microorganisms—can cause illness or even death in humans. The numbers and types of bacteria vary from one food or animal species to another, from one geographic region to another, and with production and slaughter or harvesting methods. During production, processing, packaging, transportation, preparation, storage, and service, any food may be exposed to bacterial contamination. The most common biological hazards in meat and poultry are microbiological.

Biological hazards, which are mainly bacterial, can cause either foodborne infections or intoxications. A foodborne infection is caused by a person ingesting a number of pathogenic microorganisms sufficient to cause infection as a result of their multiplication, for example, salmonellosis. A foodborne intoxication is caused by the ingestion of already formed toxins produced by some bacteria when they multiply in food, for example, staphylococcal enterotoxin.

Nine major pathogenic bacterial organisms that can cause foodborne illness from eating meat or poultry are *Salmonella, Clostridium perfringens, Listeria monocytogenes, Staphylococcus aureus, Campylobacter jejuni, Yersinia enterocolitica, Bacillus cereus, Clostridium botulinum*, and *Escherichia coli* O157:H7. Table 43.1 describes the characteristics of growth for nine pathogens associated with meat and poultry products. Table 43.2 describes examples of preventive measures for biological hazards. To thoroughly identify significant biological hazards in your establishment, you need to evaluate each specific ingredient and processing step in your operation. The following identifies and discusses the nine pathogenic microorganisms of concern.

1. *Bacillus cereus* *B. cereus* foodborne intoxication includes two recognized types of illness—diarrheal and emetic (vomiting). Foods associated with illness include boiled and fried rice, custards, cecal products meats, vegetables, and fish; food mixtures such as sauces, puddings, soups, casseroles, pastries, and salads.

TABLE 43.1

Characteristics of Growth for Nine Pathogens Associated with Meat and Poultry Products

Pathogens	Temperature of Growth (°C)	pH	Minimum A_w
Bacillus cereus	10–48	4.9–9.3	0.95
Campylobacter jejuni	30–47	6.5–7.5	
Clostridium botulinum (Types A,B,E)	3.3–46	>4.6	0.94
Clostridium perfringens	15–50	5.5–8.0	0.95
Escherichia coli O157:H7	10–42	4.5–9.0	
Listeria monocytogenes	2.5–44	5.2–9.6	
Salmonella	5–46	4–9	0.94
Staphylococcus aureus	6.5–46	5.2–9	0.86
Yersinis enterocolitica	2–45	4.6–9.6	

2. *Campylobacter jejuni* Campylobacteriosis is the illness caused by *C. jejuni*. It is also often known as campylobacter enteritis or gastroenteritis. Foods associated with illness include raw and undercooked chicken, raw milk, nonchlorinated water.

3. *Clostridium botulinum* Foodborne botulism (as distinct from wound botulism and infant botulism) is a severe foodborne disease caused by the ingestion of foods containing the potent neurotoxin formed during the growth of the organism. Botulism has a high mortality rate if not treated immediately and properly. Foods associated with disease include sausages, meat products, and seafood products, improperly canned foods, and vegetable products.

4. *Clostridium perfringens* Perfringens foodborne illness is the term used to describe the common foodborne disease caused by the release of enterotoxin during sporulation of *C. perfringens* in the gut. Foods associated with illness include meat and poultry products and gravy.

TABLE 43.2

Examples of Preventive Measures for Biological Hazards

Pathogen	Preventive Measure or Control
Bacillus cereus	Proper holding and cooling temperatures of foods; thermal processing of shelf-stable canned food.
Campylobacter jejuni	Proper pasteurization or cooking; avoiding cross-contamination of utensils, equipment; freezing; atmospheric packaging.
Clostridium botulinum	Thermal processing of shelf-stable canned food; addition of nitrite and salt to cured-processed meats; refrigeration of perishable vacuum-packaged meats; acidification below pH 4.6; reduction of moisture below water activity of 0.93.
Clostridium perfringens	Proper holding and cooling temperatures of foods; proper cooking times and temperatures; adequate cooking and avoidance of cross-contamination by unsanitary equipment or infected food handlers.
Listeria monocytogenes	Proper heat treatments; rigid environmental sanitation program; separation of raw and ready-to-eat production areas and product.
Salmonella spp.	Proper heat treatment; separation of raw and cooked product; proper employee hygiene; fermentation controls; decreased water activity; withdrawing feed from animals before slaughter; avoiding exterior of hide from contacting carcass during skinning; antimicrobial rinses; scalding procedures; disinfecting knives.
Staphylococcus aureus	Employee hygiene; proper fermentation and pH control; proper heat treatment and postprocess product-handling practices; reduced water activity.
Yersinia enterocolitica	Proper refrigeration; heat treatments; control of salt and acidity; prevention of cross-contamination.

5. *Escherichia coli O157:H7* Hemorrhagic colitis is the acute disease caused by *E. coli* O157:H7. Foods associated with illness: undercooked or raw hamburger (ground beef) has been implicated in many documented outbreaks and in other sporadic cases; other meat products, raw milk, untreated water.

6. *Listeria monocytogenes* Listeriosis is the general group of disorders caused by *L. monocytogenes.* Foods associated with illness include cole slaw, cooked poultry, cooked meat, and raw milk, supposedly pasteurized fluid milk, cheeses (particularly soft-ripened varieties). Its ability to grow at temperatures as low as 3°C permits multiplication in refrigerated foods.

7. *Salmonella* spp. *S. typhi* and the paratyphoid bacteria are normally septicemic and produce typhoid or typhoid-like fever in humans and are pathogenic only for humans. Other forms of salmonellosis generally produce milder symptoms. The organism is found in the intestinal tracts of warm-blooded animals. Foods associated with illness include raw and cooked meats, poultry, eggs (and exterior of egg shells), untreated water, raw milk and dairy products, fish, shrimp, frog legs, yeast, sauces and salad dressing, etc.

8. *Staphylococcus aureus* Staphylococcal food poisoning (staphylococcal enterotoxicosis; staphylococcal enterotoxemia) is the condition caused by the enterotoxins that some strains of *S. aureus* produce. Foods associated with illness: meat and meat products; poultry and egg products; egg, tuna, ham, chicken, potato, and macaroni salads; sandwich fillings; milk and dairy products, etc.

9. *Yersinia enterocolitica* Yersiniosis is the disease caused by pathogenic species in the genus *Yersinia*. The disease is a gastroenteritis with diarrhea and/or vomiting, and fever and abdominal pain. Foods associated with illness include meats, oysters, fish, milk, and chitterlings. Zoonotic agents are biological hazards that cause disease in animals and can be transmitted and cause disease in humans. The following lists some zoonotic hazards:

1. *Trichinella spiralis* is a nematode parasite whose larval from encysts primarily in the striated muscle of pigs, horses, rats, bears, and other mammals. Infection in humans results in "flu-like symptoms" (diarrhea, fever, stiffness, muscle pain, respiratory distress, etc.) And heavy infection may lead to death. Foods associated with illness include raw and undercooked pork, bear, and equine meat.
2. *Taenia saginata* is a human tapeworm whose larval form (*Cysticercus bovis*) encysts in the tissues of cattle. Foods associated with illness include raw or undercooked beef.
3. *Taenia solium* is a human tapeworm whose larval form (*Cystricercus cellulosae*) encysts in the tissues of pigs, dogs, and humans. Cysts in humans are most common in the subcutaneous tissues, eye, and the brain. Foods associated with illness include raw or undercooked pork.
4. *Toxoplasma gondii* is a protozoan parasite that encysts in the tissues of a variety of mammalian hosts including pigs. Human infection may result in "flu-like" symptoms in adults, late-term abortions in pregnant women, or serious congenial infections in children. Foods associated with illness include raw or undercooked pork.
5. *Balantidium coli* is a protozoal organism. Foods associated with illness include raw, undercooked pork (fecal contamination).
6. *Cryptosporidium* spp. Foods associated with illness include inadequately treated water and raw or undercooked veal or beef.

43.2.2 Chemical Hazards

While biological hazards are of great concern because contaminated foods can cause widespread illness outbreaks, chemical hazards may also cause foodborne illnesses, although generally affecting fewer people. Chemical hazards may also cause foodborne illnesses. Chemical hazards fall into two categories:

1. Naturally occurring poisons or deleterious substances are those that are natural constituents of foods and are not the result of environmental, agricultural, industrial, or other contamination. Examples include aflatoxins, mycotoxins, and shellfish toxins.

2. Added poisonous or deleterious substances are those which are intentionally or unintentionally added to foods at some point in growing, harvesting, storage, processing, packing, or distribution. This group of chemicals includes pesticides, fungicides, insecticides, fertilizers, and antibiotics, as well as direct and indirect food additives. This group also includes chemicals such as lubricants, cleaners, paints, and coatings.

Based on these two categories, chemical hazards can originate from five general sources:

1. Agriculture chemicals: pesticides, herbicides, animal drugs, fertilizers, etc.
2. Plant chemicals: cleaners, sanitizers, oils, lubricants, paints, pesticides, etc.
3. Naturally occurring toxicants: products of plant, animal, or microbial metabolisms such as aflatoxins, etc.
4. Food chemicals: preservatives, acids, food additives, sulfiting agents, processing aids, etc.
5. Environmental contaminants: lead, cadmium, mercury, arsenic, polychlorinated biphenyls (PCBs).

Table 43.3 describes the types of chemical hazards. To identify any chemical hazards, you first need to identify any chemical residues that might be in the animal. To do this, think about the following:

1. The types of drugs and pesticides routinely used in raising the animals which are the source of your meat and poultry ingredients.
2. Feeds and supplements fed to the animals.
3. Environmental contaminants that the animals may have come into contact with. This includes both naturally occurring contaminants and added contaminants.
   - Pesticides used on plants that may end up as residues in the animal.
   - The source of the water the animals were allowed to drink. You can use the following preventive measures to help ensure that animals entering your establishment are free of harmful residues.
   - Require that the animals have been raised in conjunction with the January 1994 FDA Compliance Policy Guidelines.
   - Require written assurances from suppliers for each lot of animals, stating that the animals are free of illegal residues.
   - Set your own maximum allowable residue limits for specific drugs, pesticides, and environmental contaminants in animal urine or tissues as targets to ensure that FDA and environmental protection agency (EPA) tolerances are met.
   - Ensure that trucks used to ship the animals do not have chemical hazards that could contaminate the animals.

TABLE 43.3

Types of Chemical Hazards

Location	Hazard
Raw materials	Pesticides, antibiotics, hormones, toxins, fertilizers, fungicides, heavy metals, PCBs. Color additives, inks, indirect additives, packaging materials.
Processing	Direct food additives—preservatives (nitrite), flavor enhancers, color additives. Indirect food additives—boiler water additives, peeling aids, defoaming agents.
Building and equipment maintenance	Lubricants, paints, coatings.
Sanitation	Pesticides, cleaners, sanitizers.
Storage and shipping	All types of chemicals, cross contamination.

For many years, the FSIS has conducted a National Residue Program to monitor the occurrence of residues from hazardous chemicals in meat and poultry products. Under a HACCP regime, frontline responsibility for control of residues from animal drugs or environmental contaminants will move from the government to the industry, although the agency will continue to verify that these controls and preventive measures are effective. Companies that slaughter livestock and poultry will probably find the FSIS National Residue Program Plan to be a useful document. The plan contains lists of compounds that might leave residues in the tissues of animals or birds, and provides some information on their relative risk through the rankings in the Compound Evaluation System. It provides information on the compounds that FSIS has included in its annual testing program. It also provides information on the methods that are used to test for the compounds. Another FSIS document, the Domestic Residue Data Book, presents the results of FSIS testing. These data can help a HACCP team understand the overall hazard presented by various residues, although each company should gather information about the residue control performance of its own suppliers.

Most establishments use chemicals during processing and to keep their operations sanitary. Yet you need to be aware that chemical hazards can occur at any of the following points:

- Prior to receiving chemicals at your establishment.
- Upon receiving chemicals.
- At any point where a chemical is used during processing.
- During storage of chemicals.
- During the use of any cleaning agents, sanitizers, lubricants, or other maintenance chemicals.
- Prior to shipment of the finished product.
- In trucks used to ship finished product.

Some of the measures you can use to prevent chemical hazards are:

- Use only approved chemicals.
- Have detailed product specifications for chemicals entering your plant.
- Maintain letters of guarantee from suppliers.
- Inspect trucks used to ship finished products.
- Properly label and store all chemicals.
- Properly train employees who handle chemicals.

Table 43.4 describes some examples of preventive measures for chemical hazards.

TABLE 43.4

Examples of Preventive Measures for Chemical Hazards

Hazard	Preventive Measure
Naturally occurring substances	Supplier warranty or guarantee; verification program to test each supplier's compliance with the warranty or guarantee.
Added hazardous chemicals	Detailed specifications for each raw material and ingredient; warranty or letter of guarantee from the supplier; visiting suppliers; requirement that supplier operates with a HACCP plan; testing program to verify that carcasses do not have residues.
In-process chemicals	Identify and list all direct and indirect food additives and color additives; check that each chemical is approved; check that each chemical is properly used; record the use of any restricted ingredients.

43.2.3 Physical Hazards

A physical hazard is any physical material not normally found in a food which causes illness or injury to the individual using the product. Physical hazards include a variety of foreign materials or objects, such as glass, metal, and plastic. However, foreign objects that cannot cause illness or injury are not hazards, even though they may not be esthetically pleasing to your customers. A number of situations can result in physical hazards in finished products. They include, but are not limited to:

- Contaminated raw materials.
- Poorly designed or poorly maintained facilities and equipment. An example would be rust particles and paint chips falling from overhead structures onto exposed product.
- Improper procedures or improper employee training and practices. For example, by using the wrong cutting technique during the cut-up/prefabrication process, employees could cut off and leave pieces of their rubber gloves in the product.

Measures you can take to prevent physical hazards include, but are not limited to

- Make sure your plant specifications for building design and operation are accurate and updated regularly.
- Make sure your letters of guarantee for ingredients and product supplies are accurate and updated regularly.
- Perform random visual examinations of incoming product and materials.
- Use magnets and metal detectors to help find metal fragments that would be a physical hazard.
- Use stone traps and bone separators to remove these potential physical hazards.
- Keep equipment well maintained.
- Train employees to identify potential problems.

Table 43.5 describes types of physical hazards, and Table 43.6 provides examples of preventive measures for physical hazards.

Table 43.7 describes some examples of regulatory limits for biological, chemical, and physical hazards.

43.2.4 Controls and Critical Limits for Biological, Chemical, and Physical Hazards

When all significant biological, chemical, and physical hazards are identified along with their points of occurrence, the next task is to identify measures to prevent the hazards from compromising the safety of the finished product.

Preventive measures or controls can be defined as physical, chemical, or other factors that can be used to remove or limit an identified hazard. When considering preventive measures or controls, a limit must be established—this is the criterion that must be met to ensure safety. For example, proper heat treatment will control some pathogenic bacteria, and it is thus crucial to know what time–temperature combinations

TABLE 43.5

Types of Physical Hazards

Hazard	Source or Cause
Glass	Bottles, jars, light fixtures, utensils, gauge covers, thermometers.
Metal	Nuts, bolts, screws, steel wool, wire, meat hooks.
Stones	Raw materials.
Plastics	Packaging materials, raw materials.
Bone	Raw material, improper plant processing.
Bullet/BB shot/needles	Animals shot in field, hypodermic needles used for infections.
Jewelry	Pens/pencils, buttons, careless employee practices.

TABLE 43.6

Examples of Preventive Measures for Physical Hazards

Hazard	Preventive Measure
Foreign objects in raw materials	Supplier's HACCP plan; use of specifications, letters of guarantee; vendor inspections and certification; in-line magnets; screens, traps, and filters; in-house inspections of raw materials.
Foreign objects in packaging materials, cleaning compounds, etc.	Supplier's HACCP plan; use of specifications, letters of guarantee; vendor inspections and certification; in-house inspections of materials.
Foreign objects introduced by processing operations or employee practices.	In-line metal detectors; visual product examinations; proper maintenance of equipment; frequent equipment inspections.

constitute proper heat treatment for various products; these time–temperature combinations are the critical limits. Another example of a preventive measure for a biological hazard is the chlorination of poultry chiller water to prevent cross-contamination of carcasses with *Salmonella*.

With identified physical hazards, the most common preventive measures may be visual examinations of the product or the use of a metal detector. Chemical hazards associated with raw materials may be controlled through detailed product specifications, letters of guarantee, or purchase specifications.

43.2.5 Specific Risk Materials

In addition to all the regulations, the FSIS has issued special requirement concerning certain Specific Risk Materials or SRM from cattle and their handling and disposition.

The following materials from cattle are SRM, except when they are from cattle from a country that can demonstrate that its bovine spongiform encephalopathy (BSE) risk status can reasonably be expected to provide the same level of protection from human exposure to the BSE agent as prohibiting SRM for use as human food does in the United States:

> The brain, skull, eyes, trigeminal ganglia, spinal cord, vertebral column (excluding the vertebrae of the tail, the transverse processes of the thoracic and lumbar vertebrae, and the wings of the

TABLE 43.7

Some Examples of Regulatory Limits

Hazard	Regulatory Limit	Regulatory Citation
Biological: Microbial growth due to temperature abuse-poultry chilling.	All poultry must be chilled immediately after processing to a temperature of 40°F or less.	§381.66
Chemical: Excess chemicals contact product	Chemicals used are approved for the intended use and at appropriate amounts.	§318.7
Chemical: Chemical hazard from packaging materials	Edible products must be packaged in container that will not adulterate product or be injurious to health. Packaging materials must be covered by a letter of guaranty.	§317.24
Biological: Trichinae in pork	Products containing pork muscle tissue must be effectively heated, refrigerated, or cured to destroy any possible live trichinae.	§318.10
Biological: Pathogens in ready to eat products	For destruction of pathogens that may survive a dry heat process. One of the time/temperature combinations for *cooked* beef, *roast* beef, and *cooked* corned beef; e.g., 143°F\61.7°C minimum temperature at minimum time of 6 min.	§318.17
Physical: Extraneous material found on post chill examination of poultry carcasses.	Sampled carcasses observed for conformance with postchill criteria, including unidentified foreign material.	§381.76

sacrum), and dorsal root ganglia from cattle 30 months of age and older and the distal ileum of the small intestine and the tonsils from all cattle.

SRM are inedible and prohibited for use as human food. SRM must be removed from the carcasses of cattle, segregated from edible materials, and disposed of in accordance with regulations. The spinal cord from cattle 30 months of age and older must be removed from the carcass at the establishment where the animal was slaughtered.

43.2.5.1 Use of the Small Intestine for Human Food

The small intestine from all cattle may be used for human food if:

1. It is derived from cattle that were inspected and passed in an official establishment in the United States or in a certified foreign establishment in a country listed as eligible to export meat and meat products to the United States.
2. The distal ileum is removed by a procedure that removes at least 80 in. of the uncoiled and trimmed small intestine as measured from the cecocolic junction and progressing proximally toward the jejunum or by a procedure that the establishment demonstrates is effective in ensuring complete removal of the distal ileum.
3. If the conditions are not met, the entire small intestine must be removed from the carcass, segregated from edible materials, and disposed of in accordance with regulations.

The requirements do not apply to materials from cattle from countries that can demonstrate that their BSE risk status can reasonably be expected to provide the same level of protection from human exposure to the BSE agent as prohibiting SRM for use as human food does in the United States.

43.2.5.2 SRM: Removal, Segregation, and Disposition

Procedures for the removal, segregation, and disposition of SRM are

1. Establishments that slaughter cattle and establishments that process the carcasses or parts of cattle must develop, implement, and maintain written procedures for the removal, segregation, and disposition of specified risk materials. These procedures must address potential contamination of edible materials with SRM before, during, and after entry into the establishment. Establishments must incorporate their procedures for the removal, segregation, and disposition of SRM into their HACCP plans and/or other prerequisite programs.
2. Establishments that slaughter cattle and establishments that process the carcasses or parts of cattle must take appropriate corrective action when either the establishment or FSIS determines that the establishment's procedures for the removal, segregation, and disposition of SRM, or the implementation or maintenance of these procedures, have failed to ensure that SRM are adequately and effectively removed from the carcasses of cattle, segregated from edible materials, and disposed of in accordance with regulations.
3. Establishments that slaughter cattle and establishments that process the carcasses or parts of cattle must routinely evaluate the effectiveness of their procedures for the removal, segregation, and disposition of SRM in preventing the use of these materials for human food and must revise the procedures as necessary whenever any changes occur that could affect the removal, segregation, and disposition of specified risk materials.
4. Recordkeeping requirements are:
   a. Establishments that slaughter cattle and establishments that process the carcasses or parts of cattle must maintain daily records sufficient to document the implementation and monitoring of the procedures for the removal, segregation, and disposition of the materials and any corrective actions taken.

b. Records may be maintained on computers, provided that the establishment implements appropriate controls to ensure the integrity of the electronic data.
c. Records must be retained for at least 1 year and must be accessible to FSIS. All such records must be maintained at the official establishment for 48 h following completion, after which they may be maintained off-site, provided such records can be made available to FSIS within 24 h of request.

43.2.5.3 Equipment for Cutting Specified Risk Materials

If an establishment that slaughters cattle, or that processes the carcasses or parts from cattle, does not segregate the carcasses and parts from cattle 30 months of age and older from the carcasses and parts from cattle younger than 30 months during processing operations, it must

1. Use dedicated equipment to cut through specified risk materials.
2. Clean and sanitize equipment used to cut through SRM before the equipment is used on carcasses or parts from cattle younger than 30 months of age.

If an establishments that slaughters cattle, or that process the carcasses or parts from cattle, segregates the carcasses and parts of cattle 30 months of age and older from cattle younger than 30 months of age during processing operations, and processes the carcasses or parts from the cattle younger than 30 months first, it may use routine operational sanitation procedures on equipment used to cut through SRM.

43.2.5.4 Shipping SRM

Slaughter establishments may ship beef carcasses or parts that contain vertebral columns from cattle 30 months of age and older to another federally inspected establishment for further processing if the establishment shipping these materials:

1. Maintains control of the carcasses or parts while they are in transit or ensures that the carcasses or parts move under FSIS control.
2. Ensures that the carcasses or parts are accompanied by documentation that clearly states that the carcasses or parts contain vertebral columns from cattle that were 30 months of age and older at the time of slaughter.
3. Maintains records that identify the official establishment that received the carcasses or parts.
4. Maintains records that verify that the official establishment that received the carcasses or parts removed the portions of the vertebral column designated as SRM and disposed of them in accordance with regulations.

43.2.6 Red Meat (Beef) Slaughter Hazards

Table 43.8 presents examples in the slaughter of beef for three criteria:

1. Processing steps
2. Biological, chemical, or physical hazards
3. Control or preventive measures

In the first column of Table 43.8 each processing step is shown, you will find an “X” in the next three columns to tell you if there is a biological hazard in column 2, a chemical hazard in column 3, or a physical hazard in column 4. Column 5 describes the hazard(s), and the last column lists some relevant

TABLE 43.8

Red Meat Slaughter: Beef

Red Meat Slaughter-Beef: Examples of Processing Steps	B	C	P	Description of Biological, Chemical, or Physical Hazards for the Process Steps	Controls or Preventive Measures
Receiving AND holding		X		Residues present in edible tissues above tolerances.	Residue certification presented for live animal(s).
Skinning	X			Micro contamination of carcass surface due to contaminated outside hide surface—contamination of carcass from floor—cross-contamination.	Skinning procedures are accomplished without hair or visible fecal contamination of the carcass—Careful employee practices—Udder and puzzle removal are accomplished without contamination of edible product.
Evisceration	X			Cross-contamination from broken viscera.	Esophagus is tied to prevent escape of stomach contents—Bung is dropped with sanitized knife and bagged to prevent escape of feces—Viscera are removed intact.
Final wash	X			Growth of pathogens through insufficient wash.	Final wash: Temperature: 90–100°F Pressure: 345–2070 kPa (50–300 psi)—Steam Pasteurization: Temperature: 195°F or greater at surface Dwell time: 5–15 s in cabinet.
Chilling	X			Growth of pathogens.	Surface temperature ≤40°F as soon as possible—Carcasses spaced a minimum of 1 in. apart.
Receiving-packaging materials and nonbeef supplies		X		Contamination from deleterious chemicals present in the packaging materials.	Letters of guarantee on file for all packaging materials/nonpoultry supplies used by the establishment.
Storage-nonbeef supplies			X	Contamination of stored packing materials/supplies from foreign material.	Examine to ensure no visible foreign material on/in nonpoultry supplies or packaging materials.

controls or preventive measures. Table 43.8 should be used in conjunction with the process flow diagram developed by your HACCP team for your plant's beef slaughter process.

43.2.7 Red Meat (Swine) Slaughter Hazards

Table 43.9 presents examples in the slaughter of swine for three criteria:

1. Processing steps
2. Biological, chemical, or physical hazards
3. Control or preventive measures

In each processing step shown in the first column in Table 43.9, you will find an "X" in the next three columns to tell you if there is a biological hazard in column 2, a chemical hazard in column 3, or a physical hazard in column 4. Column 5 describes the hazard(s), and the last column lists some relevant controls or preventive measures. Table 43.9 should be used in conjunction with the process flow diagram developed by your HACCP team for your plant's swine slaughter process.

TABLE 43.9

Red Meat Slaughter: Swine

Red Meat Slaughter-Swine: Examples of Processing Steps	B	C	P	Description of Biological, Chemical, or Physical Hazards for the Process Steps	Controls or Preventive Measures
Scalding	X		X	Contamination from scalding medium.	Plant time/temperature limits for scalding (e.g., although it may vary with facilities, a temperature of 138–140°F is usually satisfactory).
					Carcasses should remain in scalding tanks long enough to loosen hair (excessive time or temperature results in carcass cooking).
		X		Contamination with chemicals.	USDA-FDA approved chemical concentration not to exceed manufacturer's recommendations.
Dehairing	X			Contamination and growth of microorganisms due to breaking of the skin from overexposure to the dehairer.	Time/temperature determined by plant-specific testing results to remove visible hair to an acceptable level without breaking skin.
Evisceration	X			Cross contamination from equipment/utensils.	Remove all viscera intact.
				Contamination from stomach, intestines, and/or bladder contents.	Contaminated equipment will be clean and sanitized before being used again.
				Contamination from employee handling.	Training program for all employees, to include personal hygiene, product handling procedures, and sanitary dressing procedures.
Trimming	X			Stick wound has not been removed.	Remove all visible stick-wound related defects.
Chilling	X			Growth of pathogens.	Cool surface temperature to 40°C as soon as possible.
Receiving-packaging materials and nonswine supplies		X		Contamination from deleterious chemicals present in the packaging materials.	Letters of guarantee are on file for all packaging materials/nonpoultry supplies used by the establishment.
Storage-nonswine supplies			X	Contamination of stored packing materials/supplies from foreign material.	Examine to ensure no visible foreign material on/in nonpoultry supplies or packaging materials.

43.2.8 Ingredients Hazards

Refer to Table 43.10 for ingredient hazards and ingredient-related hazards. Table 43.10 contains an alphabetical list of ingredients commonly used in making meat and poultry products. For each entry, you will find the name of the ingredient in the first column, and an "X" in the next three columns to tell you if there is a biological hazard in column 3, or a physical hazard in column 4. Column 5 describes the hazard(s), and the last column lists some relevant controls or preventive measures. Table 43.10 should be used in conjunction with the list of ingredients developed by your HACCP team for the products produced by the process under consideration. The HACCP team may find that a particular ingredient does not present the hazard identified in Table 43.10. The presence or absence of a hazard can be influenced by the ingredient source and company. Also, ingredient specifications, provided by the supplier to the

TABLE 43.10

Ingredient Hazards

Examples of Ingredient	B	C	P	Description of Biological, Chemical, or Physical Hazard for the Ingredient	Controls or Preventive Measures
Acidifiers		X		Toxicological effects if limits are exceeded.	Ingredients purchased under a letter of guarantee. Ingredients purchased based on producer/provider ingredient specifications.
Anticoagulants		X		Toxicological effect if limits are exceeded.	Ingredients purchased under a letter of guarantee. Ingredients purchased based on producer/provider ingredient specifications.
Antifoaming agents		X		Toxicological effect if limits are exceeded.	Ingredients purchased under a letter of guarantee. Ingredients purchased based on producer/provider ingredient specifications.
Antioxidants		X		Toxicological effect if limits are exceeded.	Ingredients purchased under a Letter of guarantee. Ingredients purchased based on producer/provider ingredient specifications.
Batter/breading	X		X	Growth of pathogens due to improper storage and handling.	Temperature controls for use.
				Foreign material.	Ingredient specification sheet identifying the required parameters the ingredient must meet. Where applicable, ingredients must be pathogen-free.
Beef (fresh, frozen)	X			Growth of pathogens due to improper storage and handling.	Product temperature must be 40°F or less at receiving. Product must meet establishment purchase specifications. Product must be produced under a HACCP plan.
Binders/extenders		X	X	Foreign material.	Ingredients purchased under a letter of guarantee. Ingredients purchased based on producer/provider ingredient specifications.
Bleaching agents		X		Toxicological effect if limits exceeded.	Ingredients purchased under a letter of guarantee. Ingredients purchased based on producer/provider ingredient specifications.
Blood	X			Growth of pathogens from improper handling and storage.	Ingredient specification sheet identifying the required parameters the ingredient must meet. Where applicable, ingredients must be pathogen-free. Meet appropriate temp.
Boneless beef	X		X	Growth of pathogens due to improper handling and storage.	Product temperature must be 40°F or less at receiving.
				Foreign particle contamination, e.g., metal fragments or bone.	Product must meet establishment purchase specifications.

continued

TABLE 43.10 (continued)

Ingredient Hazards

Examples of Ingredient	B	C	P	Description of Biological, Chemical, or Physical Hazard for the Ingredient	Controls or Preventive Measures
					Product must be produced under a HACCP plan.
					Visual examination of product for foreign materials.
Cooked beef	X		X	Growth of pathogens due to improper handling and storage.	Receiving temperature of product must be frozen or refrigerated at 40°F or below.
				Foreign particle contamination, e.g., metal fragments or bone particles in boneless beef.	Product must be received from an approved supplier who produces the product under a HACCP plan.
					Visual examination of product for foreign materials upon receipt.
Cooked poultry	X		X	Growth of pathogens due to improper handling and storage.	Receiving temperature of product must be frozen or refrigerated at 40°F or below.
				Foreign particle contamination, e.g., bone particles in boneless poultry.	Product must be received from an approved supplier who produces the product under a HACCP plan.
					Product must be organoleptically acceptable at receipt.
Cooked pork	X		X	Growth of pathogens due to improper handling and storage.	Receiving temperature of product must be frozen or refrigerated at 40°F or below.
				Foreign particle contamination, e.g., bone particles in boneless pork.	Product must be received from an approved supplier who produces the product under a HACCP plan.
					Product must be organoleptically acceptable at receipt.
Coloring agents (natural)		X		Toxicological effect if limits exceeded.	Ingredients purchased under a letter of guarantee.
					Ingredients purchased based on producer/provider ingredient specifications.
Coloring agents (artificial)		X		Toxicological effect if limits exceeded.	Ingredients purchased under a letter of guarantee.
					Ingredients purchased based on producer/provider ingredient specifications.
Curing agents		X		Toxicological effect if limits exceeded.	Ingredients purchased under a letter of guarantee.
					Ingredients purchased based on producer/provider ingredient specifications.
Curing accelerators		X		Toxicological effect if limits are exceeded.	Ingredients purchased under a letter of guarantee.
					Ingredients purchased based on producer/provider ingredient specifications.
Dairy products	X		X	Growth of pathogens due to improper handling and storage.	Temperature control.
				Foreign material.	Ingredient specification sheet identifying the required parameters the ingredient must meet.

TABLE 43.10 (continued)

Ingredient Hazards

Examples of Ingredient	B	C	P	Description of Biological, Chemical, or Physical Hazard for the Ingredient	Controls or Preventive Measures
					Where applicable, ingredients must be pathogen-free.
Eggs or egg products	X		X	Growth of pathogens due to improper handling and storage.	Temperature control.
				Foreign particle contamination, e.g., shell particles in broken eggs.	Ingredient specification sheet identifying the required parameters the ingredient must meet.
					Where applicable, ingredients must be pathogen-free.
Emulsifying agents		X		Toxicological effects if limits exceeded.	Ingredients purchased under a letter of guarantee.
					Ingredients purchased based on producer/provider ingredient specifications.
Flavoring agents		X		Toxicological effects if limits exceeded.	Ingredients purchased under a letter of guarantee.
					Ingredients purchased based on producer/provider ingredient specifications.
Fruits		X	X	Contamination from agricultural chemicals.	Ingredient specification sheet identifying the required parameters the ingredient must meet.
				Foreign material.	
Honey	X		X	Contamination from inherent microorganisms.	Ingredient specification sheet identifying the required parameters the ingredient must meet.
				Foreign particle contamination, e.g., dirt, insect parts.	
Legumes (dry)			X	Foreign particle contamination, e.g., rocks.	Ingredient specification sheet identifying the required parameters the ingredient must meet.
Mechanically deboned product	X		X	Growth of pathogens due to improper handling and storage.	Product temperature must be 40°F or less at receiving.
				Foreign particle contamination, e.g., bone particles.	Product must meet establishment purchase specifications.
					Product must be produced under a HACCP plan.
Mold inhibitors		X		Toxicological effect if improper amounts used.	Ingredient specification sheet identifying the required parameters the ingredient must meet.
Mushrooms	X	X	X	Contamination from inherent microorganisms.	Ingredient specification sheet identifying the required parameters the ingredient must meet.
				Contamination from agricultural chemicals.	Where applicable, ingredients must be pathogen-free.
				Foreign material.	
Nuts	X	X	X	Contamination from inherent microorganisms.	Ingredient specification sheet identifying the required parameters the ingredient must meet.
				Contamination from agricultural chemicals.	
				Foreign particle contamination, e.g., broken shells.	

continued

TABLE 43.10 (continued)

Ingredient Hazards

Examples of Ingredient	B	C	P	Description of Biological, Chemical, or Physical Hazard for the Ingredient	Controls or Preventive Measures
Packaging materials			X	Toxicological effects.	Use only FDA-approved packaging materials. Each lot of packaging material must be accompanied by a letter of guarantee in which the manufacturer attests to compliance with FDA requirements.
Phosphates		X		Toxicological effect if limits are exceeded.	Ingredients purchased under a letter of guarantee. Ingredients purchased based on producer/provider ingredient specifications.
Poultry (fresh, frozen)	X			Growth of pathogens due to improper handling and storage.	Product temperature must be 40°F or less at receiving. Product must meet establishment purchase specifications. Product must be produced under a HACCP plan.
Pork (fresh, frozen)	X			Growth of pathogens due to improper handling and storage.	Product temperature must be 40°F or less at receiving. Product must meet establishment purchase specifications. Product must be produced under a HACCP plan.
Proteolytic enzymes—aspergillus oryzae, aspergillus, flavusoryzae group, bromelin, ficin, papain.				Toxicological effects if limits exceeded.	Ingredients purchased under a letter of guarantee. Ingredients purchased based on producer/provider ingredient specifications.
Partially defatted products	X		X	Growth of pathogens due to improper handling and storage. Foreign particle contamination, e.g., metal, plastic.	Product temperature must be 40°F or less at receiving. Product must meet establishment purchase specifications. Product must be produced under a HACCP plan.
Seafood (fresh, frozen)	X	X		Growth of pathogens due to improper handling and storage. Environmental contamination.	Product temperature must be 40°F or less at receiving. Product must meet establishment purchase specifications. Product must be produced under a HACCP plan.

TABLE 43.10 (continued)

Ingredient Hazards

Examples of Ingredient	B	C	P	Description of Biological, Chemical, or Physical Hazard for the Ingredient	Controls or Preventive Measures
Spices/herbs—sterilized, unsterilized	X			Contamination from microorganisms inherent to the ingredient.	Ingredient specification sheet identifying the required parameters the ingredient must meet.
				Contamination from agricultural chemicals.	
				Foreign material.	
Sweeteners—saccharin, citric acid, malic acid, monoisopropyl citrate, phosphoric acid, monoglyceride citrate.				Toxicological effects if limits exceeded.	Ingredients purchased under a letter of guarantee.
					Ingredients purchased based on producer/provider ingredient specifications.
Tenderizing agents		X		Toxicological effects if limits exceeded.	Ingredients purchased under a letter of guarantee.
					Ingredients purchased based on producer/provider ingredient specifications.
Variety meats	X			Growth of pathogens due to improper handling, storage, or cleaning.	Product temperature must be 40°F or less at receiving.
					Product must meet establishment purchase specifications.
					Product must be produced under a HACCP plan.
Vegetables	X	X	X	Growth of pathogens due to improper handling and storage.	Ingredient specification sheet identifying the required parameters the ingredient must meet.
				Contamination from agricultural chemicals.	
				Foreign material.	

establishment, may give details on the material/ingredient being sold, including statements that the materials/ ingredients are food grade and are free of harmful components. For example, the ingredient specifications for dried legumes might state that there will be fewer than five small rocks or stones per 10 pound bag and that no harmful pesticides were used in the growing process.

43.2.9 Processing Steps Hazards

Refer to Table 43.11 for processing steps hazards and controls.

This section contains a list of processing hazards and controls commonly used in making meat and poultry products. They are listed in alphabetical order. For each processing step, shown in the 1st column, you will find an ''X'' in the next three columns to tell you if there is a Biological hazard (2), Chemical hazard in (3), or Physical hazard (4). Column 5 describes the hazard(s), and the last column lists some relevant controls or preventive measures. Table 43.11 should be used in conjunction with the process flow diagram developed by your HACCP team for the products produced during the process under consideration.

TABLE 43.11

Processing Step Hazards

Processing Steps	B	C	P	Description of Biological, Chemical, or Physical Hazards for the Process Steps	Controls or Preventive Measures
Acidifying (see also pickling, brining)	X			Survival of pathogens due to final pH > 4.6.	Shelf-stable nonheat-treated acidified product must obtain a pH of 4.6 or lower.
Aging (meats)	X			Growth/survival of pathogens from inappropriate storage temperatures and humidity (inadequate product water activity (a_w)).	The temperature of the aging room will not exceed 40°F.
				Growth of pathogens due to rise in the pH due to development of surface molds.	Product temperature does not exceed 40°F throughout the aging process.
					The aging process will not exceed 7 days.
Boning	X			Contamination by pathogens in product accumulations (e.g., cutting boards, conveyor belts, utensils and other equipment).	Careful employee practices to make sure that there is no contamination of the product.
				Cross-contamination of product by equipment/utensils contaminated with pathogens when cutting through a nonapparent lesion (e.g., abscesses).	Equipment and utensils are washed and sanitized immediately when contaminated and each time the employee leaves the working station.
				Contamination from bones, cartilage/extraneous material.	All hot water sanitizers are maintained at 180°F.
					Processing room temperature is maintained at 50°F, or a midshift cleanup is performed within 5 h after operations begin.
					A boneless beef re-inspection procedure will be established using specifications outlined by FSIS.
Cooling	X			Growth of pathogens due to improper temperatures.	Cooked product will be cooled according to established procedures.
				Germination of spore-forming pathogens due to slow chilling (e.g., *C. perfringens*).	
Cooking	X			Survival of pathogens due to improper procedures.	Time/temperature combinations are adequate to destroy the pathogens of concern.
Drying (meat)	X			Bacterial growth due to inadequate control over time, temperature, and humidity.	A water activity will be specified that in conjunction with other barriers will inhibit growth of pathogenic microorganisms (e.g., for shelf stable sausage a_w of 0.91 and a pH of 4.6).
Filling	X			Recontamination by pathogens in product accumulations.	Product will be protected from contamination during the filling process, and product temperature/time will be maintained at or below the maximum determined to inhibit growth of pathogenic microorganisms.
				Growth of pathogens due to temperature abuse.	

TABLE 43.11 (continued)

Processing Step Hazards

Processing Steps	B	C	P	Description of Biological, Chemical, or Physical Hazards for the Process Steps	Controls or Preventive Measures
		X		Contamination from lubricants.	No lubricants or other chemical contaminants will be allowed in or on the product.
Formulation	X			Contamination by employee handling.	Careful employee practices used at all times to make sure that there is no contamination of product.
				Incorrect formulation.	Ingredient packages will be clean and intact.
				Contamination through damaged packages.	Ingredients will be added to product according to requirements outlined 9CR §318.7.
		X		Excessive addition of restricted ingredients/additives could be toxic to the consumer.	Restricted ingredients will be added to product according to requirements outlined in the 9CFR §317.8.
Freezing (meats)	X			Survival of parasites due to improper time/temperature application.	Rapid cooling and freezing.
				Growth of pathogens due to temperature abuse.	
Grinding	X			Contamination by employee handling.	Careful employee practices to make sure that there is no contamination of product.
				Recontamination by pathogens in product accumulations.	Product will not be allowed to accumulate at the end of the grinder.
					The temperature of the grinding room will be maintained at 50°F.
Grinding		X		Contamination from lubricants.	Food grade lubricants will be used on areas of the machinery where a potential for product contamination exists.
			X	Contamination from extraneous material.	All boneless product will be re-inspected before being loaded into the grinder.
Handling and inspecting of empty containers and packaging materials.	X	X	X	Recontamination through damaged or soiled containers/packaging material.	Packaging materials and empty containers will be protected from contamination during their storage and handling.
					No materials or containers that appear to be contaminated with hazardous foreign material will be used.
Mechanical separating	X			Growth of pathogens.	Product holding and cooling requirements outlined in 9CFR 318.18 will be followed.
			X	Contamination from bone, cartilage fragments.	The finished product will meet the standards outlined in 9CFR 319.5 for bone particles and calcium.

continued

TABLE 43.11 (continued)

Processing Step Hazards

Processing Steps	B	C	P	Description of Biological, Chemical, or Physical Hazards for the Process Steps	Controls or Preventive Measures
				Contamination from extraneous material.	
Packaging (also see modified atmosphere packaging, vacuum packaging seaming, sealing).	X	X	X	contamination from packaging material.	Closure and/or machine specifications sufficient to ensure adequate barrier formation.
				Contamination through damaged containers.	
			X		No detectable foreign material will be allowed in or on the product or immediate product containers.
Peeling	X			Contamination by pathogens in product accumulations.	Careful employee practices to make sure that there is no contamination of product.
				Contamination from employee handling.	Product will not be allowed to accumulate in/on peeling equipment.
			X	Contamination from harmful extraneous material.	Peeling equipment will be maintained in a proper operating condition. No foreign material in the finished product.
Receiving	X			Contamination through damaged containers.	Product must be received in sound containers and at temperatures appropriate for the type of product.
				Growth of pathogens due to inappropriate storage conditions (temperature, humidity).	
				Growth of pathogens due to temperature abuse.	
				Contamination from receiving equipment (pumps, hoses).	
		X		Cross-contamination from nonfood chemicals.	Product must be received in sound containers and be accompanied by a letter of guarantee from the supplier if such letter is not on file.
		X		Contamination from hazardous extraneous material (wood, nails from pallets, plastic pieces).	Product must be received in sound containers and be accompanied by a letter of guarantee from the supplier if such letter is not on file.
Retorting	X			Inadequate application of scheduled process.	A thermal process specific to the product, container type and size, and retorting system must be in use. The initial product temperature and any critical factors specified for the thermal process must also be controlled. Specified retort come up procedures will be followed.
Reworking	X			Contamination by employee handling.	Careful employee practices to make sure that there is no contamination of product.

TABLE 43.11 (continued)

Processing Step Hazards

Processing Steps	B	C	P	Description of Biological, Chemical, or Physical Hazards for the Process Steps	Controls or Preventive Measures
				Contamination by pathogens in product accumulations.	Room temperature of storage coolers will not exceed 40°F.
			X	Contamination from foreign material.	Careful employee practices to make sure that there is no contamination of product.
Shipping	X			Growth due to improper temperatures.	Product will not be shipped unless it is 40°F or less.
					Product will not be loaded into transport vehicles if the trailer temperature exceeds 40°F.
			X	Contamination from hazardous extraneous material through damaged packages.	All product packages will be intact before shipping.
					All transport vehicles will be cleaned after each use and before loading of product.
Thawing	X			Growth of pathogens due to improper temperatures.	Thawing room temperature will not exceed 50°F.

43.3 HACCP Applications

Each establishment that processes meat and poultry is required by law to develop and implement an HACCP plan or system. The first step is to form an HACCP team. This team will develop the plan, using the seven principles as a guide. The first approach is Principle 1: Hazards and Controls, as discussed below. It is important to realize that the discussion is preliminary information. More details have to be complemented by the team. We will summarize our discussion.

43.3.1 Principle 1: Hazards and Controls

The basic information is designed to help a plant's HACCP team conduct a hazard analysis by providing both general and detailed information on hazards associated with meat and poultry products and by listing some of the controls that can be used to prevent or manage those hazards. When using this information, it is very important to remember that it is not all-inclusive: There may be other hazards associated with ingredients or processes; there may be other control measures. The examples assembled here are to help plant HACCP teams think through all the hazards that could affect their product and know about various controls that can be used.

The discussion describes some of the biological (including microbiological), chemical, and physical hazards generally recognized and associated with meat and poultry products. This section can serve as a resource when the HACCP team begins the hazard analysis. It is probably useful to read through this general information early in the process of developing the HACCP plan. This will help the team form an idea of what is meant by a given hazard.

This is followed by information on generally recognized preventive measures used in the meat and poultry industry to control biological, chemical, and physical hazards. This section also has examples of regulatory critical limits associated with some preventive measures.

Next is a list of processing steps, hazards, and controls for beef and swine slaughter. This section should be used with the process flow diagram developed by the HACCP team. This is followed by a

presentation of presents hazards and controls organized according to ingredients, including both meat and poultry ingredients and other ingredients used in meat and poultry production. This section should be used with the list of ingredients developed by the HACCP team.

The last section describes the hazards and controls for different steps, stages or methods in processing meat and poultry.

43.3.2 Principles 2 through 7

This chapter has not provided details on how to fulfill HACCP principles 2 through 7. They cover:

- Identify critical points where hazards can be controlled, and prevented.
- Monitor the production process and make corrective actions where necessary.
- Recordkeeping.
- Verification completes the system.

The FSIS has released thousands of documents on how to accomplish the seven principles, all available at its website (http://www.fsis.usda.gov).

43.3.3 HACCP Plan Checklist

You can use the HACCP Plan Checklist provided in Table 43.12 to ensure that your HACCP plan adequately addresses all seven HACCP principles. When completing the checklist, if you answer ''NO'' to any question, you reevaluate that section of the HACCP plan and make whatever modifications are necessary. Some modifications may require the assistance of recognized HACCP experts. Any time you make major changes to the HACCP plan based upon product or process modifications, it would be advisable to review the checklist to ensure that the revisions are acceptable. You can keep the HACCP Plan Checklist as part of your HACCP plan for future reference and to provide documented evidence that your HACCP plan addresses all seven HACCP principles.

TABLE 43.12

HACCP Plan Checklist

ESTABLISHMENT NO.		
PRODUCT/PROCESS		
DATE		
HACCP PLAN CHECKLIST		
A. DESCRIBE THE PRODUCT	YES	NO
1. Does the HACCP plan include:		
a. The producer/establishment and the product name?		
b. The ingredients and raw materials used along with the product receipt or formulation?		
c. The packaging used?		
d. The temperature at which the product is intended to be held, distributed, and sold?		
e. The manner in which the product will be prepared for consumption?		
2. Has a flow diagram for the production of the product been developed that is clear, simple, and descriptive of the steps in the process?		
3. Has the flow diagram been verified for accuracy and completeness against the actual operating process?		
B. CONDUCT A HAZARD ANALYSIS	YES	NO
1. Have all steps in the process been identified and listed where hazards of potential significance occur?		
2. Have all hazards associated with each identified step been listed?		

TABLE 43.12 (continued)

HACCP Plan Checklist

3. Have safety concerns been differentiated from quality concerns?		
4. Have preventive measures to control the identified hazard been identified, if they exist, and listed?		
C. IDENTIFY CRITICAL CONTROL POINTS	YES	NO
1. Has the CCP Decision Tree been used to help determine if a particular step is a CCP for a previously identified hazard?		
2. Have the CCPs been entered on the forms?		
3. Have all significant hazards identified during the hazard analysis been addressed?		
D. ESTABLISH CRITICAL LIMITS	YES	NO
1. Have critical limits been established for each preventive measure at each CCP?		
2. Has the validity of the critical limits to control the identified hazard been established?		
3. Were critical limits obtained from the regulations, processing authority, etc?		
4. Is documentation attesting to the adequacy of the critical limits maintained on file at the establishment?		
E. ESTABLISH MONITORING PROCEDURES	YES	NO
1. Have monitoring procedures been developed to assure that preventive measures necessary for control at each CCP are maintained within the established critical limits?		
2. Are the monitoring procedures continuous or, where continuous monitoring is not possible, is the frequency of monitoring sufficiently reliable to indicate that the hazard is under control?		
3. Have procedures been developed for systematically recording the monitoring data?		
4. Have employees responsible for monitoring been identified and trained?		
5. Have employees responsible for reviewing monitoring records been identified and trained?		
6. Have signatures of responsible individuals been required on the monitoring records?		
7. Have procedures been developed for using the results of monitoring to adjust the process and maintain control?		
F. ESTABLISH CORRECTIVE ACTIONS	YES	NO
1. Have specific corrective actions been developed for each CCP?		
2. Do the corrective actions address:		
a. Reestablishment of process control?		
b. Disposition of affected product?		
c. Procedures to correct the cause of noncompliance and to prevent the deviation from recurring?		
3. Have procedures been established to record the corrective actions?		
4. Have procedures been established for reviewing the corrective action records?		
G. ESTABLISH RECORDKEEPING PROCEDURES	YES	NO
1. Have procedures been established to maintain the HACCP plan on file at the establishment?		
2. Do the HACCP records include:		
Description of the product and its intended use?		
Flow diagram for the process, indicating CCPs?		
Preventive measures?		
Critical limits?		
Monitoring system:		
Corrective action plans for deviations from critical limits?		
Recordkeeping procedures for monitoring?		
Procedures for verification of the HACCP system?		
H. ESTABLISH VERIFICATION PROCEDURES	YES	NO
1. Have procedures been included to verify that all significant hazards were identified in the HACCP plan when it was developed?		
2. Have procedures been included to verify that the critical limits are adequate to control the identified hazards?		
3. Are procedures in place to verify that the HACCP system is functioning properly?		
4. Are procedures in place to reassess the HACCP plan and system on a regular basis or whenever significant product, process or packaging changes occur?		

44

Shelf-Stable Processed Meat Products[*]

Y. H. Hui

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44.1 Introduction

This chapter provides basic information on various types of shelf-stable meat products and the principles for controlling the food safety hazards specific to these products.

In the United States, the Food Safety Inspection Service (FSIS), a unit of the Department of Agriculture (USDA), is responsible for the safety of meat and meat produces. In accordance with the following, please note that, for this chapter:

1. The text and tables have been collated from multiple USDA public documents (legal, regulatory, and training), distributed in USDA'S website, http://www.fsis.usda.gov.
2. The figures were originally adapted from a series of USDA continuing educational documents, *USDA HACCP Generic Models on Shelf-Stable Processed Meat Products.* They are available at USDA's website, http://www.fsis.usda.gov.

It must be emphasized that, in this chapter:

1. All information have been designed by the USDA for training purposes only and is not meant to detail the actual manufacturing processes.
2. All abbreviations are standard ones used in law, science, and technology as issued by FSIS.
3. All terms with legal implications apply only in the United States.

[*] The information in this chapter has been modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. With permission.

The language format used in this chapter represents supervisor to employees or instructors to students. Despite having originated from FSIS educational materials, a food establishment will find that such format can be easily adapted to company's in-service training.

44.2 Types of Products

First we must define what we mean by shelf-stable products and distinguish them from nonshelf-stable products. Nonshelf-stable products must be refrigerated (or frozen) until consumption to prevent the growth of both pathogenic and spoilage microorganisms. Shelf-stable products, on the other hand, can be stored under ambient temperature and humidity conditions and, if the package integrity is maintained during storage, shipping, display at retail, and in the home, will not spoil or become unsafe throughout the manufacturer's specified shelf-life. Ready-to-eat (RTE) shelf-stable products can be stored unrefrigerated (e.g., at temperatures over 50°F or 10°C) and are safe to eat without additional preparation.

Products can be made shelf stable in a variety of ways. In very broad terms, meat and poultry products are made shelf stable either by "canning" or by "drying." Within the two broad categories, there are numerous variations on how shelf stability is achieved.

In this chapter, we will utilize the terms commercial sterility or commercially sterile for "canned" products and use shelf stable to refer to the dried products. Nevertheless, it should be recognized that all these products are shelf stable, that is, they can be held safely without temperature control.

For example, flow charts and processing stages are presented for dry sausage (Figures 44.1–44.4), dry ham (Figures 44.5–44.8), and canned beef stew (Figures 44.9–44.12).

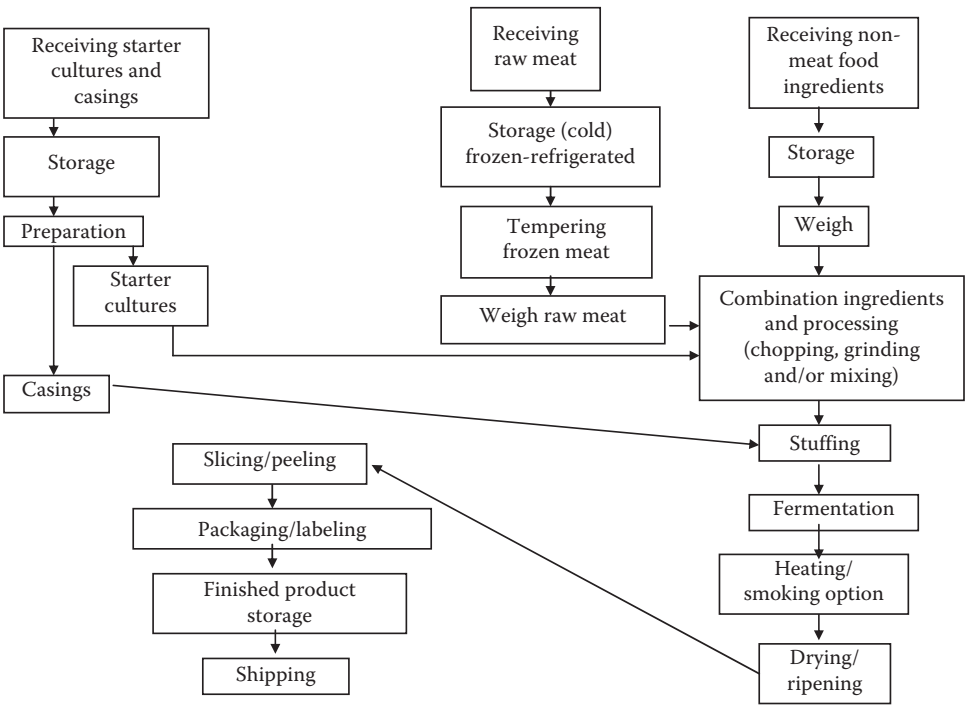

FIGURE 44.1 Fermented dry sausage: Flow diagram. (Modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. With permission.)

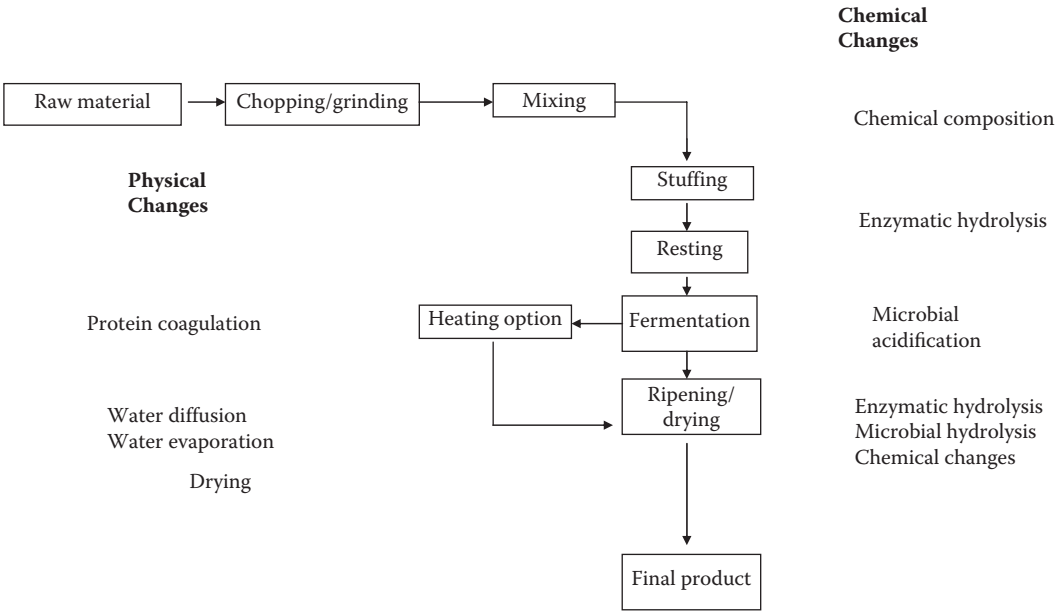

FIGURE 44.2 Changes during processing dry-fermented sausage. (Modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. With permission.)

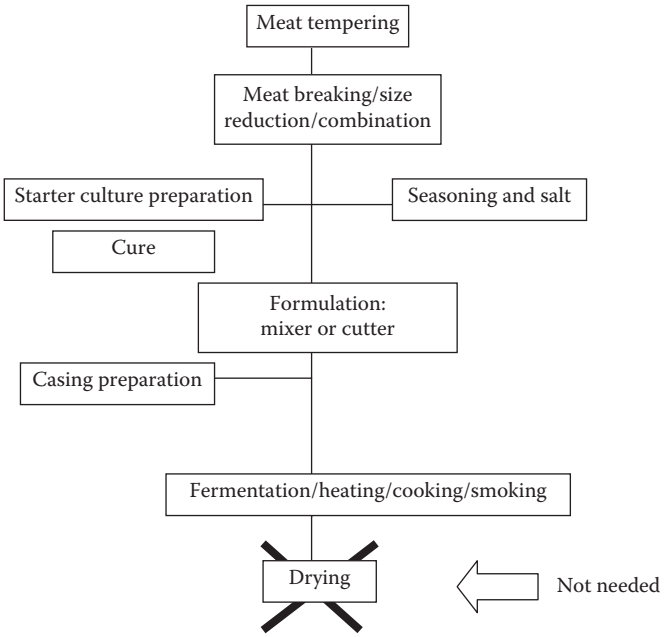

FIGURE 44.3 Fermented semidry sausage flow diagram. (Modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. With permission.)

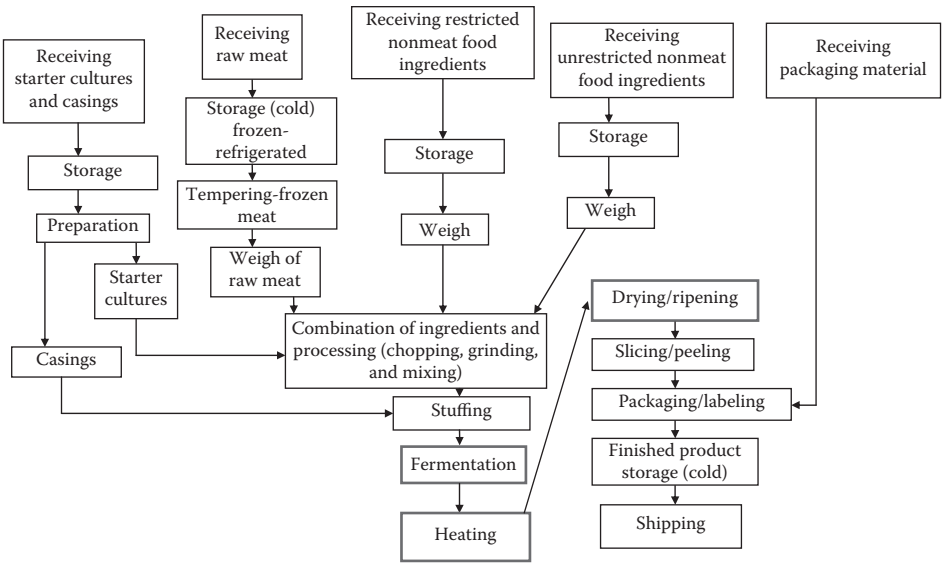

FIGURE 44.4 Critical stages: Safety in fermented sausages. *Note*: This flow diagram is for teaching purposes only. For dried whole muscle meats, particularly dry-cured products, the initial salt level and application to all exposed meat surfaces is the most critical point in the operation, along with holding the salted product at relatively low temperatures until the critical brine content is achieved uniformly. (Modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. With permission.)

44.3 Canned Products

FSIS defines a canned product as "a meat or poultry food product with a water activity (a_w) above 0.85 which receives a thermal process either before or after being packed in a hermetically sealed container." These products will remain stable and retain their organoleptic quality for several years, even when held at room temperature. When most products are "canned," they are treated with heat to make them shelf stable or, to use another term, commercially sterile. FSIS considers shelf stability and commercial sterility to be the same with respect to canning and canned products. The FSIS canning regulations define shelf stability as "the condition achieved by application of heat, sufficient, alone or in combination with

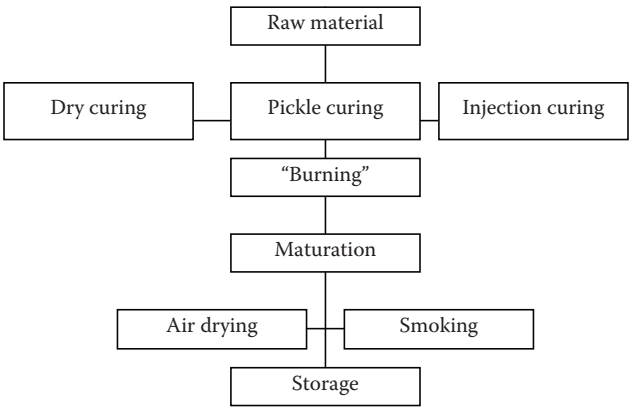

FIGURE 44.5 Whole muscle process flow diagram. (Modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. With permission.)

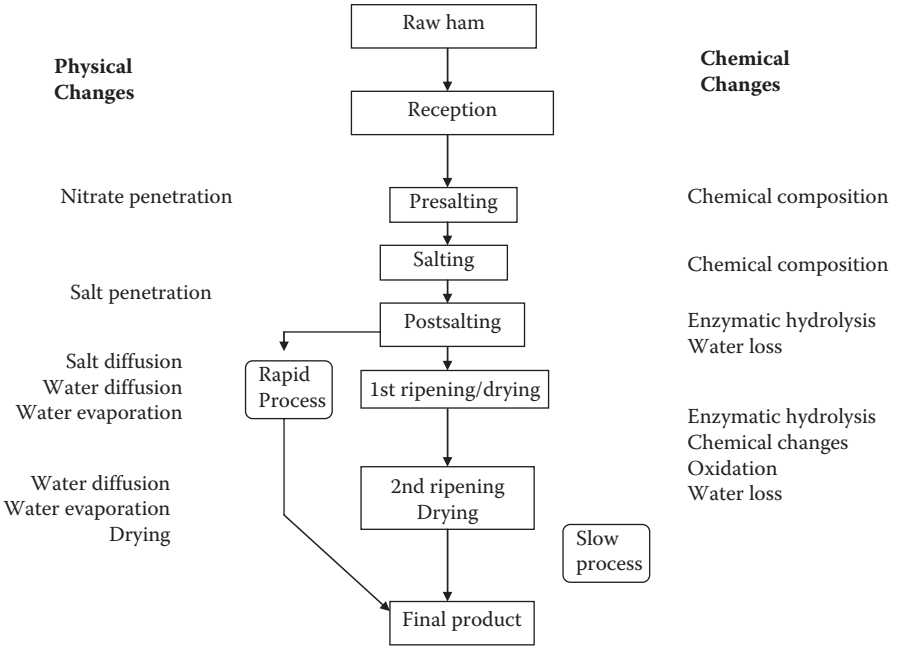

FIGURE 44.6 Changes during processing: Dry Ham. (Modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. With permission.)

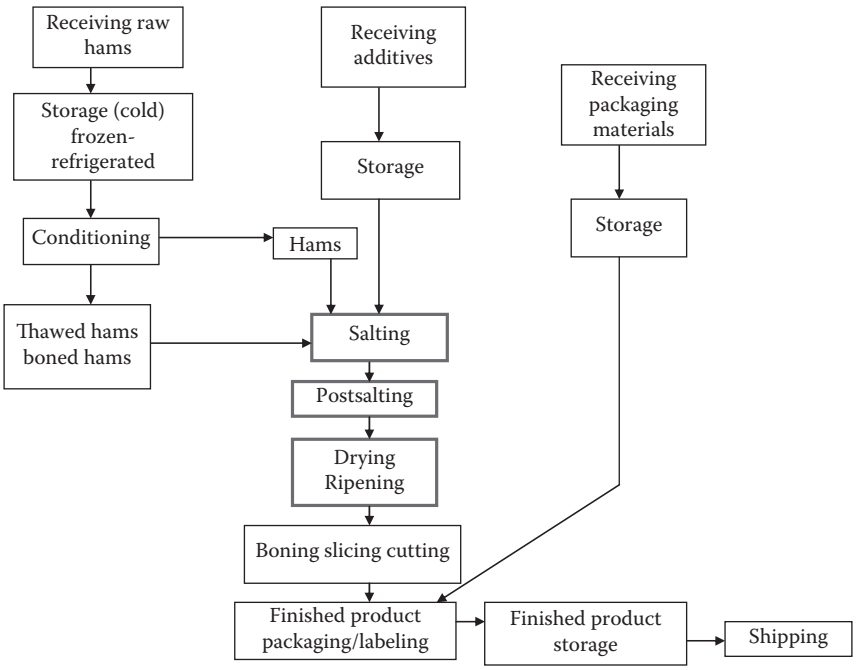

FIGURE 44.7 Critical stages: Safety in dry-cured Hams. *Note*: This flow diagram is for teaching purposes only. (Modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. With permission.)

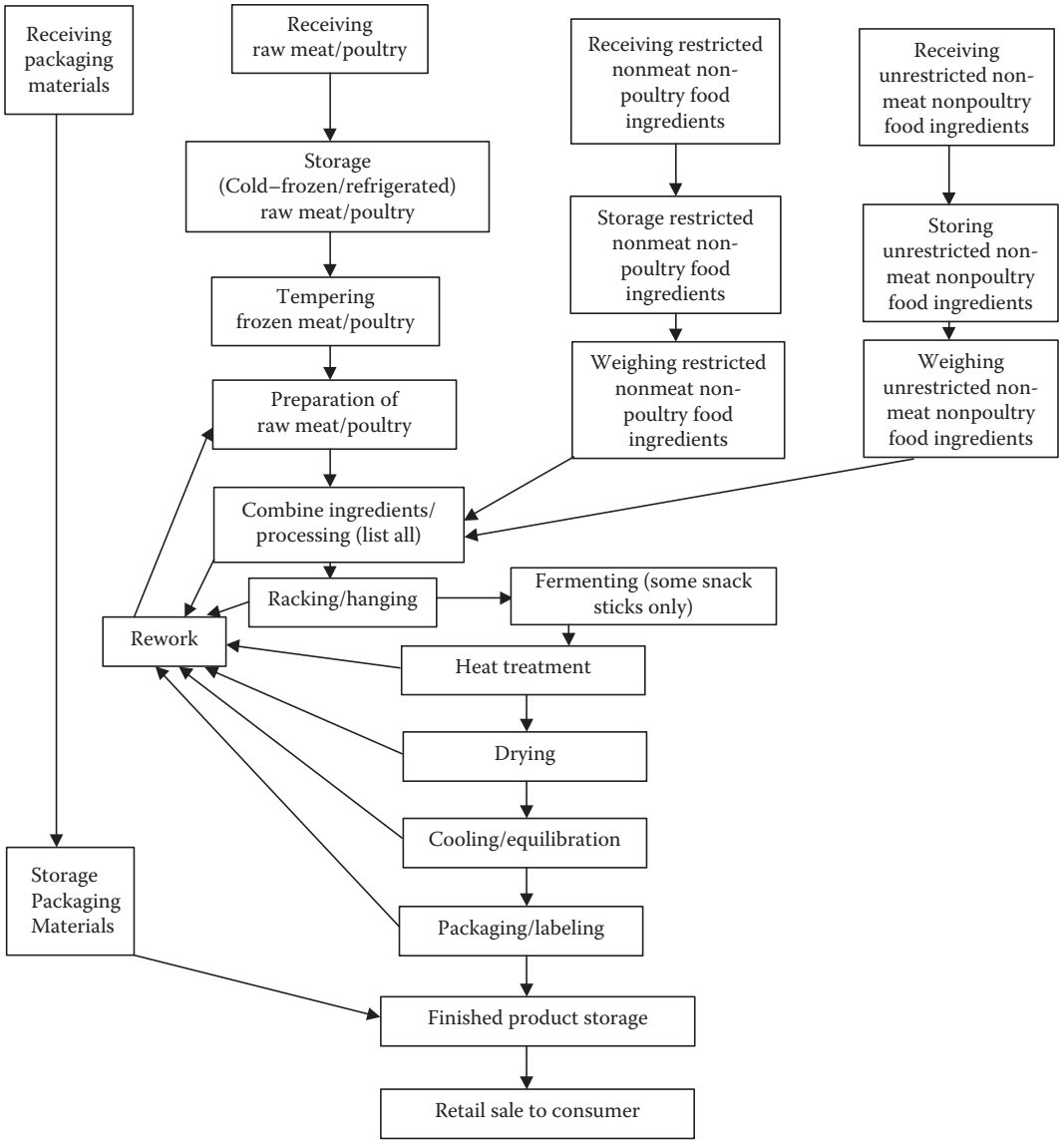

FIGURE 44.8 Process flow diagram of beef jerky. (Modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. With permission.)

other ingredients and/or treatments, to render the product free of microorganisms capable of growing in the product at nonrefrigerated conditions (over 50°F or 10°C) at which the product is intended to be held during distribution and storage. Shelf-stability and shelf-stable are synonymous with commercial sterility and commercially sterile, respectively."

Canned products are often referred to as "low-acid canned foods" (LACF) or "acidified low-acid foods," depending on whether the pH is above 4.6 (for LACF) or is 4.6 or below (for acidified foods). An acidified low-acid food is a canned product that has been formulated or treated (by addition of an acid or acid food) so that every component has a pH of 4.6 or below within 24 h after the completion of the thermal process. (A third category of products not applicable to meat and poultry products is acid foods, which have a natural pH ≤ 4.6.)

1. Common name?	Beef stew
2. How is it to be used?	Product is ready-to-eat; typically heated before consumption. Intended for persons without special dietary requirements or problems
3. Type of package?	Metal, double-seamed ("sanitary") can
4. Length of shelf life, at what temperature?	2–3 Years under cool (e.g., 75°F or lower), dry conditions; must be protected from freezing
5. Where will it be sold?	Retail
Consumers?	General public
Intended use?	Heat and consume
6. Labeling instructions?	No special instructions
7. Is special distribution control needed?	None required

FIGURE 44.9 Canned beef stew: Product description. (Modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. With permission.)

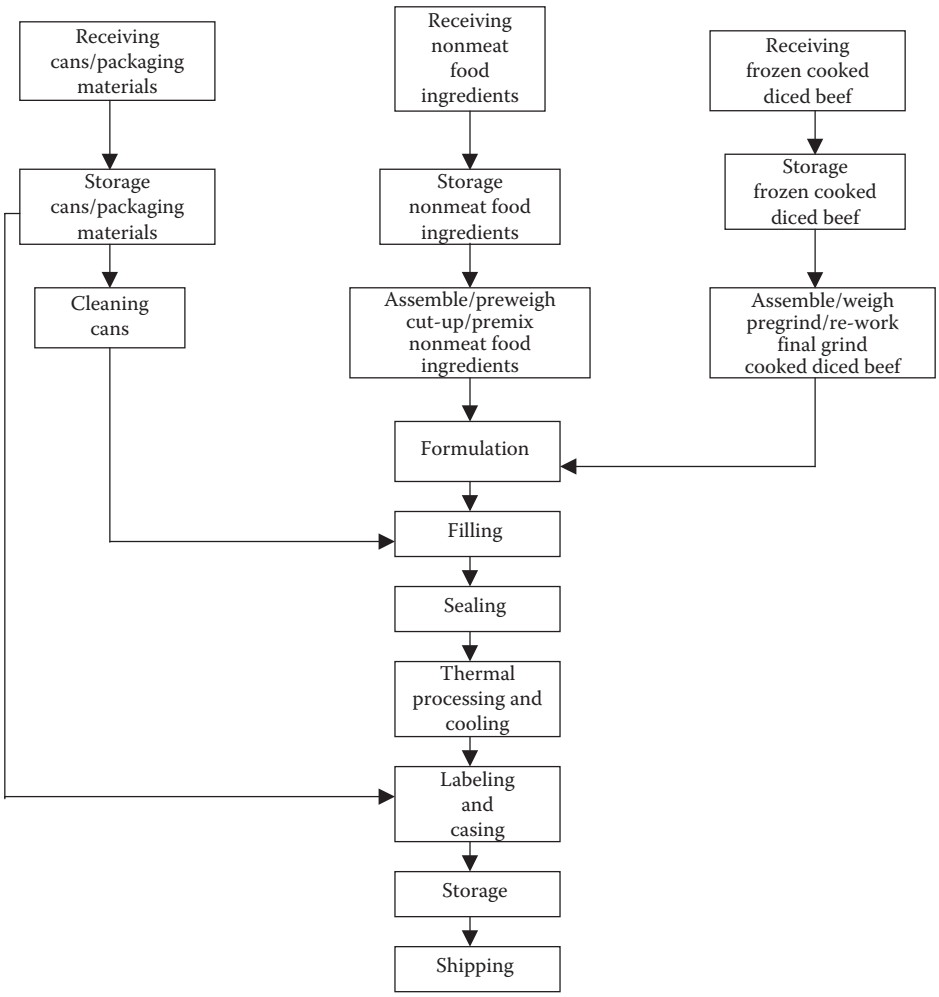

FIGURE 44.10 Canned beef stew: Process flow diagram. (Modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. With permission.)

Process Step	Food Safety Hazard	Reasonably Likely to Occur?	Basis	If Yes in Column 3, What Measures Could Be Applied to Prevent, Eliminate, or Reduce the Hazard to an Acceptable Level?	Critical Control Point
Receiving—frozen cooked diced beef	Biological: *listeria monocytogenes*	Yes	Growth of pathogens due to improper temperature and handling.	Measure and record temperature of incoming lots. Check container integrity.	1B
	Chemical—antibiotic and pesticide residues.	No	Supplied by inspected establishments.		
	Physical—foreign materials	No	Pieces of broken glass, metal, or plastic has been found in product in the past.	Visual examination	2P
Receiving—nonmeat food ingredients	Biological—bacterial spores *clostridium botulinum*	No	The bacterial spore load is controlled at the establishment by the thermal process sufficient to destroy > 10^{12} spores of *clostridium botulinum.*	Proper application of the thermal process	
	Chemical—pesticides	No	Plant records show that there has not been a problem in the past.		
	Physical—wood, metal in dried beans, potatoes, etc.	Yes	Pieces of broken glass, metal, or plastic have been found in ingredients in the past.	Visual examination and metal detectors used at receiving.	3P
Receiving—cans/packaging materials	Biological—none				
	Chemical—none				
	Physical—foreign material	Yes	Wood, metal, or glass may get on the cans during storage and shipping if protective packaging or containers are damaged.	Control can be applied most effectively at process steps where cans are inverted and cleaned.	
Storage—frozen cooked diced beef	Biological—pathogens	No	Pathogens are not reasonably likely to grow in this product if temperature is maintained at or below a level sufficient to preclude their growth.		
	Chemical—none				
	Physical—none				

Storage—nonmeat food ingredients	Biological—none Chemical—none Physical—none				
Storage—cans/packaging materials	Biological—none Chemical—chemicals	No	Packing materials stored in a location that does not allow chemical contamination.		
	Physical—foreign material	No	Packing materials stored in a location that does not allow foreign material contamination.		
Assemble/weigh pregrind/re-work final grind—cooked diced beef	Biological—none Chemical—none Physical—none				
Assemble/preweigh cut-up/premix nonmeat food ingredients	Biological—none Chemical—none Physical—none				
Cleaning cans	Biological—none				
	Chemical—none				
	Physical—foreign material	Yes	Foreign material in container.	Control of cleaning operation. Use of removable can twists.	4P
Formulation	Biological—improper formulation may allow survival of *clostridium botulinum* spores.	Yes	Improper application of thermal process may allow the survival of *clostridium botulinum* spores.	Operational formulation controls as defined by a process authority.	5B
	Chemical—none				
	Physical—none				
Filling	Biological—improper fill	Yes	If the containers are not filled per the processing authority's recommendations, the thermal process may be inadequate.	Operational filling controls.	6B

FIGURE 44.11 Canned beef stew: Hazard analysis. (Modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. With permission.)

Process Step	Food Safety Hazard	Reasonably Likely to Occur?	Basis	If Yes in Column 3, What Measures Could Be Applied to Prevent, Eliminate, or Reduce the Hazard to an Acceptable Level?	Critical Control Point
	Chemical—none				
	Physical—none				
Sealing	Biological—none				
	Chemical—none				
	Physical—none				
Thermal processing and cooling	Biological—survival of *clostridium botulinum* spores due to inadequate process.	Yes	Improper application of thermal process may allow the survival of *clostridium botulinum* spores.	Operational thermal processing controls.	7B
	Chemical—none				
	Physical—none				
Labeling and casing	Biological—none				
	Chemical—none				
	Physical—none				
Storage	Biological—none				
	Chemical—none				
	Physical—none				
Shipping	Biological—none				
	Chemical—none				
	Physical—none				

FIGURE 44.11 Continued.

CCP# and Location	Critical Limits	Monitoring Procedures and Frequency	HACCP Records	Verification Procedures and Frequency	Corrective Actions
1B Receiving—Frozen Cooked Diced Beef	Temperature within plant specifications. Meat must be received at 10°F or below to maintain in frozen state.	Receiver will check the temperature of each load of meat received. Results will be recorded, initialed, signed and dated in the Product Temperature Receiving Log.	Product Temperature Receiving Log Thermometer Calibration Log Corrective Action Log	Receiving supervisor will observe receiving employee taking product temperature or will take product temperature once per shift Receiving supervisor will review Product Temperature Receiving Log, Corrective Action Log, Calibration Log, and Verification records once per shift. QA will check all thermometers used for monitoring and verification for accuracy daily and calibrate to within 2°F accuracy as necessary.	Receiver will hold meat that exceeds 10°F and notify supervisor. Any rejected meat will be returned to supplier. Supplier history will be reviewed. Condemned meat will be denatured at the plant.
2P Receiving—Frozen Cooked Diced Beef	No visible hazardous foreign material (e.g., glass); no visible metal contamination.	Receiver will visually examine a random sample from each lot received for foreign material.	Receiving Log Corrective Action Log	QA supervisor will review Receiving Log twice per shift. QA supervisor will observe visual inspection of incoming product by receiver once per shift.	Receiver will ensure that all meat received is from establishments on company approved list. Supplier history will be reviewed and if there is a trend in supplier inability to meet the critical limit, the supplier will no longer be used. If hazardous foreign material is detected in or on the meat, QA will identify and control affected product for disposition; condemn or return controlled product to supplier. QA will take action to prevent reoccurrence.
3P Receiving - Nonmeat Food Ingredients	No visible hazardous foreign material (e.g., glass); no metal contamination 31/32 inch.	Receiver will examine each lot for foreign material using metal detector and visual examination. QA will examine a random sample from each lot for foreign material using	Receiving Log Metal Detector Log Corrective Action Log	Maintenance supervisor will verify metal detectors are functioning. QA will verify that the metal detectors are functioning as intended by running a seeded sample through the metal detectors twice per shift (once in the AM and once in the PM).	Receiving supervisor will control and segregate affected product. Maintenance personnel will identify and eliminate the problems with the metal detectors. Preventive maintenance program will be implemented.

FIGURE 44.12 Canned beef stew: HACCP plan. * Time elapsed will not always be specified as critical factor by a Processing Authority. (Modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. With permission.)

CCP# and Location	Critical Limits	Monitoring Procedures and Frequency	HACCP Records	Verification Procedures and Frequency	Corrective Actions
		metal detector and visual examination.			QA will run a seeded sample through the metal detectors after repair.
4P Cleaning Cans	No visible hazardous foreign material (e.g., glass, metal).	Can washer operator will visually examine cans as they exit washer to ensure unit is operating properly and cans are adequately cleaned. QA will randomly visually check cans as they exit washer at a frequency determined using a statistical randomization chart. Daily check to determine that can twists are installed & working properly.	Can Washer Log Corrective Action Log	QA supervisor will review Can Washer Log and Corrective Action Log twice per shift. QA supervisor will observe can washer operator. Maintenance will verify can twists are properly installed and operating on a weekly basis.	If can washer or can twist malfunctions, operator will stop line, remove uncleaned cans, and notify plant designee. When proper functioning is restored, cans removed will be examined by QA, then recycled through washer. QA will take action to prevent reoccurrence. Maintenance will make appropriate repairs to cleaners or twists and alter maintenance schedule as required.
5B Formulation	Criteria as specified by the Processing Authority (e.g. maximum sauce viscosity value).	Head formulation cook will check ingredient characteristics, quantities, sauce viscosity, and conformance with specified formulation procedure for each batch prepared. Plant designee will check the time elapsed from assembly to commercial sterilization for each batch to determine that it meets limits specified by the processing authority.*	Formulation Log Process Deviation Log Corrective Action Log	QA will review the formulation log twice a week to verify that every batch is properly formulated. QA will audit to verify sampling techniques and accuracy of records; determine if the critical limit corresponds to the plant records; check to see if critical limits are adequate for hazard; assure corrective actions are adequate, document findings.	Head formulation cook will not pass batch for transfer to the filler that has not been formulated correctly or has exceeded the time specification. If possible, rejected batches will be reformulated. Otherwise, the product will be condemned. QA will take action to prevent reoccurrence.

6B Filling	Criteria as specified by the Processing Authority (e.g. maximum fill weight).	Fill operator will ensure that filled containers are run through an automatic over/under check weigher set to reject above the weight limit once per hour. Also, the "toppers" on the seamer will be set to produce headspace in excess of prescribed minimum.	Weight/Headspace Log Process Deviation Log Corrective Action Log	QA will review the records and twice weekly verify the accuracy and measure sample weights and headspaces daily to ensure that weight and headspace standards are met. QA will audit to verify sampling techniques and accuracy of the records; determine if the critical limit corresponds to the plant records; check to see if critical limits are adequate for hazard; assure corrective actions are adequate, document findings. Weekly calibration of filler.	Production foreman and QA will ensure that all rejected containers are emptied and contents reworked or condemned. QA will take action to prevent reoccurrence.
7B Thermal Processing and Cooling	Criteria as specified by the Processing Authority (e.g. minimum product initial temperature, minimum retort temperature, minimum process time, etc.).	Retort operator will monitor and record thermal processing conditions at intervals determined to be sufficient by the processing authority to ensure that the process schedule is properly applied, including process application, venting procedures.	Process Operating Log Recorder Charts Process Deviation Log Corrective Action Log	QA will review the logs and charts within one working day after the thermal process. QA will audit to verify sampling techniques and accuracy of records; determine if the critical limit corresponds to the plant records; check to see if critical limits are adequate for hazard; assure corrective actions are adequate, document findings. Quarterly calibration of retort.	If a process deviation occurs, the plant designee will apply a filed alternate process schedule appropriate for the situation or hold the product pending a processing authority's evaluation. QA will take action to prevent reoccurrence. Product will be reworked if applicable using the alternate process schedule.

FIGURE 44.12 Continued.

Although we refer to these commercially sterile products as "canned," as you will see, the products may be in cans, glass jars, plastic containers, laminated pouches, paperboard containers, etc. that are sealed to prevent the entry of microorganisms. These products may be made commercially sterile by placing them in the container, sealing it, and treating the product with high heat under pressure using a retort, or pressure vessel. However, if the pH of the product is low enough (at or below 4.6), the thermal process may be delivered with lower heat and no pressure (e.g., in an atmospheric cooker or pasteurizer). Important considerations include

- Types of retorts that used for meat and poultry products.
- The role of pH in manufacturing commercially sterile products.
- Acidified versus low-acid products.
- Impact of curing agents on thermal processes.

Commercially sterile uncured meat and poultry products include canned beef stew, whole chicken, chili, meat sauces and gravies, meat spreads, soups containing meat and poultry, baby and toddler foods, and even entrees such as chicken with noodles. Some of these, such as spaghetti sauce with meat, may be acidified products. Commercially sterile-cured products include Vienna sausages, canned hams (not perishable), and canned luncheon meats.

The process of placing food in a container and heating it to make the product commercially sterile is known as conventional canning. There is another method of producing commercially sterile products known as aseptic processing. This method involves sterilizing the food and the container separately and filling. Commercially sterile products are not widely used for meat and poultry products in the United States at this time, although it is a proven technology.

44.4 Dried Products

They include:

1. Dry and semidry sausages (e.g., pepperoni, hard salami, Summer Sausage)
   a. Fermented products
   b. Products acidified with chemical acidulants
   c. Nonacidified-cooked products
2. Dried (mostly dry cured) whole muscle products, prosciutto, parma and country hams.
3. Dried whole muscle meat snacks, beef jerky products.

The major categories of dried meats are dry and semidry sausages, dried (i.e., dry cured) whole muscle meats, and dried whole muscle meat snacks. Dry and semidry sausages (e.g., pepperoni, hard salami, Summer Sausage) are probably the largest group of dried products. These products may be further broken down into fermented products, those acidified with chemical acidulants, and nonacidified cooked products. FSIS guidelines specify that shelf-stable dry sausage must have a Moisture Protein Ratio (MPR) ≤ 1.9:1 and semidry sausage must have an MPR ≤ 3.1:1 with a pH ≤ 5.0, or be commercially sterilized (unless another MPR is specified for a product). Alternatively, nonrefrigerated, semidry shelf-stable sausages are those that are fermented to a pH of 4.5 or lower (or pH 4.6 if combined with a_w of <0.91); are in an intact form or, if sliced, are vacuum packed; have an internal brine concentration of no <5%; are cured with nitrite or nitrate; and are smoked with wood. These guidelines are standards of identity; they were not developed for safety.

Dried (mostly dry cured) whole muscle products such as Prosciutto, Parma, and Country Hams are treated with salt or salt brines to achieve shelf-stability. Dried whole muscle meat snacks such as beef jerky products are brine cured and dried to a low a_w or MPR. The a_w (available moisture) of the product is the primary factor affecting shelf stability in salt-cured dried products.

44.5 Other Shelf-Stable Products

They include:

1. Pickled pigs' feet and vinegar-pickled sausages.
2. Sliced-dried beef packed in jars or sausages covered with lard packed in cans.
3. Canned bacon.
4. Freeze-dried or dehydrated meat.
5. Snack foods such as fried pork skins and pork cracklings.

There are additional shelf-stable products that do not fit easily into the two broad classifications of canned or dried. Some products found in cans or jars are neither conventionally canned nor aseptically packed. For example, some products such as pickled pigs' feet and vinegar-pickled sausages rely primarily on pH control and, although they may be cooked, they are not heated in the container. The shelf stability of pH-controlled products is due to a combination of the low pH, the use of acetic acid as the acidulant, control of fermentable carbohydrate, and packaging in an airtight container. Other products, such as sliced-dried beef packed in jars or sausages covered with lard packed in cans, do not receive a heat treatment in the container and are primarily a_w controlled. (Dried beef and sausages are addressed in the "dried" category; the only distinction here is that the type of packaging is similar to that of commercially sterile product.) The a_w-controlled products rely primarily on the high brine levels, but this may be combined with other factors. FSIS guidelines specify that sausages such as chorizos-packed hot in lard have an MPR of 1.8:1 and a pH of 5.5 or less, or, alternatively, a_w of 0.92. Sliced-dried beef is shelf stable if packaged under vacuum, as in a glass jar, with a brine concentration of 16% or greater or a_w of 0.86 or below. Microbial spoilage due to micrococci can occur if the vacuum is inadequate, the brine level is low or if residual nitrite is inadequate.

Shelf-stable bacon can also be found in cans (e.g., canned prefried bacon) or jars (e.g., bacon bits). The product is shelf stable if the a_w is ≤ 0.86 and it is packed under vacuum. Several processes may be used. One involves cooking bacon and filling it into the container, whereas another process involves heating the bacon in the container to an internal temperature of 156°F (68.9°C) to pasteurize the product; both rely on high-brine concentration for stability. FSIS specifies that canned pasteurized bacon have a brine concentration of 7%. Canned prefried bacon must have an M/SP (moisture divided by salt times protein) index of 0.4 or more, a brine ratio (moisture/salt) of 9.0 or less and a brine concentration (salt/moisture + salt) of 10% or more.

Shelf-stable products may also include freeze dried or dehydrated meat and poultry products (such as beef gravy mix and foods for camping) and snack foods such as fried pork skins and pork cracklings. These products are cooked (which destroys pathogens) and then dried to a level that does not support microbial growth. Meat base (beef, pork, ham) is shelf stable primarily because of high salt content (30–40%), as is beef concentrate and salt, which is made from cooked beef broth that is evaporated and then salted. We will not cover these products in detail in this course.

44.6 Hazards

The purpose of this section is to

1. Review *biological* hazards of significance to commercially sterile meat products.
2. Review biological hazards significant to shelf-stable meat products.
3. Distinguish between biological hazards and spoilage organisms.
4. Review chemical and physical hazards that are unique to commercially sterile and shelf-stable products.
5. Discuss how hazards related to shelf-stable products may differ from those for nonshelf-stable products.

44.6.1 Biological Hazards of Shelf-Stable Meat Products

Biological hazards associated with meat and poultry products in general include spore formers and nonspore formers. The spore formers include *Clostridium botulinum*, *Clostridium perfringens*, and *Bacillus cereus*. Nonspore formers (vegetative pathogens) may include, depending on the product, *Staphylococcus aureus*, *Salmonella* spp., *Campylobacter* spp., *Listeria monocytogenes*, and *Escherichia coli* O157:H7. In addition, parasites may be a hazard to be addressed, especially *Trichinella spiralis* in pork.

C. botulinum is the hazard of greatest concern to canned (commercially sterile) products because:

1. When it grows it can produce a deadly toxin or poison.
2. It can be isolated from soil or water practically everywhere in the world.
3. It is the pathogen with the greatest heat resistance due to the production of heat-resistant spores.
4. Canning foods provide an anaerobic environment favorable to growth of the organism if it has not been destroyed by the process. When processing commercially sterile meat and poultry products, if an establishment controls *C. botulinum*, the other biological hazards in meat and poultry, as well as those in other added ingredients, will also be controlled. Thus, an establishment would be expected to identify *C. botulinum* as a hazard and implement controls for it when producing commercially sterile meat or poultry products unless otherwise exempted.

In low-acid foods, high heat must be applied to kill the spores of *C. botulinum*. Thus, these foods must be heat processed under pressure; the scheduled process established by a process authority would be a critical control point or CCP. In foods with low pH (≤4.6), such as acidified meat and poultry products, the spores of *C. botulinum* cannot germinate and grow out. Controlling the pH is critical to address *C. botulinum*. These foods can be heated at lower temperatures not requiring pressure to destroy nonspore formers such as *E. coli* O157:H7. Likewise in canned cured meat and poultry products in which outgrowth of *C. botulinum* spores is controlled by formulation (e.g., salt and nitrite), the thermal process will not be designed to inactivate pathogenic spore formers. In pH-controlled products and cured products, usually the formulation step is a CCP for *C. botulinum* (pH of the product, amount of acid added, amount of nitrite and salt) and the heat step is a CCP (time and temperature) to control vegetative pathogens such as *E. coli* O157:H7 and *Salmonella* (unless the establishment elects to use the regulatory exemption).

In order to make low acid, acidified, and cured meat and poultry products commercially sterile (shelf-stable), processes are designed primarily to destroy heat-resistant organisms that could spoil the product when it is held at room temperature rather than the pathogens of concern. These spoilage organisms include spore formers such as *Clostridium sporogenes* for low acid and cured meat and poultry products and *Clostridium butyricum* for acidified products. Products that have received an insufficient heat process may or may not be unsafe, depending on whether the under-processing was sufficient to allow pathogens to survive. Also, it must be remembered that, by design, not all the lethality is included in most processes. Thus, in some under processing situations, the product will still be commercially sterile. It is important to have a processing authority that is familiar with the product involved in the design of the process and the evaluation of all deviations.

Because of the severity of the processes to provide a commercially sterile product, there is less concern about control at steps coming before the thermal process. For example, although establishments will implement programs to control the temperature of raw ingredients such as meat, even if there are problems with temperature control it is not likely that microbial growth will compromise the process (although it may compromise the quality of the product). Likewise, canning establishments are likely to have programs to control microorganisms in can cooling water; however, it is unlikely that low levels of chlorine in the water will result in unsafe product if water containing microorganisms should pass through the can seam during cooling. Thus, although these prerequisite programs address potential biological hazards, their primary impact is on product quality, not safety, and they are not considered CCPs.

44.6.2 Biological Hazards of Shelf-Stable Dried Meats

Biological hazards associated with meat and poultry products in general, as described for commercially sterile meat and poultry products, are the same for shelf-stable dried meats: *C. botulinum*, *C. perfringens*, *B. cereus*, *S. aureus*, *Salmonella* spp., *Campylobacter* spp., *L. monocytogenes*, and *E. coli* O157:H7, and parasites such as *T. spiralis* in pork.

Although *C. botulinum* is a hazard to be considered, because of the dried nature of these products vegetative pathogens—*Salmonella*, *E. coli* O157:H7, *S. aureus*, and *L. monocytogenes*—are more of a concern than with commercially sterile products. Control of biological hazards for shelf-stable dried meat products relies on multiple factors (the hurdle concept). The hurdles and the CCPs will vary, depending on the product and process, with pH and a_w of primary importance to the safety of these products. For fermented shelf-stable products, CCPs are generally at the steps for fermentation (e.g., time to pH 5.3), heating (time and temperature), and, sometimes, drying (e.g., drying room temperature and time). For nonfermented salt-cured products, the salting step (e.g., amount of salt, penetration time) is critical and for dried products the drying step (e.g., drying temperature, time, humidity) is critical. For freeze-dried products and bacon bits, the cooking step (time and temperature) is usually critical.

The success of the CCPs for these products is more dependent on the proper conduct of other procedures that may be in prerequisite programs than with commercially sterile products. For example, proper handling of meats is important to ensure low levels of pathogens that can be controlled by fermentation, salting, drying, etc. With fermented meats, storage and handling of the starter culture are important to ensure proper fermentation takes place. Cell death of the culture due to temperature abuse can result in the rate of pH reduction not being adequate to prevent toxin formation by *S. aureus*. However, starter culture failure would be detected by monitoring the pH during fermentation (the CCP). Proper handling of starter cultures to prevent failure of the fermentation process is important to prevent economic loss.

44.6.3 Chemical Hazards

Chemical hazards for commercially sterile and shelf-stable meat products do not differ from those for other food products. Potential chemical hazards for shelf-stable products include nitrite, unlabeled allergens, antibiotic residues in meats, cleaners, sanitizers, etc. However, these are not unique to shelf-stable products. Establishments producing shelf-stable products would evaluate potential chemical hazards in the same manner as other establishments.

44.6.4 Physical Hazards

Physical hazards for commercially sterile and shelf-stable meat products do not differ from those for other food products. Potential physical hazards include metal, in ground meat products, and glass, if product is packed in glass containers. Again, these are not unique to shelf-stable products, and the establishment would use the same procedures to evaluate whether the identified potential physical hazards are reasonably likely to occur in its operations.

44.7 Hazards for Shelf-Stable Products Compared to Nonshelf-Stable Products

The major difference in hazards for shelf-stable and nonstable products lies in the biological hazards that need to be addressed in the Hazards Analysis Critical Control Points (HACCP) plan and how they are controlled. For comparison purposes, let us look at canned beef stew in comparison with frozen beef stew. As noted above, the biological hazard to be addressed for canned beef stew is *C. botulinum*. This hazard will be controlled by the thermal process given to the product in the can, which will include processing time and temperature, along with other critical factors specified by the process authority. However, with frozen beef stew, *C. botulinum* cannot grow since the product is frozen. The biological hazards likely to be addressed in the HACCP plan include Salmonella and *E. coli* O157:H7 from the beef,

L. monocytogenes in from the beef and the vegetables, and *C. perfringens* from the beef and spices. The vegetative cells of these pathogens will be killed in the cooking process for the stew; spores of *C. perfringens*, which will survive the cook, will be controlled by proper cooling. Recontamination with *L. monocytogenes* will probably be addressed with sanitation that is verified by environmental monitoring. Similarly, for luncheon meats, the hazard for commercially sterile pork luncheon meat is *C. botulinum*, which will be controlled by the curing salts (sodium chloride and nitrite) and the thermal process. For refrigerated luncheon meats, the hazards are likely to include *Salmonella*, trichinae, and *L. monocytogenes*, as well as *C. botulinum*. The curing salts (along with refrigeration) will control *C. botulinum* (and *C. perfringens*) and the heat treatment will kill *Salmonella*, trichinae, and *L. monocytogenes*.

44.8 An Example: Dried Meat

The science and technology of producing dried meat products are discussed in another chapter in this book. This chapter is not a proper forum to discuss the details of the legal requirements of the manufacture of dried meat. Instead, we will present flowcharts to outline the processing of manufacturing different dried meats.

We will briefly discuss the safety concerns in beef jerkey as emphasized in FSIS compliance guideline for meat and poultry jerky produced by small and very small plants.

This guideline includes measures that small and very small establishments that process jerky products can use to achieve adequate lethality. These measures are described in the applicable processing step discussed in Section 44.8.2. Also see Figures 44.1–44.4.

44.8.1 Background

Meat or poultry jerky is a RTE, dried product, that is, generally considered to be shelf stable (i.e., it does not require refrigeration after proper processing). FSIS is concerned that producers of meat and poultry jerky may not be adequately processing jerky to achieve the lethality necessary to produce a safe product. FSIS has identified two points in jerky processing where producers may need to do a better job.

First, jerky may not be adequately heat treated to meet the lethality performance standards if the requirement for moist cooking is not achieved. Some processors use dry heat to both heat and dry their product and, thus, do not achieve adequate lethality during the heating process because the product dries prematurely, and the lethality process stops.

Secondly, FSIS has become aware that some manufacturers rely upon the maximum MPR, rather than a_w, for determining whether their process adequately dries the jerky to produce a shelf-stable product. While an MPR of 0.75:1 or less remains part of the standard of identity for jerky, a_w, as measured by laboratory analysis, should be used to verify that the jerky is properly dried. a_w is a better measure of available water for microbial growth than MPR. Minimizing available water (e.g., achieving a a_w of 0.80 or less) is critical for controlling the growth of pathogens.

44.8.2 Lethality Compliance Guidelines for Jerky

In general, jerky processing includes slicing or forming the meat or poultry, marinating the strips, heating them, and then drying them. The purpose of the heating step is to apply a lethality treatment to kill or reduce the numbers of microorganisms so that the jerky is safe for human consumption. Drying the jerky stabilizes the final product and prevents the growth of toxigenic microorganisms such as *S. aureus*. Some processors combine the heating and drying procedures into one step. However, it is critical that the heating accompanied by adequate humidity precede the drying.

If the times and temperatures in the lethality compliance guidelines are used, it is critical that the humidity criteria be rigorously followed during the cooking/heating (lethality) steps.

The following are general or common processing steps used in jerky production. Although an establishment's process may not include all these steps, the lethality treatment and drying are required to produce a safe product. The intervention step may be required for those processes that do not achieve an

adequate lethality. The steps listed as heating and drying are consecutive steps. Drying should closely follow heating. Heating is used to achieve lethality of harmful microorganisms and drying is used to stabilize the product.

Step 1: Strip preparation: Whole muscle is sliced or ground; ground product is formed into strips (some jerky is formed).

Step 2: Marination: The strips are then marinated in a solution that often contains salt, sugar, and flavoring ingredients.

Step 3: Interventions: Antimicrobial interventions before and after marinating the strips of raw product have been shown to increase the level of pathogen reduction above that achieved by heating alone. Some processes may not deliver an adequate lethality and, therefore, may require an additional intervention step to ensure product safety. Examples of such interventions are

- Preheating the meat or poultry jerky strips in the marinade to achieve a minimum internal temperature of 160°F will provide an immediate reduction of *Salmonella*. Because heating in the marinade may produce an unacceptable flavor for some products, other liquids, such as water, could be used. The times and temperatures in the lethality compliance guidelines could be used for preheating in the liquid.
- Dipping the product in 5% acetic acid for 10 min before placing it in the marinade can augment the log reduction effects of drying but not enough to eliminate pathogens. This intervention may also result in an undesirable flavor.

Step 4: Lethality treatment: The establishment must apply a treatment to control, reduce, or eliminate the biological hazards identified in the hazard analysis. For meat and poultry jerky, these hazards will most likely include the microbiological hazards from *Salmonella* spp., *L. monocytogenes*, and *S. aureus*. For beef jerky, *E. coli* O157:H7 may also be a hazard reasonably likely to occur. In recent years, several jerky products have been found to be adulterated with *Salmonella* and *E. coli* O157:H7.

For meat jerky, use of the time–temperature combinations provided in the lethality compliance guidelines should help ensure the safety of the product. These time–temperature combinations are based on experiments that were done with ground beef without added salt or sugar. Added salt, sugar, or other substances that reduce a_w will increase the heat resistance of bacteria in a product. However, time and experience have shown that the time–temperature combinations in the lethality compliance guidelines have been sufficient to produce safe products even with both salt and sugar additives but the humidity during heating is a critical factor.

For poultry jerky, to produce a safe product, producers can use the minimum internal temperatures listed in the lethality compliance guidelines of 160°F for uncured poultry or 155°F for cured poultry. They can also use the time–temperature combinations listed in the poultry time–temperature tables of the Compliance Guidelines for RTE Meat and Poultry Products. However, humidity during heating is a critical factor regardless of which compliance guideline is used. As with meat jerky, the time–temperature combinations would be sufficient to produce safe products with both salt and sugar additives if the processor uses the humidity parameters applicable to beef as described below.

Therefore, for both meat and poultry, the humidity parameters described for meat products *must* be followed if the lethality compliance guidelines are used as supporting documentation. The time–temperature tables are based on wet heat. Without humidity the product will dry, and the bacteria will become more heat resistant. As long as proper humidity is maintained, the level of pathogen reduction attained by using the lethality compliance guidelines for cooking poultry or whole beef should be sufficient to provide a safe product.

If the lethality compliance guidelines are used, the relative humidity must be maintained above 90% throughout the cooking or thermal-heating process by using a sealed oven or steam injection. This level of humidity may not be necessary if an establishment can provide documentation that its process can achieve an adequate lethality with less humidity. With adequate humidity, small mass products such as jerky should heat rapidly and attain the necessary time and temperature to meet the lethality compliance

guidelines criteria for lethality. Therefore, because of this shorter-heating time, FSIS is not incorporating the humidity criteria, "50% of the cooking time but in no case not <1 h," intended for large mass products into the compliance guidelines for jerky.

The heating temperature and humidity (e.g., steam) are critical for achieving adequate lethality. As the a_w is reduced, the heat resistance (D value) of the bacteria increases. Therefore, if adequate humidity is not maintained during heating, the time at a particular temperature to eliminate *Salmonella* will be greatly increased. It is crucial that the processor prevent drying of the product until a lethal time–temperature combination is attained. The humidity requirement must be applied during the first part of the heating process before any drying and an increase in solute concentration occurs.

The process should be monitored using wet and dry bulb thermometers as noted below. The use of wet and dry bulb measurements can be used to determine the relative humidity. For example, readings that show a difference of 2°F between the wet and dry bulbs might indicate ~94% relative humidity. Wet and dry bulb temperatures should not differ by more than 4.5°F. A temperature difference greater than 4.5°F indicates a relative humidity of ~86% and shows the needed minimum relative humidity (90%) is not being maintained.

At high altitudes, the amount of humidity in the chamber necessary to achieve a given log reduction of bacteria may need to be increased. Processing failures in the manufacture of jerky have occurred in establishments located at high altitudes. Table 44.1 provides some simple and practical measures used to meet the humidity parameters in the lethality compliance guidelines.

Step 5: Drying: After the lethality treatment, the product should be dried to meet the MPR product standard and to stabilize the finished product for food safety purposes. If the product is insufficiently dried, *S. aureus* and mold are potential hazards. These organisms are not expected to grow in properly dried products. A suggested a_w critical limit for stabilization of jerky is 0.80 or lower. This range of a_w should control growth of all bacterial pathogens of concern.

The establishment should verify the a_w to demonstrate that the product has attained the critical limit for shelf stability. a_w is the key to determining the proper level of drying. The a_w can vary greatly at any given MPR (as a result of the presence and level of different solutes, such as sugar and salt). Therefore, a laboratory test for a_w should be used to verify proper drying.

Step 6: Postdrying heat step: Heat the dried product in a 275°F oven for 10 min. This heating has the potential to reduce *Salmonella* levels by ~2 logs from the level of reduction achieved during initial heat step. This step may be needed for processes that do not result in an adequate reduction of *Salmonella* through the heating process.

TABLE 44.1

Some Simple and Practical Measures Used to Meet the Humidity Parameters in the Lethality Compliance Guidelines

Seal the oven	Close the oven dampers to provide a closed system and prevent moisture loss. Steam may be observed venting when the dampers are closed, similar to venting that occurs in a steam retort during canning.
Add humidity	Place a shallow and wide pan of hot water in the oven to provide humidity in the system. Conduct a test run to determine whether the water evaporates. Injecting steam or a fine water mist in the oven can also add humidity. Use of a wet bulb thermometer, in addition to the dry bulb thermometer, also would enable the operator to determine whether adequate humidity is being applied.
Monitor humidity	Use a wet bulb thermometer in combination with a dry bulb thermometer. A basic wet bulb thermometer can be prepared by fitting a wet, moisture-wicking cloth around a dry bulb thermometer. To maintain a wet cloth during the process, submerse an end of the cloth in a water supply. The cloth must remain wet during the entire cooking step and should be changed daily, especially if smoke is applied. The use of a wet bulb thermometer is especially important for production at high altitudes or areas of low humidity where evaporation is facilitated.

Step 7: Handling: The establishment's Sanitation Operation Procedures should ensure that the product is properly handled to prevent recontamination or cross-contamination of the meat and poultry products by the bacterial pathogens of concern.

44.8.3 Validating Custom Processes

Establishments, or their processing authorities, may develop customized processes that achieve an appropriate reduction of pathogens throughout the product. Customized processes should be based on a scientific rationale, supported by experimental data. They may be developed by using information obtained from the literature, from unpublished studies that are scientifically valid, or by comparing the methods used by the establishment with established procedures that have been validated to achieve the required $\log_{10}$ reduction of the pathogen. At a minimum, a validation study for a microbiological food safety hazard should identify the hazard, indicate the $\log_{10}$ reduction achieved for the specified pathogen, describe how the $\log_{10}$ reduction of the pathogen was achieved or determined, specify the actual processing conditions (e.g., time, temperature, and humidity), list critical ingredients (e.g., salt, sugar, and cure), and list the critical product characteristics (e.g., pH, a_w, and fat content). The processing procedures, ingredients, and product characteristics may determine the range of products to which the study applies. For example, if the test product contains additives that increase the heat resistance of *Salmonella*, the process could apply to all products that did or did not include the additives since a worst-case formulation was used in the study. On the other hand, an additive may have a bactericidal effect and thus limit the products to those that contain the additive. Alternative or custom processes must be validated.

Challenge studies are excellent means to validate processes. Validation by a challenge study is based on scientific rationale and provides the necessary data to determine the $\log_{10}$ reduction of the target pathogen. Pathogen challenge studies should be conducted in a testing laboratory and *not* in the processing plant environment. Product sampling results, based on historical data alone, should not be used to validate these procedures because they do not provide information on the incoming pathogen load and, consequently, the level of pathogen reduction achieved is unknown.

Definition

Lethality treatment. A process, including the application of an antimicrobial agent, which eliminates, or reduces the number of, pathogenic microorganisms on or in a product to make the product safe for human consumption. Examples of lethality treatments are cooking or the application of an antimicrobial agent or process that eliminates or reduces pathogenic microorganisms.

44.9 An Example: Canned Beef Stews

Canned beef is a sterile and shelf-stable meat product. This chapter is not a proper forum to discuss the science, technology, and legal requirements of the manufacture of canned beef stew. Instead, Figures 44.9–44.12 provide an overall picture of the safety production of this sterile meat product.

45

Not Shelf-Stable Processed Meat Products[*]

Y. H. Hui

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[*] The information in this chapter has been modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. Used with permission.

45.1 Introduction

In the United States, the Food Safety Inspection Service (FSIS), a unit of the Department of Agriculture (USDA), is responsible for the safety of meat and meat produces. In accordance with the following, please note that, for this chapter:

1. The text and tables have been collated from multiple USDA public documents (legal, regulatory, and training), distributed in USDA'S website, http://www.fsis.usda.gov.
2. The figures were originally adapted from a series of USDA continuing educational documents, *USDA HACCP Generic Models on Not Shelf-Stable Processed Meat Products*. They are available at USDA's website, http://www.fsis.usda.gov.

It must be emphasized that, in this chapter:

1. All information have been designed by the USDA for training purposes only and is not meant to detail the actual manufacturing processes.
2. All abbreviations are standard ones used in law, science, and technology as issued by FSIS.
3. All terms with legal implications apply only in the United States.

Products discussed in this chapter fall into three of the nine FSIS-processing categories:

- Fully cooked—not shelf stable (NSS).
- Heat treated, but not fully cooked—NSS.
- Products with secondary inhibitors—NSS.

Products produced in these three processing categories share the feature that they are NSS products. Products produced in these three processing categories differ in that they include:

1. Not Ready-to-Eat (NRTE), or
2. Ready-to-Eat (RTE) or
3. Both NRTE and RTE products

45.2 Fully Cooked-NSS (RTE)

This processing category includes all meat and poultry products that have been fully cooked, but are NSS. Fully cooked means that these products have been sufficiently cooked so that they are safe to eat as they are, with no further preparation required by the consumer. This is also known as "RTE." *Note:* many of these products are customarily eaten hot, and cooking instructions may be included on the label. This does not affect the classification of these products into this processing category. An example is the hotdog. This product receives sufficient heat treatment to be fully cooked, and does not necessarily need to be reheated by the consumer. Most consumers do, however, heat this product, and cooking instructions may be included on the label of the product. Another parameter that defines this category is that the products are NSS. Shelf stable means that the product has received a treatment that renders it safe to store without refrigeration. This does not apply to this category. These products, although fully cooked, are NSS, and must be kept refrigerated or frozen to maintain safety and quality. Again, the hotdog is a great example. It must be kept refrigerated by the consumer until it is eaten.

The finished products in this category are NSS products and require special handling to maintain their wholesome condition, that is, labeling that states the product must be kept refrigerated or kept frozen.

Some frozen RTE products produced in this processing category require reheating for palatability. These frozen products are still safe to eat without this further preparation by the consumer and are therefore still considered RTE. Some examples of finished products produced under this process category include: fully cooked hams, cooked beef, roast beef, pastrami, corned beef, hotdogs, meat loaves, meat and poultry salads, sliced luncheon meats, baked chicken, frozen entrees, and poultry rolls. These finished products are labeled in a manner that identifies them as RTE by the consumer. Product standards, processing methods, and labeling are all factors that must be considered in determining the regulatory-processing category for RTE products.

A major product group in this category is the cooked and smoked sausage. There are many different types of these sausages made; some common examples are bologna, cooked salami, polish sausage, and hotdogs.

RTE fresh or frozen entrees range from precooked chicken pieces, barbecue beef, to prepared dinners with meat or poultry along with rice, pasta, sauce, vegetables, etc. Fully cooked meat or poultry portions are combined with fully cooked sauces, vegetables, pasta, or other ingredients. These products are designed to be RTE-heated by the consumer, and may include instructions for reheating. If these products are considered fully cooked, they must be safe to eat without the reheating step.

45.3 Heat Treated but not Fully Cooked: NSS (NRTE)

The products included in this category vary quite a bit from each other. What they have in common is that they have received some type of heat treatment, but not sufficient heat treatment to result in RTE product. The finished products in this category are NSS products and require special handling to maintain their wholesome condition, that is, labeling that states the product must be kept refrigerated or kept frozen. These products also require safe handling instructions. Some finished products in this processing category may be prepared with both meat/poultry components that have received a lethality treatment in combination with nonmeat/poultry components that need to receive a lethality treatment. These multicomponent products, for example, meals, dinners, and entrees, have labeling features which are conspicuous so that intended users are fully aware that the product must be cooked for safety. The principle display panel on the label will define these products, for example, “Cook and Serve,” “Must be thoroughly cooked,” “Cook before eating,” and the product will have cooking instructions. Some examples of finished products produced under this process category include bacon, char-marked patties, partially cooked nuggets, frozen entrees or dinners, and low-temperature-rendered products. In the case of bacon, the heat is used to aid the smoking and curing process. For the char-marked patties, the heat is applied to sear grill marks on the meat, but is not meant to cook the product. Nuggets are partially cooked to help set the breading and give it a more appealing color. All of these products must be thoroughly cooked before consumption.

One of the key elements in determining if an establishment is producing products that fall under the regulatory processing category is the finished product label.

However, product standards, processing methods, as well as labeling are all factors that must be considered in determining the regulatory-processing category for NRTE products. Finished products in this category would not have received heat in combination with other treatments that change the product characteristics to promote microbial inhibiting qualities that are enhanced by antimicrobial agents, acidulants, salt, etc.

Char-marked patties are included in this category. These products received a heat treatment on the outside surface that produces a “char-mark” which imitates the marks created from cooking product on a grill. The product is still essentially uncooked, and it is important that product labelings distinguish this product from RTE products. It is crucial that this product be fully cooked by the final user, to ensure safety.

Low-temperature-rendered products are derived from the low-temperature rendering of fresh meat. The products are usually ground, heated, then treated to a process that separates some of the fat from the lean portion. The temperature used must not exceed 120°F. The product is then cooled quickly to

limit potential growth of bacteria at these warm temperatures. The heat treatment is not sufficient to eliminate pathogens or to result in a cooked appearance. The rendered product is frozen and used in further processing operations. If the raw meat trimmings had at least 12% lean meat prior to rendering, the resulting product is called partially defatted chopped (species). If the fatty trimmings (used as raw materials) contain <12% lean meat, the resulting products are called partially defatted (species) fatty tissue.

There are, of course, many other products that you may encounter which fall into this category. The common characteristic is that these products receive some heat treatment, but not enough to result in a fully cooked, RTE product.

45.4 Products with Secondary Inhibitors: NSS (NRTE or RTE)

Finished products produced under this regulatory-processing category can be NRTE or RTE meat and poultry products that have been processed in a manner that utilizes strategies which produce results that will inhibit secondarily the growth of pathogenic bacteria. Finished products in this regulatory-processing category may or may not have had heat applied to the product. The finished products in this category are NSS products and require special handling to maintain their wholesome condition, that is, labeling that states the product must be kept refrigerated or kept frozen. Some of these products also require safe handling instructions if they are NRTE (SHI). However, some products may omit statements in conflict with the product's specific handling instructions from the SHI and replace it with statements such as "refrigerate leftovers." In other words, the product may be heat treated, but not fully cooked, and a secondary inhibitor gives a cumulative effect (heat plus a food additive that affects the product) so that the product is RTE, yet it would not be RTE in the absence of the secondary inhibitor. These RTE products would not be shelf stable. An example would be the addition of salt or sugar in quantities that effectively lower the water activity (A_w) of the finished product.

A_w: Microorganisms in food need water to live and grow. The water must be in a form that is available to the microorganisms. A_w is a measurement of how much water is available in a product. The A_w can be reduced by removing water (drying) or by increasing the concentration of solutes dissolved in the water (adding salt or sugar). The A_w is a measure of the amount of water that is available to support bacterial growth in different foods. Bacteria require a certain amount of "free" water to grow. The more available the water, the faster the bacteria will grow. A_w is lowered by the addition of solutes, such as salt or sugar. These food constituents bind water molecules together, making it unavailable for use by microorganisms.

Acidity: Most bacteria grow best in a medium that is neutral or slightly acidic, and the growth of most bacteria is significantly inhibited in very acidic foods. The pH is measured on a scale from 1 to 14, with 7 being neutral. pH levels from 7 to 14 are basic, or alkaline, while those below 7 are acid. Most bacteria do not thrive in high pH (basic mediums) or low pH (acidic mediums), but prefer pH ranges close to neutral. Adding acid lowers the pH level of the product. Citric acid is a common food acidulant. Lactic acid is also used, but it imparts a fermented flavor, which may not always be a desired quality in the end product. Many foods are acidified to prevent the growth of undesirable microbes. This may be done by adding acidic ingredients, like tomatoes, or by adding the acid directly, like vinegar. The acidity of products may also be increased by the process of fermentation.

Fermentation: During this process, the pH level of the product is reduced by starter culture activity and by appropriate time/temperature factors. The starter culture is added to the meat or poultry mixture, and held in an environment optimum for their growth.

The temperature and humidity are carefully monitored. The starter culture bacteria actively reproduce, and as they do they give off lactic acid, which lowers the pH of the product. It is important that lactic acid is produced quickly, because it inhibits undesirable bacteria, like the toxin-producing *Staphylococcus.* The pH is monitored over time to determine when the process is complete. During fermentation, the establishment will probably want to achieve a pH of 5.0 or less within a certain time.

pH: The symbol pH, which stands for the hydrogen-ion concentration of a product, is an expression of the degree of acidity or alkalinity of a substance.

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Acidity Neutral Alkalinity

Most microorganisms thrive on pH near neutral, 7.0, although there are exceptions. Meat and poultry processors can control pH to limit microbial growth and give meat and poultry a longer shelf life. A pH below 7 indicates the degree of acidity, whereas a pH above 7 indicates the degree of alkalinity. The pH of fresh meat ranges between 5.3 and 6.4. Higher pH meat (6.0–6.4) spoils faster than meat in the lower pH range (5.3–5.7), because the spoilage bacteria are more active at the higher pH.

Drying: Drying is the process of dehydration in which osmosis withdraws water from the cell of the spoilage organisms, shriveling or inactivating the cells. The product must be dried to the point at which bacterial growth is slowed or the bacteria are inactivated or destroyed to create a shelf-stable product.

The process of using secondary inhibitors is a very complex system. Often several different inhibitors are used, each depending on the others to result in a safe product. For example, preservation methods that use large amounts of salts or sugars work by reducing the A_w. An ingredient that changes the pH to a level that inhibits bacterial growth is another example of a secondary inhibitor. The added ingredient is the secondary inhibitor because it does not inhibit the growth, but changes the pH of the product to the level that the pH inhibits the growth. This regulatory-processing category is only used when products do not fit into one of the other categories. The food safety system is designed around monitoring the secondary inhibitor for products to be regulated under the HACCP element.

Some examples of products that may fall into this processing category include products that are uncooked, cured, fermented, dried, salted, or brine treated, which are NSS but can be RTE or NRTE, such as sliced country-style ham, salt pork, and semidry-fermented sausage. The product standards, processing methods, and labeling are all factors that must be considered in determining the regulatory-processing category for the products.

45.5 RTE Establishment Sanitation

45.5.1 Background

What are some of the sanitation issues essential to the production of safe RTE products? In this section, we will discuss some of the establishment's responsibilities for sanitation in the RTE environment. Establishments producing RTE products have a special responsibility for sanitation because of the high risk of foodborne illness due to postlethality contamination.

Before we go further, let us define some of these terms. RTE products have received a *lethality* treatment. The lethality treatment, generally a cooking procedure, must be designed to eliminate pathogens, or harmful bacteria. This lethality treatment makes the product safe to eat by the consumer without additional preparation to achieve food safety, and we normally refer to these products as "RTE."

Many RTE processes involve handling the product after it has been subject to an initial lethality treatment (*postlethality exposure*). When the product is directly exposed to the environment, it can become cross-contaminated. *Cross-contamination* is the transfer of bacteria and possibly pathogens to the exposed RTE product after the lethality treatment. These bacteria can come from the environment, from the employees, or from the equipment. They can be transferred directly, such as when an exposed RTE product is placed on a tabletop which has bacteria on it. Often they are transferred indirectly, such as when a pallet placed on the floor in the raw area is subsequently used in the RTE area, or when an employee handles a pallet and then touches the exposed product.

Many RTE products are taken right from the package and consumed as they are, with little or no heat treatment. If any pathogens are present, they will be consumed along with the product. Thus, the risk of

these products producing foodborne illness is increased. Because of this, establishments producing these products have an increased responsibility for sanitation of the RTE-processing environment.

45.5.2 Processing Environment Sanitation

Sanitation is critical for ensuring that RTE products do not become cross-contaminated. Sanitation Standard Operating Procedures should be established to provide effective and consistent results. Effective sanitation is a complex process. A successful establishment must understand and apply the cleaning and sanitizing process and select the proper methodology and chemical agents for the particular environment and equipment being cleaned. RTE sanitation may include the following steps:

1. Dry/waterless cleaning, removing large debris
2. Prerinsing equipment
3. Foaming and scrubbing
4. Rinsing
5. Visual inspection of equipment
6. Sanitizing
7. Drying

Cleaning consists of removing the soil from the equipment and environment. The "soil" in this case is product residue, which provides nutrients for bacterial growth.

Sanitizing is the application of either heat or chemicals to substantially reduce the numbers of microorganisms to an acceptable level. In some situations, the acceptable level for a specific pathogen is zero. Sanitizing is done after cleaning, because a sanitizer cannot work effectively unless the equipment is cleaned first. Chemical solutions commonly used are chlorine, iodophors, or quaternary ammonium compounds. To be effective, the sanitizer must be applied at the appropriate temperature and concentration for the proper length of time. Heat, usually in the form of hot water, is also sometimes used to sanitize.

Rotating sanitizers is generally a good practice as it will provide greater effectiveness against bacteria. Some establishments rotate sanitizers by using one for a certain time period, and then switching to a different one. Another way to rotate sanitizers is to use them for different applications, for example, using one on equipment and a different one in boot dip stations. Alternating between alkaline-based detergents and acid-based detergents also helps avoid "soapstone" or hard-water buildups. Alternating detergents helps change the pH regularly to prevent the adaptation of bacteria to a particular environment. Therefore, rotating sanitizers can help in preventing the formation of biofilms. A *biofilm* is a thin layer of microorganisms that essentially glue themselves together to adhere to the surface of equipment or other structures. Because of the nature of this film, the bacteria are difficult to remove and are protected from chemicals used to clean and sanitize the surfaces.

45.5.3 *Listeria Monocytogenes*

Establishments are responsible for producing product that is free from any pathogen. However, one strain of the *Listeria* species, the pathogen *Listeria monocytogenes* (*Lm*), is of particular concern because it has potentially fatal consequences. *Lm* is especially pathogenic to high-risk populations, including pregnant women, newborns, elderly, and people with weakened immune systems. Because *Lm* is found in the intestines of healthy animals (including humans) and in the environment in which food-producing animals are raised and processed, for example, in soil, water, and vegetation, it can be continuously introduced into the processing environment. *Lm* can contaminate the surfaces of equipment, floors, walls, drains, overhead structures, etc.

Lm is spread very easily by direct contact with a contaminated surface. *Lm* can survive and grow in cool, damp environments such as those found in process areas, coolers, or floors. Incomplete removal of

product debris can provide nutrients and a place of attachment which allows bacterial growth. *Lm* can form biofilms on solid surfaces, such as stainless steel and rubber, and can survive adverse conditions on apparently smooth surfaces.

Lm is more heat resistant than most foodborne bacterial pathogens and also can survive freezing and drying. *Lm* is very hardy. It can survive and grow in refrigerated, packaged, RTE products and resists high salt levels, nitrite, and acid. In addition, it can grow in vacuum-packaged products. Thus, it is vitally important for an establishment to maintain strict sanitary controls to prevent the organism from contaminating products.

Foodborne illnesses and deaths have been linked to some recalled products adulterated with *Lm*. It has generally been concluded that the adulteration occurred through cross-contamination from environmental sources after cooking.

45.5.3.1 Plant Environment

Plants must focus on preventing contamination of RTE products with *Lm*. *Lm* can be introduced by contaminated food contact surfaces, by employees, or by environmental reservoirs. Cross-contamination occurs when postlethality exposed RTE product directly contacts a surface that has been contaminated with *Lm*. In order to prevent cross-contamination, establishments need to ensure that sanitation is effectively maintained, with special attention being given to those areas where product is stored or handled after a lethality treatment has been applied to the product.

Sanitizers that have proven most effective against *Lm* are quaternary ammonia compounds, chlorine solutions, and newer products containing peracetic acid.

Because this organism needs moisture to grow, keeping storage and production areas and equipment as dry as possible helps reduce the opportunity for *Listeria* to reproduce. For example, product contact equipment, and production area floors should be maintained free of standing water and kept as dry as possible.

45.5.3.2 Construction

Lm contamination has been linked with disruptions in the production process or environment. In particular, disruptive construction (e.g., breaking out walls or other activities that can generate dust) has been shown to have a clear association with *Lm* contamination of both product and the surrounding environment. *Lm* can survive in moist, enclosed areas of the environments, such as cracks in walls and floors, and in crevices around drains; often these areas are disturbed during construction.

Dust generated by construction and other disruptive activities can establish contamination on food contact and other environmental surfaces. For example, dust can travel throughout the plant on air currents or be transferred by people or equipment traveling through the construction area into other areas of the establishment. Dust from construction can be difficult to detect and control. Therefore, increased monitoring of product, food contact surfaces, and the environment is recommended during and after these disruptive events.

Some examples of disruptive construction activities include

- Removal of drains.
- Removal of floor coatings.
- Removal of a wall or ceiling that has absorbed moisture.
- Movement through an RTE area of potentially contaminated materials.
- Exposure of an area typically not accessible for cleaning.

Establishments have the responsibility to control establishment activities during construction to ensure that only safe food is produced. When construction is necessary, there are several solutions that establishments may employ. Establishments may establish negative air pressure in the construction area to ensure that air does not flow from the construction area into the plant. Temporary partitions can be

established to protect the undisturbed areas of the plant from construction dust and debris. Intense cleaning is also a control method used by establishments following the disruptive construction.

45.5.4 Possible Postlethality Contamination Sources: Direct Food Contact

Cleaning and sanitizing are very important. Pathogens can be transferred to RTE products from equipment and employee hands that have not been adequately cleaned and sanitized.

Protecting exposed RTE product after the initial lethality step as it moves throughout the plant is an important consideration in preventing postlethality contamination. Products that are inadequately packaged or covered or contained in damaged packaging could become contaminated. Packaging or covering used to protect RTE products during processing and storage should be adequate for preventing the entry of contaminants.

Personnel hygiene practices are essential to prevent RTE product cross-contamination. Pathogens could be present on the hands of workers handling the food. The hands are particularly important in transmitting foodborne pathogens. Workers with dirty hands or fingernails may contaminate the food being prepared. Any activity which may contaminate the hands must be followed by thorough hand washing. Examples of activities which may contaminate the hands include using toilet areas, picking up any item on the floor, handling boxes, and touching door handles or other hand-contact devices. The hands of employees can be contaminated by touching their nose or other body parts. Hand washing is a critical factor in reducing pathogens that can be transmitted from hands to RTE products. Many employees fail to wash their hands as often as necessary and even those who do may use flawed techniques.

Examples of postlethality contamination sources:

- Solutions to chill foods, brine solutions
- Slicers, dicers, saws
- Casing peelers
- Lugs, tubs, containers
- Hand tools
- Packaging materials and equipment
- Tables, conveyors, belts
- Door pulls and equipment controls (employee hand contact areas)
- Employee hygiene and handling practices

45.5.5 Potential Reservoirs in the Environment: Nonfood Contact

Processing activities may expose the RTE product to an environment that may lead to the product's contamination. Products must be protected during processing. Workers' shoes can carry contamination onto the floors of food preparation and storage areas. Even trace amounts of refuse or wastes in rooms used as toilets or in rooms used for storing trash or housing equipment can become sources of food contamination. Moist conditions in storage areas promote microbial growth. Sources of environmental contamination may include splash from cleaning operations, drips from overhead ventilation units, or air from an insanitary atmosphere. Bacteria can be conveyed considerable distances on air currents through fine sprays or aerosols. This could originate, for example, from a water spray directed at a drain. Ice that has been in contact with unsanitized surfaces or raw meat or poultry products may contain pathogens. If this ice contacts RTE products or food contact surfaces, it could contaminate the final product.

Examples of environmental reservoirs:

- Floors and drains
- Standing water, for example, in overhead drip pans

- Ceilings and overhead pipes
- Refrigeration units
- Wet insulation
- Equipment motor housings
- Cleaning tools: mops, brushes, squeegees, or other utensils
- Employee hands, gloves, and aprons
- Overhead rails and trolleys
- Maintenance tools
- Pallets
- Forklifts
- Any recessed or hollow material: rollers, switch boxes, motor housing
- Rusted materials
- Cracked or pitted rubber hoses or seals
- Walls that are cracked or pitted, and inadequately sealed surfaces
- Vacuum or air pressure pumps, lines, and hoses
- Ice makers
- Air filters

45.5.5.1 Considerations in Plant Design

There are important considerations in the layout of the plant and the location of postlethality processing. Cross-contamination can be avoided by separating raw meat and poultry from RTE products. Cross-contamination may also occur when raw unprepared vegetables contact RTE foods.

Air flow is another important concern. Air will flow from areas of high pressure to lower pressure. Air flow can be influenced by the location and operation of refrigeration units and other types of ventilation equipment. Air flow from a raw product area into an RTE product area could possibly carry *Lm* and contaminate the RTE product or product-contact surfaces.

An effective plant design will include sufficient ventilation to prevent the formation of condensation and control humidity.

Many different types of plant layout exist. It is the establishment's responsibility to control the processing procedures in whatever environment exists in its particular plant to ensure that only safe product is produced.

Always give consideration to the following when developing the plant design:

- Traffic between raw and RTE areas
- Physical proximity between RTE and raw products
- Air supply and flow between raw and RTE areas
- Humidity
- Overhead fixtures that harbor dirt or moisture
- Condensation
- Plumbing from drip pans

45.5.6 Sanitation Program to Control *Lm*

An environmental testing program can be a means of confirming that the establishment's controls are effective in maintaining a plant environment that will minimize the hazard of pathogens, including *Lm*.

In addition to measuring the effectiveness of a sanitation program, a correctly designed environmental testing program may

- Provide information about sources of environmental contaminants.
- Identify the extent of pathogen contamination of the environment.
- Provide information about faulty equipment design or operation.
- Identify probable postlethality cross-contamination sites.

The *design* of the testing program will vary depending on the purpose, the microorganisms tested for, the sampling method used, and the analytical methods. Some establishments will design their program for the purpose of measuring the effectiveness of the cleaning procedures; other testing programs might be designed to verify that finished product is free of a certain pathogen, for example, *Lm*. Some establishments test for organic residues. Another establishment might test for spoilage organisms, for example, yeast and mold, coliforms, or nonpathogenic bacteria. Other establishments might test for indicators of potential pathogens, such as *Listeria* spp. There are a wide variety of sampling methods used and many more are being developed. Some examples are swabs and sponges used with standard plating methods, prepared plates and other testing kits of various types, and air collection systems. The amount of time it takes test results to be available also varies from several minutes to several days, depending on the type of method used.

45.5.7 A Short Field Observation

However, this chapter is not the proper forum to show how an FSIS inspector or a quality control works. A short field observation here illustrates a reality check. Try to answer the questions raised.

You are assigned to a large RTE establishment that produces a variety of sliced, cooked ham products. Raw meats are extruded into a plastic bag, placed in a metal form, and cooked in a water bath. After cooking and cooling, the meat is removed from the bags, sliced, and sealed in 8-oz plastic film packages. From the standpoint of postlethality contamination, the slicing area is the most critical. Why is this so?

Observe the slicing process. The cooked meat is dumped out of the metal forms onto a stainless-steel table by one person. A second worker opens the bags with a knife and removes the meat from the bags, pushing the unwrapped meat toward the other side of the table. In that area, a third worker places the meat into the slicer. The slicing equipment deposits the sliced meat into the packaging machine, where it is sealed into the film packages. One can observe several potential food contact surfaces in this scenario. What are they?

This establishment is updating the equipment in one of the coolers. You decide to observe. You see that there is no product in the cooler and that construction workers wearing frocks over their street clothes are dismantling the fans, overhead refrigeration units, pipes, and drains. Other workers are replacing the material on the walls. There is debris from the construction scattered about. You observe a construction worker load some of the old materials and equipment onto a pallet and take it out through the only door. This door opens into the main hallway in this plant, used by all departments. There is no covering over the old equipment on the pallet. The other departments are working as usual. There are many concerns in this situation. Explain what they are.

45.6 Biological Hazards

During the development and design of sanitation standards and operation and the HACCP plan, the official establishment determines whether there are any biological, chemical, or physical hazards that are

reasonably likely to occur before, during, or after entry into the establishment. A food safety hazard is defined as any biological, chemical, or physical property that may cause a food to be unsafe for human consumption. What are biological hazards for nonshelf-stable meat products?

45.6.1 Examples

Potential biological hazards in meat and poultry include bacteria, toxins, viruses, protozoa, and parasites. Of the biological hazards, the most important are bacteria that can cause either food-borne infections or intoxications. Bacteria cause a large proportion of all food-borne illnesses. Bacteria that cause human illness, including disease, are termed pathogenic. Most food-borne illness can result in diarrhea, nausea, vomiting, stomach cramping, and in more serious cases, organ damage, stillborn births, and death.

The pathogens that are most likely to be found in commonly slaughtered livestock (cattle, sheep, and swine) and poultry (chicken and turkey) include *Salmonella*, *Campylobacter*, and *Lm*. In addition, *Yersinia enterocolitica* has been isolated from swine as well as being detected in the environment. *Lm* also is widespread in the environment and is often present in soil, water, and silage. Although *Escherichia coli* also is found in livestock and poultry, most forms of *E. coli* are not pathogenic. *E. coli* O157:H7 is pathogenic. The ultimate source of all of these pathogens is apparently healthy animals that may shed these bacteria in their feces. While dressing the carcasses during the slaughter process, these bacteria may be transferred from the hide and offal to the carcass-causing contamination. All these pathogens have been implicated in widely publicized food-borne disease outbreaks associated with the consumption of meat and poultry products. In the production of the NSS NRTE/RTE products, pathogenic bacteria and zoonotic agents should be considered while conducting the hazard analysis. Proper cooking, fermentation, cooling, and storage of food can destroy and/or prevent the growth of these bacteria. Zoonotic agents are biological hazards that cause disease in animals which can be transmitted to and cause disease in humans.

Some types of bacteria produce toxins in NRTE/RTE products as a by-product of their growth. Toxins of most concern are produced by *Clostridium botulinum*, *Clostridium perfringens, Bacillus cereus*, and *Staphylococcus aureus*. All are the result of the growth of bacteria and the production of toxins in foods that have been mishandled. These bacteria are common in the environment and are often found on carcasses. The environment (air, water, and soil) is the common source of these types of bacteria. An exception is *S. aureus*, which is commonly found in association with human skin, and sometimes in poultry bruises. The hazard is the toxin (e.g., enterotoxin, neurotoxin, hemotoxin) produced by the organisms. The organism may contaminate the product if improper handling occurs after the product has been cooked. Proper cooking, fermentation, cooling, and storage of food can prevent growth of these bacteria and, more importantly, the production of their toxins. However, cooking will not destroy several of these toxins once they are formed in food.

Parasites (parasitic worms) of public health importance are the beef and porktapeworms (*Cysticercus bovis* [the larvae of the human tapeworm,-*Taenia saginata*] and *Cysticercus cellulosae* [the larvae of the human tapeworm—*Taenia solium*] respectively) and the roundworm that causes trichinosis (*T. spiralis*). Federal and state inspection program personnel can observe the immature stages (cysts) of tapeworms in carcasses of animals with severe infection and when detected by government inspection personnel or plant employees such product cannot be further processed for human consumption. When the cysts are less severe or evident, infected meat may enter the human food chain after it has been appropriately treated. Humans consuming undercooked meat infected with these tapeworms become ill generally after the mature stages of the tapeworms invade the intestinal tract.

T. spiralis is an intestinal worm that produces larvae that migrate to and encyst in muscles of a number of animals, particularly swine. Humans consuming infected pork which is undercooked may get ill from the cysts which then live in the muscles of the human hosts. Pork muscle tissue may carry *T. spiralis*, better known as trichinae. *T. spiralis* is an additional biological hazard that may be addressed in the manufacturing of processed pork product, especially if the product is intended to be eaten without thorough cooking by the consumer.

Tapeworms and roundworms generally are readily destroyed at cooking temperature and time combinations less rigorous than the combinations necessary to destroy pathogenic bacteria.

Viruses can cause gastroenteritis and diseases such as hepatitis and polio in humans. The presence of viruses in food and water is generally associated with a contaminated food worker, usually in the retail or food service arena. In general, healthy animals do not serve as carriers of viruses. Viruses do not normally grow or reproduce in meat and poultry products. Meat and poultry products may serve as vectors for viruses.

Protozoa can cause diseases such as sarcocystosis, toxoplasmosis, and cryptosporidiosis. These organisms are not usually considered under food safety concerns for meat and poultry products.

45.6.2 Public Health Concerns

It is particularly important for establishments to identify and control these biological hazards in RTE products, because the consumer is not expected to cook these products further. In NSS RTE products, the lethality step is the intervention that protects the consumer from most biological pathogens. However, *Lm* can pose a food safety risk in NSS RTE products if this pathogen is not properly controlled postlethality in the processing environment. Products that are NSS heat-treated can pose a food safety risk if some pathogenic bacteria remain on the product which must be controlled. In addition, products that are NSS and produced with secondary inhibitors, especially those that may be RTE, can pose a food safety risk if the pathogenic bacteria are not prevented, eliminated, or reduced to acceptable levels as many of these products have not had heat applied but rely on other strategies to control the food safety hazards. Table 45.1 shows the types of bacteria of concern for NSS NRTE/RTE products.

There are definite public health concerns associated with bacterial toxins in NRTE/RTE products. Toxins may be produced during the growth phase of some bacteria. These are called preformed toxins, such as *C. botulinum* toxin, and when they are ingested, they may cause headaches, disorientation, neurological damage, paralysis, and possibly death. The toxins may affect the nerve endings and interfere with nerve impulses. In the case of botulism toxin, the diaphragm is affected and death results from suffocation. Other toxins are formed when the vegetative cell produces a spore. Some spore-forming bacteria form spores in the human digestive tract because the digestive juices are too harsh for the vegetative cell and the self-preservation mechanism (spore formation) is turned on. This is true for *C. perfringens*. The common form of perfringens poisoning is characterized by intense abdominal cramps and diarrhea which begin 8–22 h after consumption of foods containing large numbers of those *C. perfringens* bacteria capable of producing the food-poisoning toxin. Table 45.2 shows the types of toxin-producing bacteria of concern for NSS NRTE/RTE products.

A public health concern associated with zoonotic agents is trichinosis, a disease that results from ingesting pork cysts in undercooked pork products. Due to improvements in animal production processes, trichinosis is quite rare in this country. Once ingested with the meat, the larvae bore through the intestinal wall and travel through the blood and lymph to encyst in muscles. If there is a heavy-enough

TABLE 45.1

Types of Bacteria of Concern for NSS NRTE/RTE Products

	Organisms				
Species Harboring	***Salmonella***	***E. coli* O157:H7**	***Campylobacter***	***Lm***	***Y. enterocolitica***
Beef	+	+		+	
Lamb	+			+	
Pork[a]	+			+	+
Poultry	+		+	+	

Source: Modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. Used with permission.

[a] Trichinae control may be required in certain products.

TABLE 45.2

Types of Toxin-Producing Bacteria of Concern for NSS NRTE/RTE Products

	Organisms			
Species Harboring Organism	***S. aureus***	***C. perfringens***[a]	***C. botulism***[a]	***Bacillus***[a]
Beef	+	+	+	+
Lamb	+	+	+	+
Pork	+	+	+	+
Poultry	+	+	+	+

Source: Modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. Used with permission.

[a] Spore formers.

infestation, based on the amount of *T. spiralis* ingested, the encysting process within muscles is painful. If the worms encyst in the diaphragm, they may interfere with breathing.

45.6.3 Control Methods

It is crucial to avoid the contamination of meat and poultry whenever possible. This includes inadvertent contamination from the live animal, processing procedures and equipment, employees, and the environment. Contamination can be minimized or avoided altogether by following appropriate sanitation procedures, good manufacturing practices, and procedures for employee hygiene. The term "cross-contamination" generally refers to the transfer of organisms from a contaminated source to a previously uncontaminated surface. A particular concern is the cross-contamination of RTE foods with NRTE (raw or partially cooked) meat or poultry, or with purge from NRTE meat or poultry. It is particularly important to ensure complete separation of NRTE and RTE products.

Some bacteria, such as *Listeria* (including *Lm*), can be found in the processing environment. Although most forms of *Listeria* are not pathogenic, *Lm* is a pathogen. This emphasizes the need for adequate sanitation, not only of the equipment and product contact zones, but also the floors, walls, drains, etc. Employee hygiene, air flow, and traffic flow of people and equipment between areas used for NRTE processing and RTE processing is very important and should be strictly controlled.

Recontamination with bacteria (e.g., *Lm* and *Salmonella*) must be considered as cooked products are exposed to the environment, food contact surfaces, or raw product prior to final packaging.

Recognizing that bacteria will be present on meat and poultry, it is important to keep the overall number of bacteria very low so that pathogens can be minimized. Temperature, acidity, salt and drying, and combinations of these can be used to restrict growth of pathogens.

Temperature: The growth of most bacteria can be slowed down (controlled) by keeping the product at refrigeration temperatures (<41°F), or by freezing. Some bacteria survive freezing, and so freezing cannot be considered a method to eliminate bacteria. Holding products at higher temperatures (>130°F) also restricts the growth of the bacteria.

Acidity: Fermentation restricts the growth of bacteria of public health concern by increasing the acidity (lowering the pH) of the product. Generally, a pH of <5 will severely restrict or completely stop the growth of harmful bacteria. Some bacteria can survive in acidic conditions, and so fermentation alone cannot be relied upon to completely eliminate all harmful bacteria.

Salt and Drying: Some products contain high levels of salt. Salt and low-moisture content in a product can be effective in controlling growth of some harmful bacteria, but some organisms (e.g., *S. aureus*) survive in high-salt environments.

Most pathogenic bacteria, including *Salmonella, E. coli* O157:H7, *Lm*, *Y. enterocolitica*, and *Campylobacter*, can be fairly easily destroyed using a rather mild-cooking process—maintaining a minimum temperature within the range of 130–165°F for a specific amount of time. However, cooking at this temperature range and for the specified dwell time will not destroy the heat-resistant forms (spores) of certain bacteria, nor will some types of toxins be destroyed if they have already been

formed in the product. Thermal processing (canning) at a minimum retort temperature of >240°F for a specific amount of time is necessary to destroy most spores and toxins. These bacteria are killed by proper cooking.

Spore-forming bacteria (*C. perfringens* and *C. botulinum*) can survive cooking when in the heat-resistant spore form, and these organisms need to be considered as the products are chilled. Growth (sometimes referred to as "outgrowth") of these bacteria is slowed down by proper cooling.

The types of controls used for products in the regulatory-processing categories will vary because they represent different production processes (with or without inhibitors). For example, the controls used for some products without the use of inhibitors will include cooking (lethality), because by definition, this processing category is fully cooked and RTE. The controls used for products involving secondary inhibitors include those that affect pH level or lower A_w (e.g., added salt).

Some plants use postlethality pasteurization, which involves running the intact package through steam or hot water to bring the product surface up to a temperature sufficient for the purpose of destroying *Lm*. However, only the surfaces in contact with the outer-packaging material reach these temperatures. If the product is sliced, those sliced surfaces in contact with another sliced surface do not reach the required temperature. Likewise, hot dogs in a package would not reach the temperature required to ensure the destruction of *Lm*. So for a sliced product, or for packages with multiple pieces of the product, postlethality pasteurization will not heat all product surfaces sufficiently to ensure the destruction of *Lm*. Therefore, the antimicrobial inhibitors are currently the control method of choice. Whatever control measure is used for *Lm*, the establishment should validate their process to demonstrate that the finished product are produced and distributed under conditions that will prevent or inhibit the growth of the pathogen.

Antimicrobial sprays are an example of a control measure used by establishments. Certain antimicrobials are approved as food additives for meat and poultry products (which means their use must be declared on the label). These compounds can be formulated in the product to inhibit the growth of certain pathogens. Some antimicrobials may be used as sprays or immersion dips at room temperature. Some antimicrobials are considered incidental additives by the Food and Drug Administration (FDA). Incidental additives are substances present in foods at insignificant levels and that do not serve a technical or functional effect in that food (their use is not declared on the finished product label). These antimicrobial agents are effective against *E. coli* O157:H7, *Salmonella*, and *Lm*.

Salmonella, *E. coli* O157:H7, *Campylobacter*, and *Lm* may all be effectively controlled (destroyed) by cooking to an internal temperature of 158°F for meat and 160°F for poultry products. Time and temperature may also be combined so that a product held for longer periods of time at lower temperatures (130°F or more) may receive the same lethality benefit.

Lm raises different concerns in the hazard analysis than do the other bacteria affecting NRTE/RTE products. While *Lm* may enter the establishment on/in the commonly slaughtered species, the lethality step destroys it. But *Lm* is commonly found in the plant environment too, which is a primary concern in RTE products. The results of microbiological testing show that *Lm* can be found in vents and drains and on equipment, including product contact surfaces. It thrives in warm to cool, moist environments, which is typical in an RTE-processing area. Slicers and peelers are key locations that can serve as harborage sites or niches in which *Lm* can recontaminate fully cooked product. Both pieces of equipment provide a moist environment and contain crevices where the bacteria can accumulate. The moving parts come into direct contact with product that has already received a lethality treatment. Because of the lethality treatment, the competitive microbes have been destroyed, leaving a virtually sterile surface exposed to any contaminant that contacts it. Plants must be vigilant in equipment sanitation and keep the area as dry and free of product residue as possible. Product residue may protect bacteria from harsh cleaning or sanitizing agents. When a product picks up contaminants from other sources, it is referred to as cross-contamination. Even though the lethality step destroys *Lm* in the product, postlethality handling and processing may result in recontamination of the exposed product, and there is usually not a sufficient kill step after that *Lm* contamination is often a result of postlethality contamination.

Toxins are stable under most conditions, and so for nonthermally processed foods, preventing toxin formation is the only true control that establishments can apply. Most of the bacteria that produce toxins are spore-formers. Spore-forming bacteria pose particular problems because they may survive cooking

when in their heat-resistant spore form. The bacterial sporulation is inhibited at cooler, refrigerated temperatures, and should be considered during the chilling step of production. For this reason, stabilization is important in controlling spore-formers. For some products, proper cooking and fermentation may control toxins. Postlethality handling of product is also important in controlling contamination with *S. aureus* (e.g., employee hygiene).

For zoonotic agents, the establishment may have little to no impact on preventing this hazard from entering the plant. However, the plant can destroy the parasite in NRTE/RTE products during the production process through various processing interventions (cooking, freezing, manipulating the A_w with salt, etc.). In fully cooked products, parasites are destroyed during the lethality step.

45.7 Chemical Hazards

45.7.1 Examples

Chemical hazards that may affect NRTE/RTE products usually originate from five general sources:

1. *Agriculture chemicals:* pesticides, herbicides, animal drugs, fertilizers.
2. *Plant chemicals:* cleaners, paint, sanitizers, oils, lubricants, pesticides.
3. *Naturally occurring toxicants:* products from plant, animal, or microbial metabolisms such as mycotoxins, aflatoxins, lupin alkaloids, ergot, erucic acid, phomopsins, phytoestrogens, allergens, etc.
4. *Food chemicals:* preservatives, acids, food additives, sulfating agents, processing aids, etc.
5. *Environmental contaminants:* lead, cadmium, mercury, arsenic, tin, dioxins, or polychlorinated biphenyls.

The potential health consequences of exposure to chemicals in food can be serious, are often inadequately understood, and deserve consideration. The long-term and cumulative effects of exposure associated with chemicals in food pose special difficulties in identifying and addressing these risks. The constant introductions of new chemicals that find their way into food also continue to present management challenges. It is apparent that at least some of the identified chemical hazards are of concern because they exert particular effects. For example, pesticides such as organochlorines, industrial chemicals such as dioxins and chemicals present naturally in food such as phytoestrogens may be of concern because they have the potential to cause endocrine effects and/or interfere with the immune system. Similarly, some chemicals are of concern because of their potential to act as allergens. When food additives exceed approved amounts or when used in higher concentrations, these chemicals may create a food safety hazard. For example, nitrites and nitrates can be toxic in high concentrations. Some hazards such as lead contamination can affect a certain population—infants or young children—causing toxic effects. Lead, in addition to being a chemical hazard, may be a physical hazard.

45.7.2 Public Health Concerns

The most common well-documented public health concerns associated with chemical hazards in NRTE/RTE products relate to the short-term effects of allergens. Food allergies are responses of the immune system to naturally occurring proteins in certain foods that most individuals can eat without any adverse effect. Frequently, such reactions occur because the presence of the allergenic substances in the foods is not declared on the food label. Evidence indicates that some food allergens can cause serious reactions in sensitive individuals on ingestion of very small amounts, and therefore, the presence of an ingredient that contains an allergen must be declared on the product label. For instance, allergens that are ingested by persons who are sensitive to them can cause a range of symptoms that may affect breathing, cause skin rashes, result in digestive disorders, and in more serious cases result in death due to anaphylactic shock. The symptoms usually begin with breathing difficulties and may progress to the point of death by suffocation.

There is scientific consensus that the following foods can cause serious allergic reactions in some individuals and account for more than 90% of all food allergies. The "Big 8," or top eight foods that most people with food allergies are allergic to, are peanuts, soybeans, milk, eggs, fish, crustacean, tree nuts, and wheat gluten.

Other potential causes of adverse reactions (food intolerances) in sensitive individuals are due to ingredients such as monosodium glutamate, sulfites, lactose, and Yellow 5 (tartrazine). The adverse reactions to these substances are due to the ingredients itself or its chemical composition.

Long-term effects of chemicals are associated with exposure over a period of years (e.g., lead poisoning in infants or nitrites and nitrates, which have been determined to be carcinogenic). When product that contains excessive amounts of residual nitrites and nitrates is ingested, the health consequences may be deadly. Chemical residues have been linked through research to various types of cancers. The public health concerns associated with the long-term effects of exposure to chemicals from ingestion of food are not well understood or well documented.

45.7.3 Control Methods

Under the Federal Meat Inspection Act, the Poultry Products Inspection Act, and the Egg Products Inspection Act, under which FSIS operates, all ingredients used to formulate a meat, poultry, or egg product must be declared in the ingredients statement on product labeling. A product is *misbranded* under the Acts when it contains ingredients that are permitted, but are not declared, on product labeling.

Manufacturers are responsible for ensuring that food is not adulterated or misbranded as a result of the presence of undeclared allergens. There may be situations where these substances are added intentionally to food, but not declared on the label. Other situations involve substances unintentionally introduced into a food product and consequently not declared on the label. When an allergen, not formulated in the product, is identified as likely to occur in the food due to the firms' practices (e.g., use of common equipment, production scheduling, rework practices), then you should determine whether the establishment has identified and implemented controls to prevent potential allergen cross-contact, for example, dedicated equipment, separation, production scheduling, sanitation, and proper rework usage (like to like).

Some establishments may consider food allergens in their hazard analyses if there is a potential, but may address this issue through SSOP or other prerequisite programs (e.g., segregation and labeling).

The basic controls that the plant may apply for other chemical hazards include using chemicals as approved, using them for the intended use, using them at the appropriate amount or concentration, and properly storing the chemicals. The establishment should use in-house control of all chemicals by maintaining the identity of the chemical or by properly labeling-processed products to identify any known allergens that may be present in the food. Prevention may be achieved when the plant institutes a specification program for incoming materials, such as suppliers' letters of guarantee on packaging material or nonmeat ingredients. In order to be applicable for use in meat and poultry plants, the label on chemicals must include directions and precautions for such use as required by Federal Insecticide, Fungicide, and Rodenticide Act regulations. Accordingly, any such preparation offered for use in federally inspected meat and poultry plants must be labeled in compliance with those requirements.

Documentation substantiating the safety of a chemical's use in food-processing will vary with the nature and intended use of that chemical. For example, for a pesticide, an establishment should have documentation showing that the compound is registered with Environmental Protection Agency (EPA) and the label information for the pesticide.

For a chemical sanitizer used on food contact surfaces, an establishment should have documentation showing that the compound complies with FDA requirements (Sanitizers meeting this requirement are usually identified as "Food Grade."). Meat and poultry establishments are responsible for ensuring that all proprietary substances and nonfood compounds are safe for their intended use and used appropriately. In some cases, other Federal Agencies require that chemicals meet their regulatory requirements. For example, EPA requires that pesticides be registered with EPA, labeled as such, and used only in accordance with approved instructions. An establishment should have documentation indicating that they meet these requirements.

Also, many chemicals, such as food contact surface sanitizers and lubricants used in food-processing areas must meet FDA requirements. Again, the establishment must have documentation demonstrating this. Often, the statement "Food Grade" on the label of these and other chemicals indicates that they meet FDA requirements. Many chemical uses (antislip compounds, laundry soaps, etc.) are not approved for use by any Federal agency. An establishment will likely have labels, instructions, or letters of guarantee from the manufacturer to substantiate the safety of these chemicals in a food-processing environment. In some cases, documentation will state that the chemical use was previously approved by USDA or FSIS and that the formulation and use have not changed since that approval.

Nitrites/nitrates are interesting in that while they are needed for food safety (to prevent *C. botulinum* spores from germinating and forming the botulism toxin), in excess they can be lethal. So in the case of nitrites/nitrates, adding too little may actually be a food safety hazard because these food additives are in some products to impart a measure of food safety. Therefore, strict control over the storage and application of these chemicals is essential. A plant must have creditable supporting data for the chemicals it uses in its facility.

45.8 Physical Hazards

45.8.1 Examples

Physical hazards include a variety of materials referred to as extraneous materials or foreign particles or objects. A physical hazard can be defined as any physical material not normally found in a food that can cause illness or injury to a person consuming the product.

Physical hazards in finished products can arise from several sources, such as contaminated raw materials, poorly designed or maintained facilities and equipment, faulty procedures during processing, and improper employee training or practices.

The types of physical hazards that can be found in NRTE/RTE products include metal fragments (from nails, screws, needles, seals, knives, equipment parts, shroud pins, wire, jewelry, buckshot, etc.); glass shards (from broken jars, bottles, light bulbs, thermometers, windows, eye glasses, etc.); wood pieces or chips (from broken pallets, handles, boards, etc.); stones (from driveways, etc.); plastic pieces (from packaging material, food/candy wrappers, gloves, etc.); bone fragments (from the cut-up and fabrication of carcasses); and other foreign materials (pens, trash, etc.). In some cases, physical hazards enter the plant with the live animal (e.g., buckshot). But in many cases, these physical hazards are introduced to NRTE/RTE product as a result of events that occur during the production process (e.g., metal from a blade or wood chips from a broken pallet). The size, shape, and consistency of the foreign object should be considered in determining whether or not it is a hazard.

45.8.2 Public Health Concerns

Typical public health concerns associated with physical hazards in NRTE/RTE product include broken teeth and damage, such as tears, to the mouth, esophagus, stomach, and intestines. These physical hazards may obstruct air passages or intestines. In some cases, death may result due to suffocation or infections (intestinal blockages).

Small children are particularly susceptible to problems brought on by physical hazards since their body structures are smaller, and the physical objects may have a greater effect. RTE products pose the greatest risk when physical hazards are embedded in them, because the consumer has a lower likelihood of identifying and removing the hazard prior to consumption if they do not further handle (e.g., mix) the product.

45.8.3 Control Methods

Methods that establishments use to control physical hazards include visual observation of product as it is unboxed or mixed, sanitation procedures, SOPs for product handling, GMPs to ensure proper

maintenance and inspections of facilities and equipment, and foreign materials detection equipment (inline magnets, screens, traps, filters, etc.) used during the production process. Establishments may implement controls to prevent physical objects from entering the plant in incoming product by instituting specifications for incoming material and requiring suppliers' letters of guarantee.

45.9 Fully Cooked-NSS (RTE): Frankfurter, Bologna, Ham, Roast Beef

Space limitation does not permit detailed discussion of each product. Chapter 1 provides a good discussion for hotdogs. Here, we will show basic flow charts for ham and roast beef only without processing details (Figures 45.1–45.5).

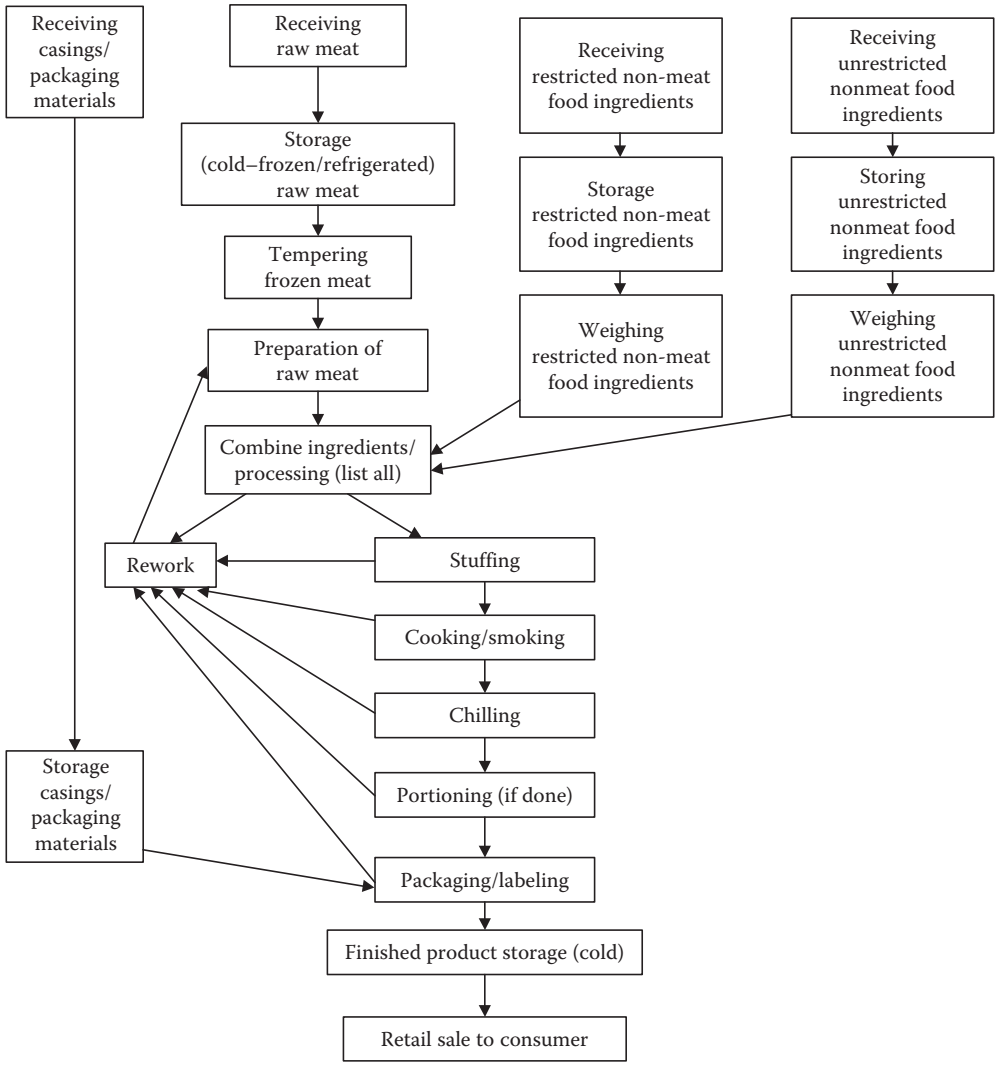

FIGURE 45.1 Process flow diagram of frankfurter and bologna. (Modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. Used with permission.)

1. Common name?	Fully cooked ham a. Bone in/semiboneless b. Boneless Fully cooked roast beef a. Solid muscle b. Restructured
2. How is it to be used?	Consumed as purchased (ready to eat)
3. Type of package?	Vacuum packed; hermetically sealed; modified atmosphere packaging (MAP); overwrap
4. Length of shelf life, at what temperature?	Varies with packaging and storage temperature: preferred refrigerated storage temperature 30–40°F
5. Where will it be sold? Consumers? Intended use?	Wholesale to distributors only
6. Labeling instructions?	Keep frozen; keep Refrigerated
7. Is special distribution control needed?	Keep frozen; keep Refrigerated

FIGURE 45.2 Product description of fully cooked ham and roast beef. (Modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. Used with permission.)

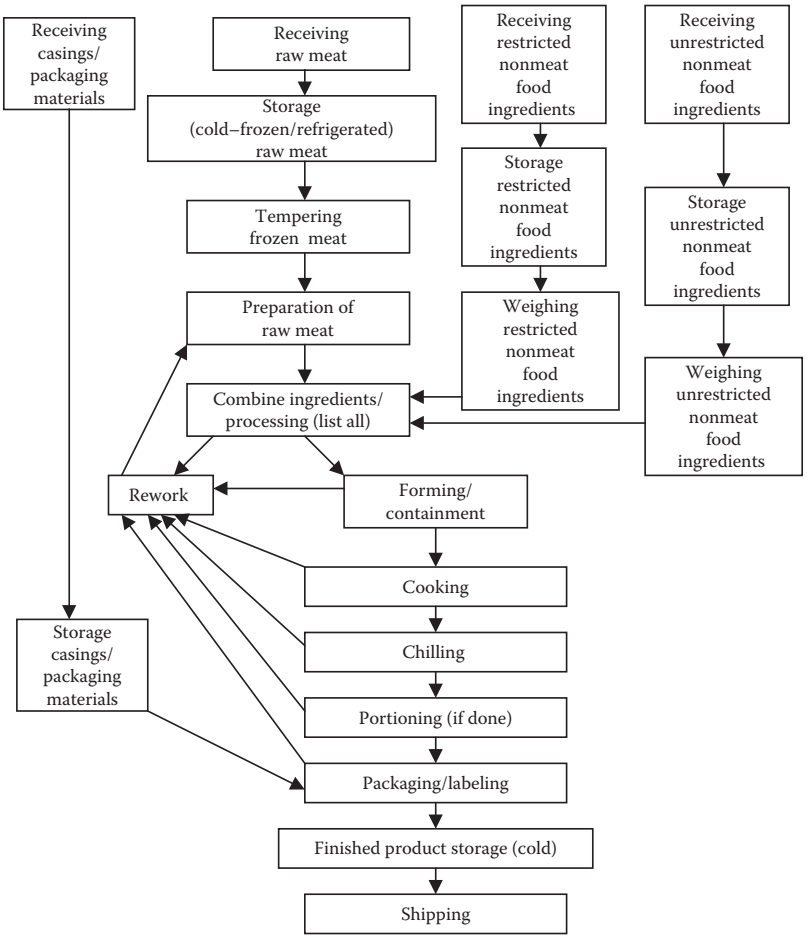

FIGURE 45.3 Process flow diagram of fully cooked ham and roast beef. (Modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. Used with permission.)

Process Step	Food Safety Hazard	Reasonably Likely to Occur?	Basis	If Yes in Column 3, What Measures Could Be Applied to Prevent, Eliminate, or Reduce the Hazard to an Acceptable Level?	Critical Control Point
Receiving—Raw Meat	Biological: Pathogens *Salmonella*, *Listeria monocytogenes*	Yes	*Salmonella* and *Listeria monocytogenes* may be present on incoming raw product.	Certification from suppliers that product has met performance standards for *Salmonella*. During processing, pathogen growth can best be controlled by appropriate cold storage; heat treatment & postheat treatment chilling.	1B
	Chemical—None				
	Physical—Foreign materials such as broken needles	No	Plant records show that there has been no incidence of foreign materials in products received into the plant.		
Receiving—Restricted and Unrestricted Nonmeat Food Ingredients; Casings/Packaging Materials	Biological—None		Letters of guaranty are received from all suppliers of casings/packaging materials.		
	Chemical—Not acceptable for intended use	No			
	Physical—Foreign materials (wood, metal, glass, etc.)	No	Plant records demonstrate that foreign material contamination has not occurred during the past several years and suppliers have remained consistent.		
Storage—Restricted and Unrestricted Nonmeat Food Ingredients; Casings/Packaging Materials	Biological—None				
	Chemical—None				
	Physical—None				
Storage (Cold–Frozen/ Refrigerated)—Raw Meat	Biological—*Salmonella*, *Listeria Monocytogenes*	Yes	Pathogens are reasonably likely to grow in this product if temperature is not maintained at or below a level sufficient to preclude their growth.	Maintain product temperature at or below a level sufficient to preclude pathogen growth.	2B
	Chemical—None				
	Physical—None				
Tempering Frozen Meat	Biological—Growth of foodborne pathogens	Yes	High temperature during tempering could result in growth of foodborne pathogens. (Recommend surface layer of product (1-in. depth) shall not exceed 40°F for more than 2 h as part of the tempering procedures for plant operation).	Subsequent cooking step is effective control.	

	Chemical—Contamination of product with cleaners, sanitizers, etc.	No	SOPs for sanitation should clearly address prevention of contamination during tempering of meat.		
	Physical	No	Production and process controls to reduce potential contamination		
Weighing Restricted and Unrestricted Nonmeat Food Ingredients	Biological—None Chemical—None Physical—None				
Preparation of Raw Meat (including the following; bone-in, semi-boneless, and boneless hams; Solid muscle and restructured roast beef—injection, tumble, massaging, mechanical tenderization, trimming)	Biological—*Salmonella*	Yes	Prolonged exposure to high ambient temperatures may result in unacceptable levels of pathogens. Potential for cross contamination.	Subsequent cooking step will eliminate this hazard.	
	Physical—None				
	Physical—Metal Contamination	Yes	Plant records show that during mechanical processing metal contamination is likely to occur.	Metal detector with functioning kick out is installed prior to packaging.	
Rework	Biological—Pathogens	No	Rework at the end of the day is condemned.		
	Chemical—None				
	Physical—None				
Combine Ingredients	Biological—None				
	Chemical—None				
	Physical—None				
Forming/Containment	Biological—None Chemical—None Physical—None				
Cooking	Biological—Pathogens *Listeria monocytogenes*, *Escherichia coli O157:H7*, *Salmonella*, *Staphylococcus aureus*, *Trichina*	Yes	Potential survival and/or growth of pathogens with the failure of the cooking.	Cook product using validated time/temperature controls.	3B

FIGURE 45.4 Hazard analysis of fully cooked ham and roast beef. (Modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. Used with permission.)

Process Step	Food Safety Hazard	Reasonably Likely to Occur?	Basis	If Yes in Column 3, What Measures Could Be Applied to Prevent, Eliminate, or Reduce the Hazard to an Acceptable Level?	Critical Control Point
	Chemical—None				
	Physical—None				
Chilling	Biological—Pathogens *Clostridium perfringens* *Clostridium botulinum* growth and toxigenesis	Yes	Heat-shocked *Clostridium* spores will become vegetative cells that proliferate. Subsequent toxigenesis in the intestine (*Clostridium perfringens*) or in the food (*Clostridium botulinum*) possible.	Proper chilling procedures are used.	4B
	Chemical—None				
	Physical—None				
Portioning (If done)	Biological—Pathogens *Listeria monocytogenes*	Yes	Potential contamination from environmental sources.	Plant will conduct midshift cleanup for each shift using a sanitizer demonstrated effective against Listeria on all product contact surfaces.	5B
	Chemical—None				
	Physical—None				
Packaging/Labeling	Biological—None				
	Chemical—None				
	Physical—Metal contamination	Yes	Plant records show that during mechanical processing metal contamination is likely to occur.	Metal detector with functioning kick out is installed prior to packaging.	6P
Finished Product Storage (Cold)	Biological—Pathogens *Listeria monocytogenes*	Yes	Psychrophilic pathogens are reasonably likely to grow if temperature is not maintained at or below a level sufficient to abate their growth.	Maintain product temperature at or below a level sufficient to abate psychrophilic pathogen growth.	7B
	Chemical—None				
	Physical—None				
Shipping	Biological—None				
	Chemical—None				
	Physical—None				

FIGURE 45.4 Continued.

CCP# and Location	Critical Limits	Monitoring Procedures and Frequency	HACCP Records	Verification Procedures and Frequency	Corrective Actions
4B Chilling (Roast Beef)	Product will be chilled from 120°F to 55°F in no more than 6 h.	QA technician will observe chilling procedures to ensure critical limits are met. Cooler temperature will be monitored & recorded continuously using temperature record charts. Charts will be reviewed for each product lot with time of observation recorded & initialed every 2 h. QA technician will select & check 5 samples per batch to ensure chilling time & temperature requirements have been met every 2 h until 55°F internal temperature is reached.	Cooler Temperature Recording Chart Product Chilling Log Thermometer Calibration Log Corrective Action Log	QA supervisor will review the product chilling log and cooler temperature recording chart once per shift. Maintenance supervisor will verify the accuracy of the cooler temperature recording chart once per shift. QA will check all thermometers used for monitoring and verification activities for accuracy daily and calibrate to within 2°F accuracy as necessary.	QA will reject or hold product dependent on time and temperature deviation. Product disposition based on advice of Processing Authority. QA will identify the cause of the deviation and prevent reoccurrence. Cooler maintenance schedule will be reviewed and any necessary repairs made.
5B Portioning	No *Listeria monocytogenes* on product contact surface.	Cleaning crew supervisor will verify that sanitizer of demonstrated effectiveness against Lm is used on all product contact surfaces at midshift clean up & record results in the midshift cleanup log.	*Listeria* Sampling Log Corrective Action Log Midshift Cleanup Log	QA will observe cleaning crew supervisor; review log results, and once per week QA will verify that appropriate sanitizer is used according to manufacturer's instructions.	QA will address positive *Listeria* samples as detailed in the FSIS issuance "*Listeria* Guidelines for Industry". Midshift procedures will be revised. All products back to last cleanup will be held. No adulterated product will be shipped.
6P Packaging	No metal fragments greater than 1/32 in.	All product will be visually examined prior to packaging after slicing.	Metal Detection Log	QA will observe packaging personnel perform visual observation and verify the entries in the log.	If metal is found, all product held for examination using metal detector. Cause of deviation is to be determined and appropriate action to prevent recurrence instituted.

FIGURE 45.5 HACCP plan of fully cooked ham and roast beef. (Modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. Used with permission.)

CCP# and Location	Critical Limits	Monitoring Procedures and Frequency	HACCP Records	Verification Procedures and Frequency	Corrective Actions
7B Finished Product Storage (Cold)	Finished product storage areas will not exceed 40°F.	Maintenance personnel will monitor finished product storage area temperatures every 2 h and records results.	Room Temperature Log Thermometer Calibration Log Corrective Action Log	Maintenance supervisor will verify the accuracy of the room temperature log once per shift. QA will check all thermometers used for monitoring and verification activities for accuracy daily and calibrate to within 2°F accuracy as necessary. QA will observe maintenance personnel check finished product storage area once per shift.	If a deviation from a critical limit occurs, the following corrective actions will be taken: 1. The cause of the temperature exceeding 40°F will be identified and eliminated. 2. The CCP will be monitored hourly after the corrective action is taken to ensure that it is under control. 3. When the cause of the deviation is identified, measures will be taken to prevent it from recurring e.g., if the cause is equipment failure, the preventive maintenance program will be reviewed and revised, if necessary. 4. If room temperature exceeds the critical limit, the Processing Authority will evaluate the product time/temperature deviation to ensure the present temperature is sufficient to preclude pathogen growth before release for shipment. If the temperature is not sufficient to preclude pathogen growth, recooking can be considered after computer modeling of *Listeria monocytogenes* growth in an assumed worst case scenario.

FIGURE 45.5 Continued.

45.10 Heat Treated but not Fully Cooked—NSS (NRTE): Bacon

Details are provided for bacon manufacture in Chapter 1. For reference purposes, Figure 45.6 describes the process flow diagram for bacon.

45.11 Products with Secondary Inhibitors—NSS (NRTE or RTE): Corned Beef, Smoked Sausage

Corned beef represents an NRTE for products with secondary inhibitors and NSS. Figures 45.7 through 45.10 use charts to summarize the safe production of corned beef.

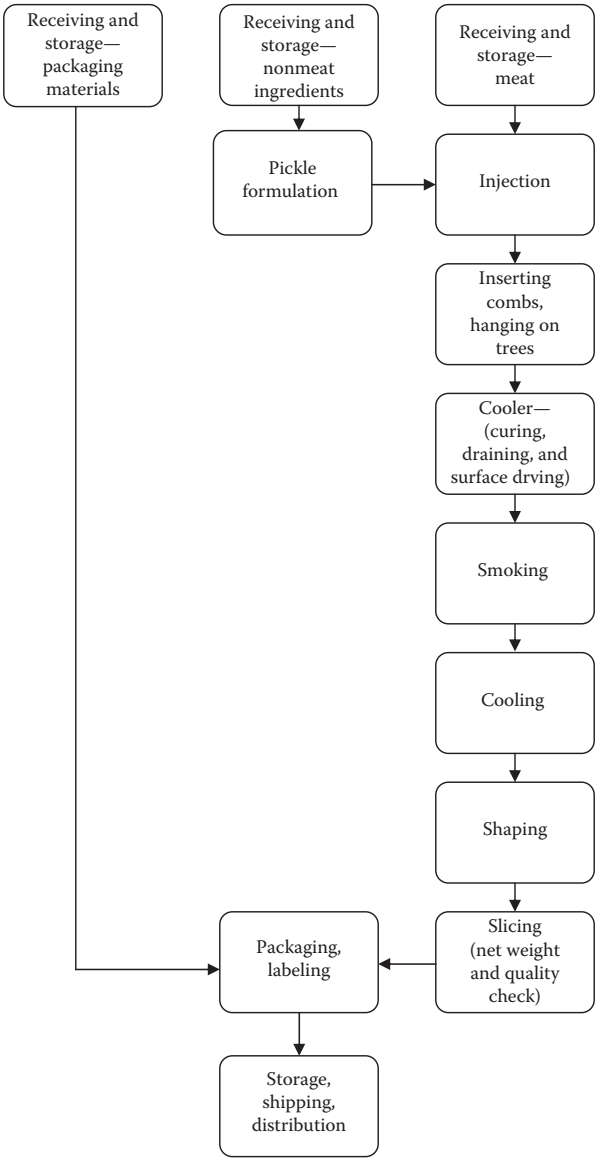

FIGURE 45.6 Process flow diagram of bacon. (Modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. Used with permission.)

1. Common name?	Corned beef
2. How is it to be used?	Cooked by purchaser
3. Type of package?	Vacuum packed
4. Length of shelf life, at what temperature?	Under refrigeration shelf Life of vacuum packaged Product is 8 days Frozen 14 days
5. Where will it be sold? Consumers? Intended use?	Wholesale to distributors only
6. Labeling instructions?	Keep refrigerated or frozen
7. Is special distribution control needed?	Keep refrigerated or frozen

FIGURE 45.7 Product description of corned or cured beef. (Modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. Used with permission.)

Sliced country ham represents an RTE product with secondary inhibitors and NSS. Figure 45.11 describes the process flow diagram for production of the product.

Smoked sausage is an RTE product with secondary inhibitors and NSS. Figures 45.12 through 45.15 use charts to summarize the safe production of the product.

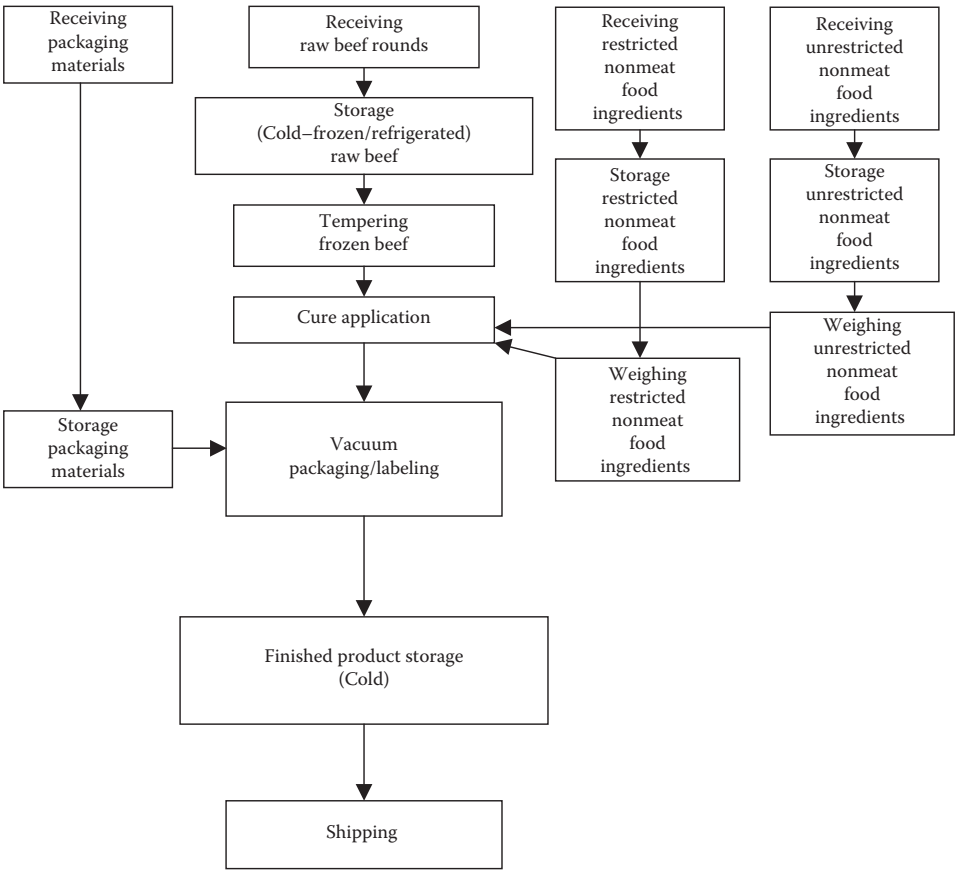

FIGURE 45.8 Process flow diagram of corned or cured beef. (Modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. Used with permission.)

Process Step	Food Safety Hazard	Reasonably Likely to Occur?	Basis	If Yes in Column 3, What Measures Could Be Applied to Prevent, Eliminate, or Reduce the Hazard to an Acceptable Level?	Critical Control Point
Receiving—Raw Beef Rounds	Biological: Pathogens *Salmonella* *E. coli* O157:H7	Yes	*Salmonella* and *E. coli* O157:H7 may be present on incoming raw product. Curing process will inhibit growth of pathogens (*E. coli*) at a further step in the process. Proper storage and handling at subsequent steps can reduce the growth of *E. coli* if present.	Certification from suppliers that product has been sampled for *Salmonella* and meets FSIS performance standards.	1B
	Chemical—None				
	Physical—Foreign materials such as broken needles	No	Plant records show that there has been no incidence of foreign materials in products received into the plant.		
Receiving—Restricted and Unrestricted Nonmeat Food Ingredients; Packaging Materials	Biological—None				
	Chemical—Not acceptable for intended use	No	Letters of guaranty are received from all suppliers of packaging materials.		
	Physical—Foreign materials (insects, glass, metal, etc.)	No	Plant records demonstrate that foreign material contamination has not occurred during the past several years.		
Storage—Restricted and Unrestricted Nonmeat Food Ingredients; Packaging Materials	Biological—None Chemical—None Physical—None				
Storage (Cold—Frozen/Refrigerated)—Raw Beef	Biological *E. coli* O157:H7	Yes	Pathogens are reasonably likely to grow in this product if temperature is not maintained at or below a level sufficient to preclude their growth.	Maintain product temperature at or below a level sufficient to preclude pathogen growth.	2B
	Chemical—None Physical—None				
Tempering Frozen Beef	Biological—None				
	Chemical—None				
	Physical—None				

FIGURE 45.9 Hazard analysis of corned or cured beef. (Modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. Used with permission.)

Process Step	Food Safety Hazard	Reasonably Likely to Occur?	Basis	If Yes in Column 3, What Measures Could Be Applied to Prevent, Eliminate, or Reduce the Hazard to an Acceptable Level?	Critical Control Point
Weighing Restricted and Unrestricted Nonmeat Food Ingredients	Biological—None Chemical—Cure Ingredients (nitrite)	Yes	Excess nitrite in a finished product may create a health hazard.	Proper control and formulation of nitrite to prevent creating a health hazard.	3C
	Physical—None				
Cure Application	Biological *Clostridium botulinum* *Clostridium perfringens* Chemical—None Physical—None	Yes	Application of cure at the proper level will inhibit the growth of pathogenic organisms.	Proper injection of cure solution to provide microbial inhibition.	4B
Packaging/Labeling	Biological *Clostridium botulinum* *Clostridium perfringens* Chemical—None Physical—None	No	Proper nitrite pump and vacuum package will allow competing organisms to grow to the exclusion of *clostridia.*	See CCP 3C and CCP 4B	
Finished Product Storage (Cold)	Biological *Salmonella*	Yes	Pathogens are reasonably likely to grow on this product if temperature is not maintained at or below a level sufficient to preclude their growth.	Maintain product temperature at or below a level sufficient to preclude pathogen growth.	5B
	Chemical—None Physical—None				
Shipping	Biological—None Chemical—None Physical—None				

FIGURE 45.9 Continued.

CCP# and Location	Critical Limits	Monitoring Procedures and Frequency	HACCP Records	Verification Procedures and Frequency	Corrective Actions
1B Receiving—Raw Beef Rounds	Supplier certification that product has been sampled for *Salmonella* must accompany shipment.	Receiving personnel will check each shipment for *Salmonella* certification.	Receiving Log Corrective Action Log	Every 2 months QA will request FSIS *Salmonella* data results from the company for at least 2 suppliers.	Will not receive product unaccompanied by *Salmonella* certification.
2B Storage (Cold–Frozen/Refrigerated—Raw Beef	Raw product storage areas will not exceed 40°F in refrigerated rooms or exceed 28°F in freezer rooms.	Maintenance personnel will check raw product storage area temperature every 2 h.	Room Temperature Log Thermometer Calibration Log Corrective Action Log	Maintenance supervisor will verify accuracy of the Room Temperature Log once per shift. QA will check all thermometers used for monitoring and verification for accuracy daily and calibrate to within 2°F accuracy as necessary.	QA will reject or hold product dependent on time and temperature deviation and use pathogen modeling to determine product disposition. QA will identify the cause of the deviation and prevent reoccurrence. Monitoring frequency will be increased until it is assured that the process step is under control.
3C Weighing Restricted and Unrestricted Nonmeat Food Ingredients	2 lb. sodium nitrite to 100 gallon pickle at 10% pump.	QA technician will monitor weighing and formulation of sodium nitrite for each batch.	Weight/Formulation Record Corrective Action Log	QA supervisor will observe QA technician perform monitoring activity once per shift. QA supervisor will check Weight/Formulation Record twice per shift. Scale will be calibrated once per week.	Improperly prepared cure ingredients will be discarded. QA will identify the cause of the deviation and prevent reoccurrence. Monitoring will be increased to include scale calibration for each batch.
4B Cure Application	120 ppm sodium nitrite at 10% pump.	Pump percentage will be monitored before and after pump to assure 10% gain. Ten pieces will be weighed before and after pump % gain calculated every 2 h.	Pump Percentage Log Corrective Action Log	QA will observe pump technician perform pump check once per lot. QA will review pump log once per shift.	Product not meeting critical limit will be held and either repumped to 10% or drained to 10%. Needle settings will be checked and adjusted as required. Pump technician will monitor every hour until assured that the CCP is under control.

FIGURE 45.10 HACCP plan of corned or cured beef. (Modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. Used with permission.)

CCP# and Location	Critical Limits	Monitoring Procedures and Frequency	HACCP Records	Verification Procedures and Frequency	Corrective Actions
5B Finished Product Storage	Product storage area will not exceed 40°F	Maintenance personnel will monitor storage room temperature once per shift.	Corrective Action Log Room Temperature Log Thermometer Calibration Log	QA will observe maintenance personnel perform storage temperature monitoring and review room temperature log once per week. Cooler thermometers will be calibrated once per week by maintenance supervisor.	If room temperature exceeds 40°F product will be held and internal temperature taken. Internal product temperature and amount of time product and room temperature deviated will determine product disposition. Product temperature will be monitored when room temperature is monitored until CCP is under control. Cause of deviation will be identified. Maintenance will repair thermometer units or cooling coils as required based on cause of deviation.

FIGURE 45.10 Continued.

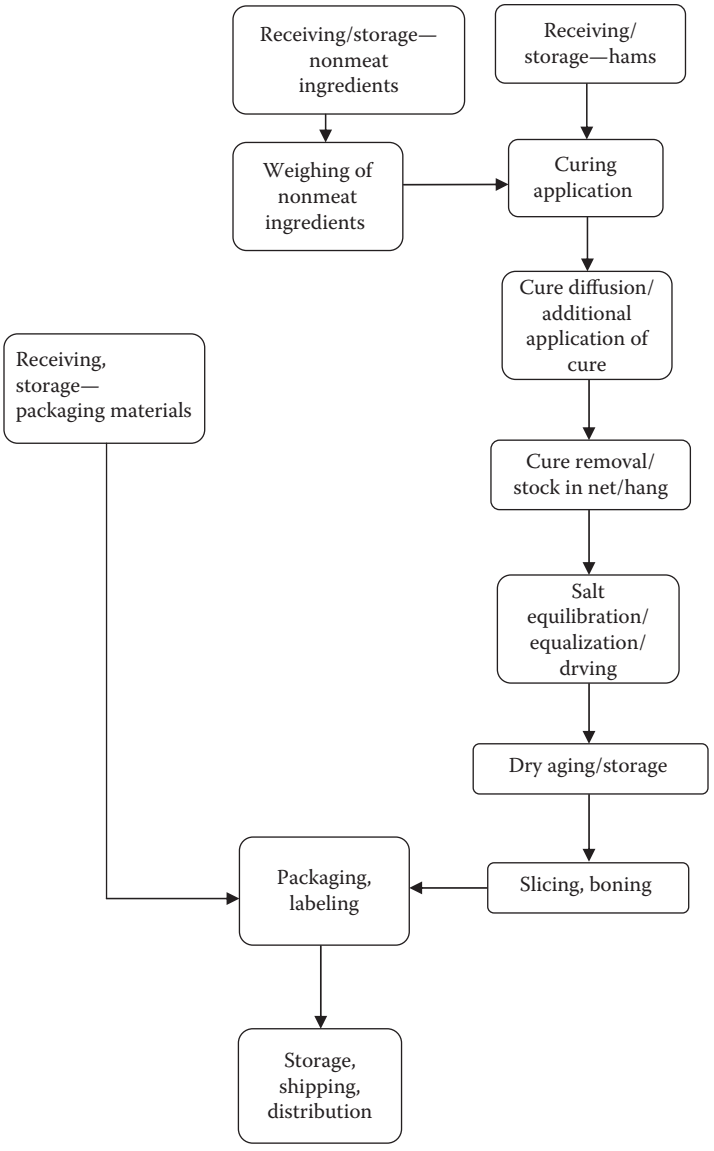

FIGURE 45.11 Process flow diagram of sliced country ham. (Modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. Used with permission.)

1. Common name?	Smoked sausage, partially cooked chicken patties
2. How is it to be used?	Entrees, nuggets, snack foods, salads
3. Type of package?	Bulk-packed (e.g., plastic bag, vacuum packed)
4. Length of shelf life, at what temperature?	3–6 Months at 0°F or below; 7 days at 40°F
5. Where will it be sold? Consumers? Intended use?	Wholesale to distributors only
6. Labeling instructions?	Keep frozen; keep refrigerated
7. Is special distribution control needed?	Keep frozen; keep refrigerated

FIGURE 45.12 Product description of smoked sausage. (Modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. Used with permission.)

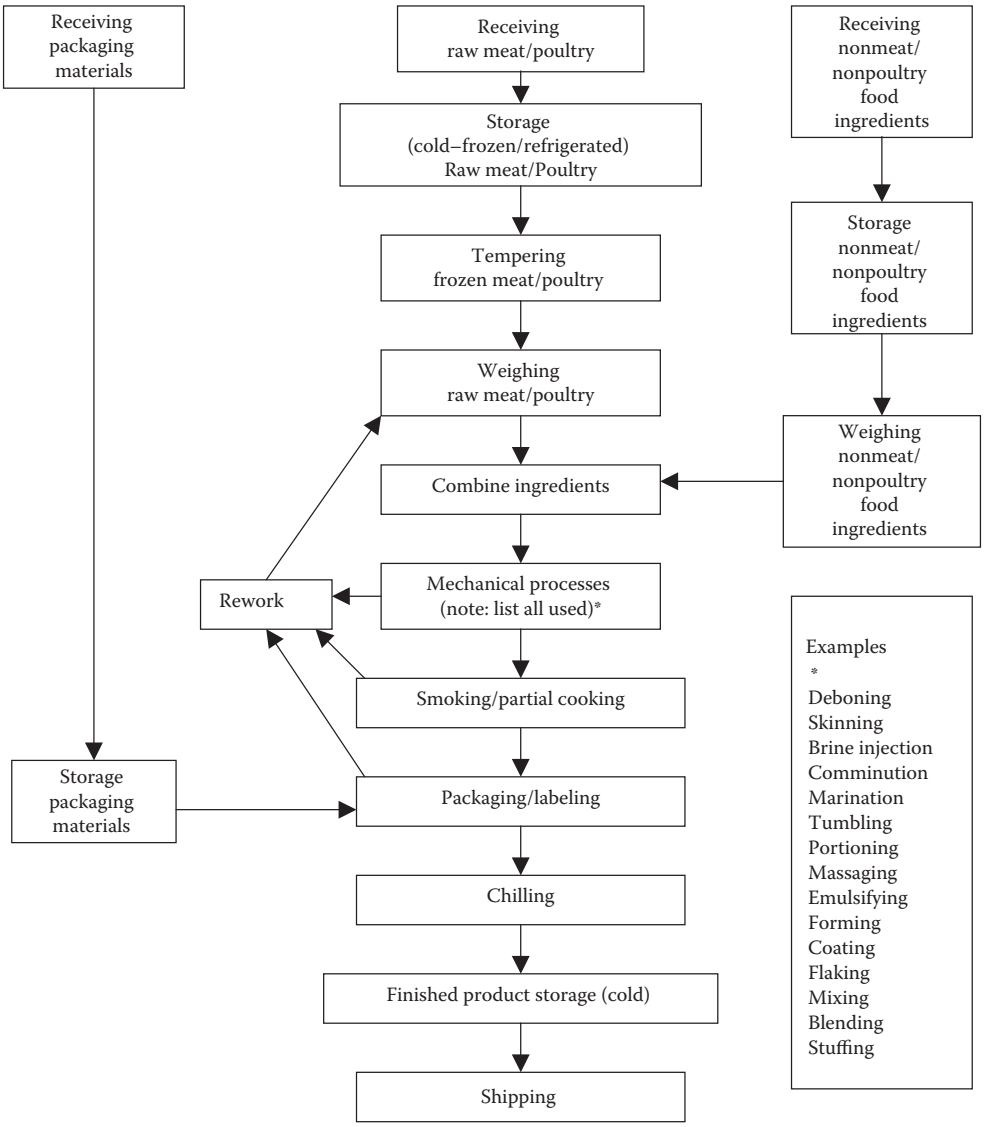

FIGURE 45.13 Process flow diagram of smoked sausage. (Modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. Used with permission.)

Process Step	Food Safety Hazard	Reasonably Likely to Occur?	Basis	If Yes in Column 3, What Measures Could Be Applied to Prevent, Eliminate, or Reduce the Hazard to an Acceptable Level?	Critical Control Point
Receiving—Raw Meat/Poultry	Biological: Pathogens *Salmonella* *Trichina* Chemical—None	Yes	*Salmonella* may be present on incoming raw product.	Certification from suppliers that product has been sampled for *Salmonella* meets performance standards	1B
	Physical—Foreign materials such as broken needles, bone/bone fragments	Yes	Bone/bone fragments (size/shape) that would represent a hazard to consumers have been found in incoming product.	Supplier specification that all product must have been run through a bone detector/bone collector in process or if whole muscle product received use of a bone detector/bone collector in process.	
Receiving —Nonmeat/ Nonpoultry Food Ingredients; Packaging Materials	Biological—None Chemical—Not acceptable for intended use	No	Letters of guaranty are received from all suppliers of packaging materials.		
	Physical—Foreign materials (wood, metal, glass, etc.)	No	Plant records demonstrate that foreign material contamination has not occurred during the past several years.		
Storage —Nonmeat/ Nonpoultry Food Ingredients; Packaging Materials	Biological—None Chemical—None Physical—None				
Storage (Cold —Frozen/ Refrigerated) —Raw Meat/ Poultry	Biological *Salmonella* *Listeria monocytogenes*	Yes	Pathogens are reasonably likely to grow in this product if temperature is not maintained at or below a level sufficient to preclude their growth and coolers are not maintained to hold temperatures.	Maintain product temperature at or below a level sufficient to preclude or abate pathogen growth.	2B
	Chemical—None Physical—None				

FIGURE 45.14 Hazard analysis of smoked sausage. (Modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. Used with permission.)

Process Step	Food Safety Hazard	Reasonably Likely to Occur?	Basis	If Yes in Column 3, What Measures Could Be Applied to Prevent, Eliminate, or Reduce the Hazard to an Acceptable Level?	Critical Control Point
Tempering Frozen Meat/Poultry	Biological—None Chemical—None Physical—None				
Weighing Nonmeat/ Nonpoultry Food Ingredients	Biological—None Chemical—None Physical—None				
Weighing Raw Meat/Poultry	Biological—None Chemical—None Physical—None				
Combine Ingredients	Biological—None Chemical—None Physical—None				
Mechanical Process	Biological—None Chemical—None				
* See list on flow chart on page 21	Physical—Metal Contamination	Yes	Plant records show that during mechanical processing metal contamination is likely to occur	Metal detectors are installed prior to packaging.	3P
Smoking/Partial Cooking	Biological—None	No	Rework at the end of the day is condemned.		
	Chemical—None Physical—None				
Rework	Biological—Pathogens	Yes	*Trichina* has historically occurred in raw pork products.	Labels that clearly indicate this is a raw product, along with cooking instructions and a safe food-handling statement.	4B
	Chemical—None Physical—None				

Packaging/ Labeling	Biological—Pathogens -parasitic (*Trichina*) Chemical—None Physical—None				
Chilling	Biological—Pathogens	Yes	Pathogens are reasonably likely to grow if improper chilling procedures are used.	Proper chilling procedures are used.	5B
	Chemical—None Physical—None				
Finished Product Storage (Cold)	Biological—Pathogens	Yes	Pathogens are reasonably likely to grow in this product if temperature is not maintained at or below a level sufficient to preclude their growth.	Maintain product temperature at or below a level sufficient to preclude pathogen growth.	6B
	Chemical—None Physical—None				
Shipping	Biological—None Chemical—None Physical—None				

FIGURE 45.14 Continued.

CCP# and Location	Critical Limits	Monitoring Procedures and Frequency	HACCP Records	Verification Procedures and Frequency	Corrective Actions
1B Receiving—Raw Meat/Poultry	Supplier certification that product has been sampled for *Salmonella* must accompany shipment.	Receiving personnel will check each shipment for *Salmonella* certification.	Receiving Log Corrective Action Log	Every 2 months QA will request FSIS *Salmonella* data results from company for at least 2 suppliers.	Will not receive product unaccompanied by *Salmonella* certification. Product found not meeting *Salmonella* performance standards will not be accepted. If supplier has failed 2 consecutive sets of *Salmonella* tests, the supplier will not be used until they certify that the results of a sample set meets baseline standard.
2B Storage (Cold–Frozen/Refrigerated —Raw Meat/Poultry	Raw product storage areas shall not exceed 40°F.	Maintenance personnel will check raw product storage area temperature every 2 h.	Room Temperature Log Thermometer Calibration Log Corrective Action Log	Maintenance supervisor will verify accuracy of the Room Temperature Log once per shift and observe temperature checks. QA will check all thermometers used for monitoring and verification for accuracy daily and calibrate to within 2°F accuracy as necessary.	Product internal temperature will be determined. QA will reject or hold product dependent on time/temperature deviation. Volume or manner of storage will be adjusted if determined to contribute to the deviation. Cooling curve or Process Authority will be used to make product disposition determination. QA will identify the cause of the deviation and prevent reoccurrence. Cooler will be repaired & maintenance schedule revised if necessary.
3P Mechanical Processes	No metal particles to exceed 1/32 in.	Maintenance personnel will check the metal detector with a seeded sample once per shift and determine that kick out device is functioning as intended.	Metal Detection Log Corrective Action Log	Maintenance supervisor will verify metal detector is functioning and perform routine maintenance as required by manufacturer. QA will verify that the metal detector is functioning as intended by observing maintenance test with seeded sample once every other day.	Mechanical separation line supervisor will control and segregate affected product. Maintenance personnel will identify and eliminate the problem with the metal detector. Preventive maintenance program will be implemented and revised as required. QA will run seeded sample through metal detector after repair. All potentially contaminated product will be visually examined & metal removed. Product will be x-rayed prior to rework or shipment.

4B Packaging/Labeling (*Trichina*)	Product must clearly be labeled as raw or partially cooked and requiring cooking. Cooking instructions must be on the package. Safe food-handling statement must be part of the label.	Packaging line supervisor will select 2 packages of each type of product produced at the start & finish of each lot and ensure labeling requirements are met.	Labeling Log Corrective Action Log	QA will observe packaging line supervisor perform monitoring activity once per shift. QA will select 3 labels intended for use from label storage area twice weekly to ensure label accuracy. QA will check labels once a day on packaged product to ensure label accuracy on packaged product.	QA will segregate and hold all affected product. QA will ensure that proper labeling is applied to all affected product prior to shipment. Labeling equipment will be adjusted if required. Label manufacturer will provide certification that all required features are printed properly. Incoming labels will be examined to assure that they are correct.
5B Chilling	Internal product temperature <70°F within 3 h and will reach <41°F within an additional 6 h after reaching <70°F (Total chilling time of 9 h).	QA technician will observe chilling handling procedures to ensure critical limits are met. Product coolers will be monitored and recorded continuously on temperature recording charts. QA technician will randomly select 5 samples of each type of product at 3 and 9 h from start of chilling and determine internal temperature.	Product Chilling Log Product Coolers Temperature Recording Charts Thermometer Calibration Log Corrective Action Log	Once per shift the QA supervisor will review the Product Chilling Log and observe temperature monitoring activity. Maintenance supervisor will verify accuracy of the product cooler temperature recording charts once per shift. QA will check all thermometers used for monitoring and verification for accuracy daily and calibrate to within 2°F accuracy as necessary.	QA will reject or hold product dependent on time and temperature deviation. Internal product time/temperature curve will be determined & used to make product disposition. Product will be condemned or fully cooked. QA will identify the cause of the deviation and prevent reoccurrence. Cooler will be repaired if required & maintenance schedule revised if required.

FIGURE 45.15 HACCP plan for smoked sausage. (Modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. Used with permission.)

CCP# and Location	Critical Limits	Monitoring Procedures and Frequency	HACCP Records	Verification Procedures and Frequency	Corrective Actions
6B Finished Product Storage (Cold)	Finished product storage areas will not exceed 40°F.	Maintenance personnel will check finished product storage areas temperatures every 2 h.	Room Temperature Log Thermometer Calibration Log Corrective Action Log	Maintenance supervisor will verify the accuracy of the room temperature log once per shift. QA will check all thermometers used for monitoring and verification activities for accuracy daily and calibrate to within 2°F accuracy as necessary. QA will observe maintenance personnel check finished product storage area once per shift.	If a deviation from a critical limit occurs, the following corrective actions will be taken: 1. The cause of the temperature exceeding 40°F will be identified and eliminated. 2. The CCP will be monitored hourly after the corrective action is taken to ensure that it is under control. 3. When the cause of the deviation is identified, measures will be taken to prevent it from recurring e.g., if the cause is equipment failure, preventive maintenance program will be reviewed and revised, if necessary. 4. If room temperature exceeds the critical limit, the processing authority will evaluate the product temperature to ensure the temperature is sufficient to preclude pathogen growth before release for shipment. If temperature is not sufficient to preclude pathogen growth, product will be cooked in the establishment to ensure destruction of pathogens or condemned.

FIGURE 45.15 Continued.

46

Processing Raw Products: Safety Requirements in the United States[*]

Y. H. Hui

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46.1 Introduction

In the United States, the Food Safety Inspection Service (FSIS), a unit of the Department of Agriculture (USDA), is responsible for the safety of meat and meat produces. In accordance with the following, please note that, for this chapter:

1. The text and tables have been collated from multiple USDA public documents (legal, regulatory, and training), distributed in USDA'S website, http://www.fsis.usda.gov.
2. The figures were originally adapted from a series of USDA continuing educational documents, *USDA HACCP Generic Models on Raw Ground and Not Ground Meat Products*. They are available at USDA's website, http://www.fsis.usda.gov.

It must be emphasized that, in this chapter:

1. All information have been designed by the USDA for training purposes only and is not meant to detail the actual manufacturing processes.
2. All abbreviations are standard ones used in law, science, and technology as issued by FSIS.
3. All terms with legal implications apply only in the United States.

FSIS regulation lists processing categories that group products by major processing parameters. They are

1. Slaughter—all species
2. Raw product—ground
3. Raw product—not ground

Table 46.1 shows examples of products in each process category.

This chapter reviews all three categories of the raw products, with an emphasis on safety intervention methodologies. We cover the slaughter process steps pertaining to food safety and methods of intervention in the following section.

46.2 Slaughter Process

We first study the technical methods for controlling bacterial levels during the slaughter process.

46.2.1 Antimicrobial Interventions

Currently, numerous antimicrobial interventions are used in slaughter processes. In this section, we look at the most common ones and their effectiveness as shown in scientific studies. It should be noted that

TABLE 46.1

Examples of Products in Each Process Category

Slaughter	Raw—Not Ground	Raw—Ground
Beef carcass	Beef forequarter	Ground beef
Veal carcass	Veal shanks	Ground beef patties
Pork carcass	Pork loin, boneless	Hamburger
Lamb carcass	Lamb rib chops	Beef patty mix
Goat carcass	Chicken parts	Ground pork
Chicken, whole	Turkey breast cutlets	Ground lamb
Turkey, whole	Beef trimmings	Ground chicken
Duck, whole	Mechanically separated pork	Ground Turkey
Squab	Beef liver	Italian sausage
Rock Cornish hen	Mechanically tenderized beef roasts	Fresh pork sausage
Ratite carcass (ostrich, rhea, emu)	Beef steaks, tenderized with enzyme solution	Breakfast sausage links
Equine carcass	Boneless skinless chicken breasts marinated in seasoning solution	Turkey bratwurst
Other exotic species carcass	Uncooked, corned beef brisket	Fresh chorizo

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antimicrobial interventions are not a substitute for sanitary-dressing procedures. In discussing the various interventions, we use the beef slaughter process as our model for red meat and the young chicken slaughter process as our model for poultry. We begin by discussing beef slaughter interventions.

Until recently, knife trimming and carcass washing with plain water were the primary means by which the industry addressed meat contaminants. However, the occurrence of foodborne disease outbreaks and scientific advances over the years have shown that trimming and washing alone will not achieve the level of food safety that regulators and consumers expect from meat products. In response to food safety concerns, the industry and scientific community, with encouragement from FSIS, have introduced numerous antimicrobial interventions to the beef slaughter process.

46.2.2 Steam Vacuum System

Steam vacuum systems are designed to remove small visible spots of contamination from carcass surfaces. The system is a hand-held apparatus that uses a hot-water spray (185°F) in a vacuum nozzle, with steam sprayed above and below the vacuum head. The hot water sprayed onto a carcass kills 90% or more of the bacteria and detaches contamination such as ingesta or feces, which is then vacuumed off. Many establishments utilize the steam vacuum system at multiple points in the slaughter process. For example, there may be a steam vacuum location after each part of the carcass is skinned.

The following items may be considered as guidelines for the use of the steam vacuum system. Establishments may develop alternate parameters and should have supporting documentation to validate the use of such parameters.

1. Fecal and ingesta contamination *less than* 1 in. in its greatest dimension may be removed by the steam vacuum system; however, contamination 1 in. *or greater* in its greatest dimension should be trimmed by a knife.
2. Accurate temperature and vacuum readings should be provided.
3. Water or steam temperatures should be maintained at a minimum of 180°F.
4. The vacuum pressure at the carcass surface should be sufficient to remove the steam and water from the vacuum area to prevent dripping.
5. The outer surface of the vacuum head should be continuously sanitized by the 180°F minimum steam during its use.

46.2.3 Carcass Washes Followed by Organic Acid Rinses

After hide removal, the carcass may be subjected to a preevisceration wash and subsequent organic acid rinse. The use of a carcass spray immediately after hide removal serves to remove bacteria before they have the opportunity to attach themselves to the carcass surface and begin to grow. The subsequent organic acid rinse then provides a significant kill step for any bacteria that remain on the carcass surface. This intervention is also commonly applied after the slaughter process is complete and before the carcasses enter the cooler. The organic acids commonly used are acetic acid and lactic acid, although citric acid is also approved for this purpose. The concentration of the organic acid is between 1.5% and 2.5% and it may be applied as a mist, fog, or a small droplet rinse. (The establishment may use a concentration >2.5% with supporting documentation.) Studies have shown that washing followed by an organic acid rinse is significantly more effective in reducing bacterial numbers than washing alone.

46.2.4 Other Antimicrobial Chemicals

Some other chemicals utilized as an antimicrobial rinse in beef slaughter include the following:

Acidified sodium chlorite (Sanova®): It has been shown to have a significant kill rate for *Escherichia coli* O157:H7*, Listeria, Campylobacter, Salmonella*, and other bacteria. Applied at ambient temperature by spraying at 500–1200 ppm, it is being used in several establishments across the country.

Lactoferrin: It is applied up to 2% of a water-based antimicrobial spray in the beef slaughter process. Lactoferrin has been shown to be effective against more than 30 foodborne pathogens, including *E. coli* O157:H7*, Salmonella*, and *Listeria*.

46.2.5 Hot-Water Rinses

High-temperature water (>160°F) sprayed on the carcass as the last step prior to chilling has been shown to be effective in substantially reducing the number of *E. coli* O157:H7 and *Salmonella*.

46.2.6 Steam Pasteurization

Steam pasteurization is a process by means of which the carcasses are placed in a slightly pressurized, closed chamber at room temperature and sprayed with steam that blankets and condenses over the entire carcass, raising the surface temperature to 185°F and killing 95–99% of all bacteria. Carcasses are then sprayed with cold water.

46.2.7 Multiple Hurdle Approach

Studies have shown that, rather than rely on any one intervention, it is more effective to use the "multiple hurdle" approach to pathogen control. In using this approach, an establishment will utilize multiple interventions at various steps of the process to achieve the maximum reduction in bacterial numbers on the carcass. For example, a beef slaughter establishment may utilize the steam vacuum at multiple locations as the carcass is dehided; rinse the carcass at preevisceration with water followed by an antimicrobial spray, then wash with water, steam pasteurize, and rinse again with an antimicrobial spray prior to chilling.

Some commercial applications have combined these different interventions to provide an enhanced antibacterial effect. For example, a beef slaughter establishment may use a process called thermal organic rinse (TOR), which utilizes organic acid heated to 131°F to provide greater bacterial reductions. Other systems utilize a hot-water wash followed immediately by an organic acid spray.

46.3 Hazard Analysis of the Beef Slaughter Process

We use the beef slaughter process as an illustration (see Figures 46.1 through 46.4).

1. Common name?	Beef; beef variety meats
2. How is it to be used?	Carcasses; variety meats
3. Type of package?	Carcasses—none; variety meats—50 pound boxes
4. Length of shelf life, at what temperature?	7 Days at 40°f
5. Where will it be sold? Consumers? Intended use?	Wholesale to distributors only
6. Labeling instructions?	Keep refrigerated
7. Is special distribution control needed?	Keep refrigerated

FIGURE 46.1 Slaughter beef: product description. (Modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. Used with permission.)

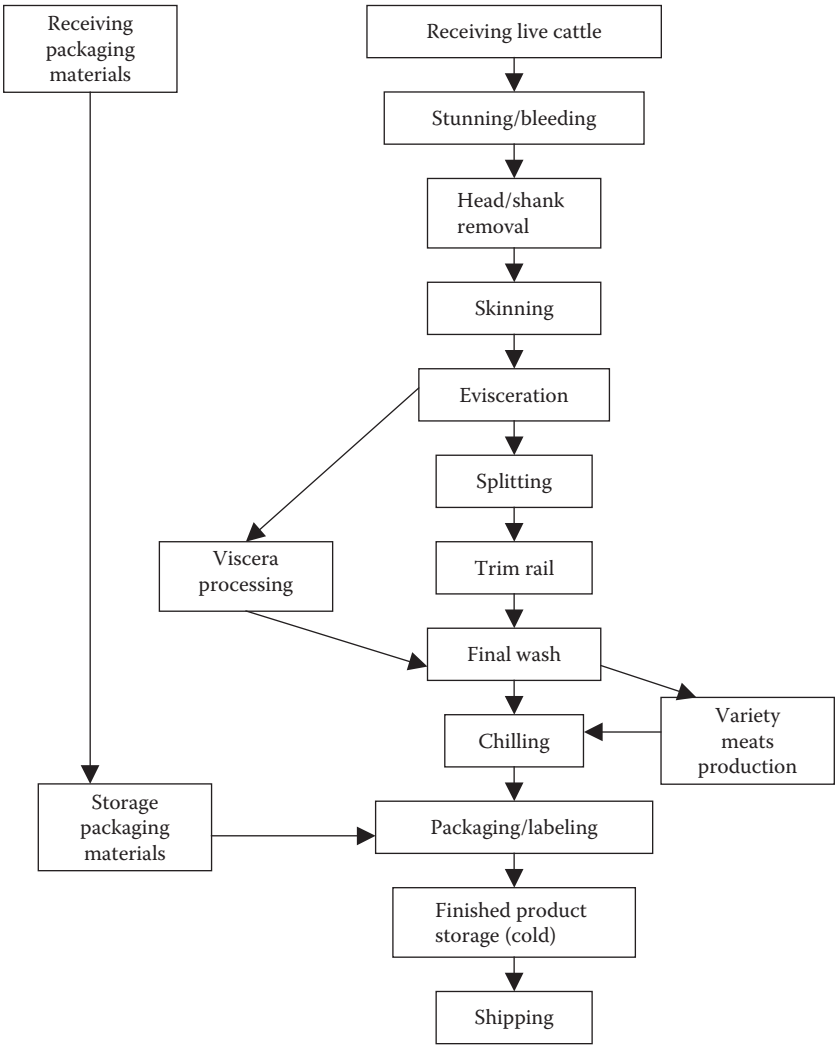

FIGURE 46.2 Slaughter beef: process flow diagram. (Modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. Used with permission.)

Process Step	Food Safety Hazard	Reasonably Likely to Occur?	Basis	If Yes in Column 3, What Measures Could Be Applied to Prevent, Eliminate, or Reduce the Hazard to an Acceptable Level?	Critical Control Point
Receiving—Live Cattle * *Note*: Since residues can be a hazard reasonably likely to occur in cull cows (dairy and beef) and bob veal calves, plants should make chemical (residues) a CCP with these animals.	Biological—*Escherichia coli O157:H7*	No	Sanitary-dressing procedures prevent contamination.		
	Chemical—Residues	No	Plant/FSIS records demonstrate residues have not been a past problem.		
	Physical—Foreign materials such as broken needles	No	Cattle are purchased from feedlots having QA procedures to prevent foreign materials such as broken needles from remaining in animals.		
Stunning/Bleeding Head/Shank Removal	Biological—None				
	Chemical—None				
	Physical—None				
Skinning	Biological—Pathogens—Contamination from the Hide —*E. coli O157:H7*	Yes	Contamination from the hide is a known source of pathogens. Potential contamination could occur at this step.	Will be controlled at the final wash (antimicrobial) step.	
	Chemical—None				
	Physical—None				
Evisceration	Biological—Pathogens —Contamination from the gastrointestinal tract —*E. coli O157:H7*	Yes	Potential contamination could occur at this step.	Will be controlled at the final wash (antimicrobial) step.	
	Chemical—None				
	Physical—None				
Splitting	Biological—None				
	Chemical—None				
	Physical—None				

Trim Rail	Biological—Pathogens —*E. coli O157:H7*	No	This step in the process used to remove incidental contamination that might have occurred in previous steps.		
	Chemical—None				
	Physical—None				
Viscera Processing	Biological—Pathogens —Contamination from the Gastrointestinal Tract —*E. coli O157:H7*	No	Contamination from the gastrointestinal tract is a known source of pathogens; however, plant records demonstrate that contamination has not been a problem in the past.		
	Chemical—None				
	Physical—None				
Final Wash (Antimicrobial)	Biological—Pathogens (Contamination from the Hide and/or Gastrointestinal Tract)	Yes	Appropriate step to reduce pathogens.	An acceptable antimicrobial wash (rinse) is applied to the carcasses.	1B
	Chemical—None				
	Physical—None				
Variety Meats Production	Biological—None				
	Chemical—None				
	Physical—None				
Chilling (All products)	Biological—Pathogens —*E. coli O157:H7*	Yes	Pathogens are reasonably likely to grow if improper chilling procedures are used.	Proper chilling procedures are used.	2B
	Chemical—None				
	Physical—None				
Packaging/Labeling	Biological—None				
	Chemical—None				
	Physical—None				

FIGURE 46.3 Slaughter beef: hazard analysis. (Modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. Used with permission.)

Process Step	Food Safety Hazard	Reasonably Likely to Occur?	Basis	If Yes in Column 3, What Measures Could Be Applied to Prevent, Eliminate, or Reduce the Hazard to an Acceptable Level?	Critical Control Point
Finished Product Storage (Cold)	Biological—Pathogens —*E. coli O157:H7*	Yes	Pathogens are reasonably likely to grow if temperature is not maintained at or below a level sufficient to preclude their growth.	Maintain product temperature at or below a level sufficient to preclude pathogen growth.	3B
	Chemical—None				
	Physical—None				
Shipping	Biological—None				
	Chemical—None				
	Physical—None				

FIGURE 46.3 Continued.

CCP# and Location	Critical Limits	Monitoring Procedures and Frequency	HACCP Records	Verification Procedures and Frequency	Corrective Actions
1B Final Wash (antimicrobial)	No visible contamination on carcasses Antimicrobial concentration in sanitizing cabinet will be maintained between 0.5 and 2.5%. Solution pressure at nozzles in sanitizing cabinet will be maintained above 35 PSI.	Quality Assurance monitors washing/antimicrobial equipment use every 2 h to ensure that distance, volume, and pressure meet critical limit and results are recorded, dated, and initialed.	Washing Equipment Monitoring Log Antimicrobial Intervention Monitoring Log	Once per shift the QA supervisor will review all Logs and observe the operation and monitoring at the CCP.	QA will identify the cause of the deviation and prevent reoccurrence.
	Pressures in carcass wash will be maintained bet. 100–350 PSI.	Quality Assurance evaluates 25% of carcasses for visible fecal contamination.	Washing Equipment Calibration Log Corrective Action Log	Maintenance supervisor will verify accuracy (calibration) of the washing and antimicrobial intervention equipment once per shift.	If concentration is outside limits, QA will identify the cause of deviation and make corrections to return concentration to within prescribed limits. Also, preventive actions will be taken to reduce the likelihood of a recurrence. Product produced below critical limit will be identified and sprayed w/a 0.2% antimicrobial solution in the cooler. Product produced above critical limit will be identified, held (exposed to carcass spraying in the cooler), and sampled until a representative sample determines that the level of residual antimicrobial on carcasses shows no significant difference between carcasses sprayed within limits and ones sprayed above the upper limit. When there is no difference, carcass will be released for fabrication. If PSI drops below 100, QA will identify cause of deviation and require corrective action to return the pressures to within prescribed limits.

FIGURE 46.4 Slaughter beef: HACCP plan. (Modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. Used with permission.)

CCP# and Location	Critical Limits	Monitoring Procedures and Frequency	HACCP Records	Verification Procedures and Frequency	Corrective Actions
					Also, preventive actions will be taken to reduce the likelihood of a recurrence. Product produced outside critical limit will be identified and subjected to carcass AQL reinspection. If carcasses pass they will proceed to fabrication. If the lot fails AQL, carcasses will be reworked and reinspected using AQL criteria. QA will stop production when the wash/antimicrobial intervention falls outside critical limits. All affected carcasses back to the last acceptable check will be visually inspected and reworked if visible fecal contamination is observed. Maintenance will correct problems found in equipment adjustment. ** *Note:* Most establishments will be washing beef carcass with a hand held at pressure 50–75 PI, which is the pressure of the system-delivering water to the establishment. When pressure drops, slaughter operations are stopped. The antimicrobial application in many small/very small plants will be a hand-operated garden sprayer containing a 2% acid solution. Other than the concentration, there are no critical limits.
2B Chilling (All Products)	All carcasses will begin chilling within 1 h from bleedout. All variety meats will begin chilling within 1 h after removal from carcass.	QA technician will observe chilling handling procedures to ensure critical limits are met.	Carcass Chilling Log Variety Meats Chilling Log	Once per shift the QA supervisor will review the Carcass Chilling Log and Variety Meats Chilling Log and observe the taking and recording a carcass and variety meat temperatures and cooler temp.	QA will reject or hold product dependent on time and temperature deviation. Process Authority or cooling curves will be used to determine specific corrective action or rejection.

		Carcass and variety meats coolers will be monitored and recorded continuously on temperature recording charts.	Carcass Cooler Temperature Recording Chart Variety Meats Cooler Temperature Recording Chart	Maintenance supervisor will verify accuracy of the carcass cooler temperature and variety meats cooler temperature recording charts once per shift.	QA will identify the cause of the deviation and prevent reoccurrence.
	Temperature of 40°F or less will be reached within 24 h on all products.	QA technician will select and check 10 carcasses and 5 samples of each type of variety meats produced after 24 h chilling to ensure a temperature of 40°F or less has been reached. Carcass lots will be monitored to assure that 1 h requirement is met by tracking 10 carcasses per lot. All results are recorded with the actual value, dated and initialed/signed.	Thermometer Calibration Log Corrective Action Log	QA will check all thermometers used for monitoring and verification for accuracy daily and calibrate to within 2°F accuracy as necessary.	Cooler maintenance will be adjusted to prevent reoccurrence and repairs made if necessary.
3B Finished Product Storage (Cold)	Finished product storage areas will not exceed 40°F.	Maintenance personnel will check finished product storage areas temperatures every 2 h and record cooler temperature in the room temperature log and initial and date.	Room Temperature Log	Maintenance supervisor will verify the accuracy of the room temperature log once per shift.	If a deviation from a critical limit occurs, the following corrective actions will be taken:

FIGURE 46.4 Continued.

CCP# and Location	Critical Limits	Monitoring Procedures and Frequency	HACCP Records	Verification Procedures and Frequency	Corrective Actions
			Thermometer Calibration Log	QA will check all thermometers used for monitoring and verification activities for accuracy daily and calibrate to within 2°F accuracy as necessary.	1. The cause of the temperature exceeding 40°F will be identified and eliminated. 2. The CCP will be monitored hourly after the corrective action is taken to ensure that it is under control.
			Corrective Action Log	QA will observe maintenance personnel check finished product storage area once per shift.	3. When the cause of the deviation is identified, measures will be taken to prevent it from recurring e.g., if the cause is equipment failure, preventive maintenance program will be reviewed and revised, if necessary. 4. If room temperature exceeds the critical limit, the processing authority will evaluate the product temperature to ensure the temperature is sufficient to preclude pathogen growth before release for shipment. If temperature is not sufficient to preclude pathogen growth, product will be cooked in the establishment to ensure destruction of pathogens or condemned.

FIGURE 46.4 Continued.

During the beef slaughter process, there are potential food safety hazards that the establishment could judge reasonably likely to occur. Potential food safety hazards are considered to exist at a step if the hazards could be introduced or enhanced at the step. There are certain steps in the slaughter process that inherently carry a high degree of risk. For these steps, it would be difficult for the establishment to support a decision that a hazard would be not likely to occur. Keep in mind that this flowchart is just an example and that processes and hazards will vary among establishments.

Keep in mind that for every hazard identified in the previous flowchart, the establishment must determine if the hazard is reasonably likely to occur in its operation. If a hazard is identified as likely to occur in the operation, there must be a critical control point (CCP) somewhere in the process to address the hazard. The CCP does not have to be at the location at which the hazard is identified.

Now let us have a look at specific steps in the beef slaughter process and the food safety considerations that the establishment could use in performing a thorough hazard analysis.

46.3.1 Receiving/Holding Cattle

Cattle under 30 months of age do not pose as great a risk for the specified risk material (SRM); cattle 30 months or older do pose a risk and need to be segregated. All cattle, regardless of age, must have tonsils and the distal ileum removed from human food channels. Is this hazard reasonably likely to occur?

The answer is "no" for this practice. The establishment may judge that this hazard is not reasonably likely to occur because it does not purchase cattle over 29 months and removes all tonsils and distal ileum. Purchase specifications may be in place to certify the age of the animal. It may have a prerequisite program to check cattle to ensure that there are no older animals.

The answer is "yes" for this practice. The establishment may slaughter older animals and therefore needs a CCP to address the hazard of SRM. There could be a CCP here for segregating the animals and slaughtering the older ones at the end of the shift and keeping the carcasses as a group.

When cattle arrive at the slaughterhouse, they carry mud, manure, bedding, and other materials that contain a load of microorganisms on their hides and hooves, and may carry microorganisms internally as well. Pathogens such as *E. coli* O157:H7 may be among the microorganisms; therefore, cattle may pose a biological hazard at this point. Is this hazard reasonably likely to occur?

The answer is "no" for this practice. The establishment may judge that this hazard is not reasonably likely to occur because of sanitary procedures to address pathogens carried by animals during receiving and holding. The establishment should have supporting documentation to support this decision. Documents could be written to include control measures applied to prevent a significant hazard at receiving (e.g., proper feed withdrawal, washing of animals).

The answer is "yes" for this practice. The establishment may judge that this hazard is likely to occur for a variety of reasons. There must be a CCP to address it, which may be at this location or it may be further in the process. The establishment may choose to address this hazard here if an intervention exists at receiving that would eliminate, prevent, or reduce the hazard to an acceptable level. For example, a chemical dehairing and wash methodology might be used as an intervention at receiving if it could be shown effective in reducing pathogens. The establishment may choose to address the hazard later in the process. For example, the establishment may address this hazard at the preevisceration antimicrobial rinse.

Cattle may pose a chemical hazard if presented for slaughter with violative levels of chemical residues. The chemicals present in live animals may include antibiotics, pesticides, and environmental contaminants among others. Is this hazard reasonably likely to occur?

The answer is "no" for this practice. The establishment may judge that the chemical hazard is not likely to occur in its process because it has not been a problem historically in the type of cattle it slaughters. For example, FSIS monitoring has shown that feedlot animals have a very low incidence of residues. Establishments may confirm this with their own data from residue testing over a period of time. Establishments may judge that the chemical hazard is not likely because it requires producers to adhere to a quality assurance program, including strict controls for chemicals. Establishments should be able to provide supporting documentation for their decisions.

The answer is "yes" for this practice. Establishments slaughtering classes of cattle that have historically had residue violations may judge that it is likely to occur in its process. For example, bob veal and cull cows have had a higher rate of violative chemical residues.

Cattle may be received that pose a physical hazard due to the presence of foreign material, such as needles or shot. Is this hazard reasonably likely to occur?

The answer is "no" for this practice. Establishments may judge that this hazard is not likely to occur in their process because it has not been a problem historically, or the establishment may choose to obtain animals from suppliers that adhere to a quality assurance program that prevents it. Again, the establishment must be able to support this decision with scientific or technical documentation.

The answer is yes for this practice. If the establishment has a history of foreign material, such as lead shot, it may be prudent in choosing to address it with a CCP somewhere in the process. For example, the establishment may use a metal detector step in boning that is used to address this hazard.

46.3.2 Stunning/Sticking/Bleeding

If the potential for hide contaminants being introduced into tissues is judged negligible at stunning, sticking, and bleeding, the establishment may choose not to identify any hazards at this step. Some establishments may use standard sanitation operational procedures (SSOP) to justify their decision.

46.3.3 Head Removal/Dehiding

The hide is one of the most significant sources of pathogens; therefore, the step of dehiding could be judged to pose a biological hazard. In addition, head removal may result in the spread of ingesta contamination if the esophagus is not closed properly. Is this hazard reasonably likely to occur?

The answer is "no" for this practice. If the establishment judges that this hazard is not likely to occur in its process, it should have documentation to support this decision. Some establishments may justify this decision based on the sanitation operation procedure that they have in place to prevent the transfer of hide contaminants to the carcass.

The answer is "yes" for this practice. If judged to be a likely occurrence in its process, the establishment must address it with a CCP somewhere in the process. For example, the establishment may address it at a steam pasteurization step prior to chilling.

46.3.4 Evisceration/Viscera Processing

Evisceration may result in carcass contamination with feces or ingesta containing pathogens, and so this step may be judged to be a biological hazard by the establishment. Is this hazard reasonably likely to occur?

The answer is "no" for this practice. The establishment may judge that it is not likely to occur in its process; however, there should be supporting documentation to justify the decision as well as demonstrate how the distal ileum is removed.

The answer is "yes" for this practice. If judged likely to occur in its process, the establishment must address it with a CCP somewhere further in the process. For example, the establishment may address this hazard at an antimicrobial rinse prior to chilling.

46.3.5 Splitting/Trimming

The splitting and trimming step may present a carcass with pathogens that could be spread by the processes, and therefore may pose a biological hazard at this point. Keep in mind that FSIS will enforce slaughter food safety standards for feces, ingesta, and milk at the rail inspection station just past the trimmers. SRM is of concern in beef. Is this hazard reasonably likely to occur?

The answer is "no" for this practice. If no, the establishment should have supporting documentation to justify the decision. It may address prevention of cross-contamination by the splitting saw and trimmers. The establishment may be able to show support for the determination that since all visible contaminants are trimmed at this point it is not reasonably likely to occur.

To address SRM, the plant may have a procedure that requires the carcasses to be split on either side of the vertebral column and sanitize the splitting saw between each carcass. Therefore, the plant determines that this is not a hazard reasonably likely to occur.

The answer is "yes" for this practice. If "yes," the establishment must address the hazard somewhere in the process. For example, the establishment may designate the antimicrobial rinse prior to chilling to address this hazard. If the plant determines that SRMs are reasonably likely to occur, it must be addressed in an acceptable manner.

46.3.6 Final Carcass Wash

The final wash step may spread pathogens on the carcass surface; therefore, this step may pose a biological hazard. Is this hazard reasonably likely to occur?

The answer is "no" for this practice. The establishment should have supporting documentation to justify the decision.

The answer is "yes" for this practice. The establishment must address the hazard somewhere in the process. For example, the establishment may designate the antimicrobial rinse prior to chilling to address this hazard.

46.3.7 Chilling

At the chilling step, carcasses may still have pathogens on them that could multiply if not controlled and a biological hazard may result. Is this hazard reasonably likely to occur?

The answer is "no" for this practice. The establishment should have supporting documentation to justify the decision.

The answer is "yes" for this practice. The establishment must address the hazard somewhere in the process. For example, the establishment may do so at chilling to ensure the proper lowering of product temperatures immediately after slaughter to inhibit the growth of pathogens.

46.3.8 Product Storage

Product storage may pose a biological hazard since the product may still contain some pathogens that could multiply. Is this hazard reasonably likely to occur?

The answer is "no" for this practice. The establishment should have supporting documentation to justify the decision.

The answer is "yes" for this practice. The establishment must address the hazard, for example, the establishment may have a finished product storage to maintain proper storage temperature to inhibit growth of pathogens.

Keep in mind that this hazard analysis is only an example and it is not meant to represent what the hazard analysis in any particular establishment will look like.

46.4 Processing Raw Product—Not Ground

The raw product, not-ground-processing, category includes all raw products that are not ground in their final form. Some examples are beef trimmings, tenderized cuts (e.g., injected or marinated), steaks, roasts, chops, poultry parts, fabricated products, and edible by-products (e.g., livers, gizzards).

In the United States, all meat-processing establishments must comply with federal requirements to protect the public from injury and fraud. Of all the requirements, the most important ones are practices, sanitation, and hazards management. Let us use raw beef trimmings and tenderized and Figures 46.5 through 46.8 to follow our discussion.

1. Common name?	Beef trimmings, tenderized (mechanically) beef cuts
2. How is it to be used?	Beef trimmings—for further processing into ground or processed products. Tenderized beef cuts—for further processing into breaded products or sold as raw-tenderized beef cuts
3. Type of package?	Bulk-packed (e.g., plastic bag, vacuum packed)
4. Length of shelf life, at what temperature?	3–6 Months at 0°F or below; 7 days at 40°F
5. Where will it be sold? consumers? intended use?	Wholesale to distributors only
6. Labeling instructions?	Keep frozen; keep refrigerated
7. Is special distribution control needed?	Keep frozen; keep refrigerated

FIGURE 46.5 Raw beef product description: beef trimmings, tenderized beef cuts. (Modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. Used with permission.)

46.4.1 Receiving

The first step in these processes is receiving. Carcasses or parts are received either from other establishments, or from the slaughter department. After meat ingredients are received, they are stored in freezers or coolers until use. Any packaging materials or ingredients, if used, are also received and stored prior to use.

46.4.1.1 Written Purchase Specifications

They are developed by some establishments to ensure that a consistent product is received. Specifications are formal agreements between the supplier and the purchaser, and may include quality aspects, such as portions of lean and fat, and safety factors such as laboratory testing for pathogens.

46.4.1.2 Refrigeration

It achieves several purposes. Carcasses are chilled after slaughter for a specified period, allowing them to become firm enough to cut. Refrigeration reduces moisture loss from the product. It slows down metabolic and enzymatic activities within the meat tissues that would lead to product deterioration.

Refrigeration is also an important food safety factor. It slows the growth of microorganisms, including spoilage bacteria and pathogens. Continuous refrigeration is essential to control microbial growth. The temperature and the holding time of the raw materials affect the multiplication of microorganisms. Meat products must be maintained at refrigeration temperatures adequate to control spoilage and growth of pathogens.

Chiller or cooler temperatures substantially retard the growth of most pathogens. Chiller storage is temporary, however, because even at these temperatures, the spoilage organisms will continue to grow, although at a very slow rate. Freezers halt the growth of all bacteria. Products kept frozen will maintain safety and quality for longer periods of time.

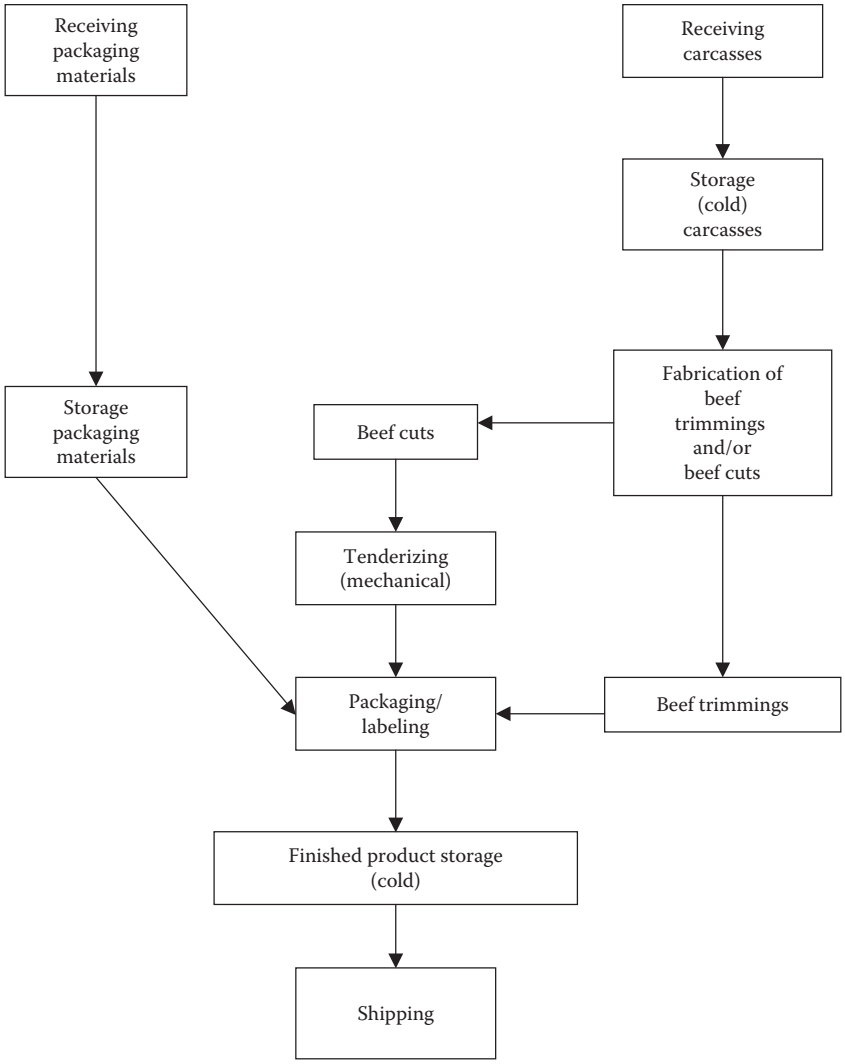

FIGURE 46.6 Raw beef process flow diagram: beef trimmings, tenderized beef cuts. (Modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. Used with permission.)

46.4.2 Sanitation

Sanitation procedures are essential in processing raw products. Any contaminated product contact surface could transfer bacteria, potentially including pathogens, to product. Sanitation procedures should prevent cross-contamination from equipment, personnel, traffic, air flow, tables, and floors. Establishments that process more than one species should implement controls to prevent cross-contamination between different species.

46.4.3 Fabrication

Fabrication refers to creating the various cuts from the carcass to produce particular types of products. Primal or wholesale cuts are made first. Their names usually identify where the meat comes from on the

Process Step	Food Safety Hazard	Reasonably Likely to Occur?	Basis	If Yes in Column 3, What Measures Could Be Applied to Prevent, Eliminate, or Reduce the Hazard to an Acceptable Level?	Critical Control Point
Receiving—Carcasses	Biological: Pathogens—microbial	Yes	*Salmonella and Escherichia coli 0157:H7* may be present on incoming raw product.	Certification from suppliers that product has been sampled for *Salmonella* and meets standard set by FSIS, and that it has been subjected to *E. coli* sampling without presumptive positive results.	1B
	Salmonella, Escherichia coli 0157:H7				
	Chemical—None				
	Physical—Foreign materials such as broken needles	No	Plant records show that there has been no incidence of foreign materials in products received into the plant.		
Receiving—Packaging Materials	Biological—None				
	Chemical—Not acceptable for intended use	No	Letters of guaranty are received from all suppliers of packaging materials.		
	Physical—Foreign materials	No	Plant records demonstrate that foreign material contamination has not occurred during the past several years.		
Storage—Packaging Materials	Biological—None				
	Chemical—None				
	Physical—None				

Storage (Cold)—Carcasses	Biological—Pathogens	Yes	Pathogens are reasonably likely to grow in this product if temperature is not maintained at or below a level sufficient to preclude their growth.	Maintain product temperature at or below a level sufficient to preclude pathogen growth.	2B
	Salmonella				
	Escherichia coli O157:H7				
	Chemical—None				
	Physical—None				
Fabrication of Beef Trimmings and/or Beef Cuts; Tenderizing (Mechanical) Beef Cuts	Biological—None				
	Chemical—None				
	Physical—Metal contamination from injection needles and machine wear.	Yes	Plant records show that during the fabrication and tenderizing process metal contamination is likely to occur.	Metal detector prior to packaging and/or visual examination and maintenance of tenderizers	
Packaging/Labeling	Biological—None				
	Chemical—None				
	Physical—Metal contamination	Yes	Metal contamination that may have come into the establishment with raw product or occurred during the fabrication process.	Functional metal detector is on-line in the packaging/labeling area to remove product with metal contamination.	3P
Finished Product Storage (Cold)	Biological—Pathogens	Yes	Pathogens are reasonably likely to grow in this product if temperature is not maintained at or below a level sufficient to preclude their growth.	Maintain product temperature at or below a level sufficient to preclude pathogen growth.	4B

FIGURE 46.7 Raw beef hazard analysis: beef trimmings, tenderized beef cuts. (Modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. Used with permission.)

Process Step	Food Safety Hazard	Reasonably Likely to Occur?	Basis	If Yes in Column 3, What Measures Could Be Applied to Prevent, Eliminate, or Reduce the Hazard to an Acceptable Level?	Critical Control Point
	Salmonella E. coli 0157:H7				
	Chemical—None				
	Physical—None				
Shipping	Biological—None				
	Chemical—None				
	Physical—None				

FIGURE 46.7 Continued.

CCP# and Location	Critical Limits	Monitoring Procedures and Frequency	HACCP Records	Verification Procedures and Frequency	Corrective Actions
1B Receiving —Carcasses	Supplier certification that product meets FSIS performance standard for *Salmonella* must accompany shipment.	Receiving personnel will check each shipment for *Salmonella* certification.	Receiving Log	Every 2 months QA will request the most recent FSIS *Salmonella* data results from at least 2 suppliers.	Product not meeting specifications will not be accepted for entry and supplier history examined. If supplier has exceeded specifications, the source will no longer be used until performance standards are met.
			Corrective Action Log		
2B Storage (Cold) —Carcasses	Raw product storage area shall not exceed 40°F.	Maintenance personnel will check raw product storage area temperature every 2 h.	Room Temperature Log	Maintenance supervisor will verify accuracy of the Room Temperature Log once per shift.	QA will reject or hold product, and dependent on time/temperature deviation will make a disposition. Maintenance will review cooler service records and placement of carcasses.
			Thermometer Calibration Log		
			Corrective Action Log	QA will check all thermometers used for monitoring and verification for accuracy daily and calibrate to within 1°F accuracy as necessary.	Product >50°F for 2 h will be diverted to a cooking process. Determination on product <50°F for <2 h will be made using Process Authority or pathogen growth modeling.
					QA will identify the cause of the deviation and prevent reoccurrence.
					Cooler unit will be repaired if necessary.

FIGURE 46.8 Raw beef HACCP plan: beef trimmings, tenderized beef cuts. (Modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. Used with permission.)

CCP# and Location	Critical Limits	Monitoring Procedures and Frequency	HACCP Records	Verification Procedures and Frequency	Corrective Actions
3P Packaging/ Labeling	No metal particles exceed 1/32 in.	Packaging line supervisor will check the metal detector using a seeded sample every 2 h to determine limits are not exceeded.	Metal Detection Control Log	QA, outside the packaging unit, will verify that the metal detector is functioning as intended by running the seeded sample through the metal detector twice per shift (once AM, once PM). QA will observe monitoring to assure that product from kick out is placed on hold.	Packaging supervisor will control and segregate affected product.
	All contaminated product is removed from system by functioning kick out mechanism.		Corrective Action Log		Maintenance personnel will identify and eliminate any problems with the metal detector or kick out mechanism. Preventive maintenance program will be implemented. QA will run seeded sample through detector after repair. All potentially contaminated product will be run through functional metal detector prior to shipment. All product rejected by detector will be reworked after visual examination.
4B Finished Product Storage (Cold)	Finished product storage areas will not exceed 40°F.	Maintenance personnel will check finished product storage areas temperatures every 2 h.	Room Temperature Log	Maintenance supervisor will verify the accuracy of the room temperature log once per shift.	If a deviation from a critical limit occurs, the following corrective actions will be taken:

Thermometer Calibration Log	QA will check all thermometers used for monitoring and verification activities for accuracy daily and calibrate to within 1°F accuracy as necessary.	1. The cause of the temperature exceeding 40°F will be identified and eliminated. 2. The CCP will be monitored hourly after the corrective action is taken to ensure that it is under control. 3. When the cause of the deviation is identified, measures will be taken to prevent it from recurring e.g., if the cause is equipment failure, preventive maintenance program will be reviewed and revised, if necessary. 4. If room temperature exceeds the critical limit, the processing authority will evaluate the product temperature to ensure the temperature is sufficient to preclude pathogen growth before release for shipment. If temperature is not sufficient to preclude pathogen growth, product will be cooked in the establishment to ensure destruction of pathogens or condemned.
Corrective Action Log	QA will observe maintenance personnel check finished product storage area once per shift.	

FIGURE 46.8 Continued.

animal, such as the loin, the shoulder, etc. The plant typically uses large mechanized saws to fabricate the carcass into primal cuts.

Retail cut names indicate what part of the primal cut the meat comes from, for example, rib roast or round steak. Retail cuts may be made with a saw, especially if they include bone. Primal parts are often boned before cutting into retail cuts to produce boneless items.

Establishments that produce portion-controlled retail cuts for hotels (H), restaurants (R), and institutions (I) are often called HRI operations.

46.4.4 Tenderization

Tenderization is another procedure used in some plants. All cuts can be tenderized, but this is typically applied to cuts from lower-quality grades and less tender cuts of higher-graded carcasses. There are several methods for tenderizing the meat. They include aging, use of enzyme solutions, and use of mechanical tenderizers. Mechanical tenderizers typically press many thin blades through the meat pieces, cutting the muscle fibers. Whenever cuts are made into a piece of meat, any bacteria on the surface of the meat, or on the equipment, will be spread onto the cut surfaces. This is particularly significant during mechanical tenderization, when many cutting blades are inserted into the center of the meat, potentially drawing bacteria down from the surface.

46.4.5 Marination

Marination is the process of soaking, massaging, tumbling, injecting, or otherwise combining a liquid solution with the meat or poultry product. Products are marinated to improve taste, tenderness, color, juiciness, or other sensory attributes. Some examples of ready-to-cook marinated products include lemon-herb-flavored boneless chicken breast, beef strips for fajitas, or seasoned pork roast.

46.4.6 Curing

There are also some uncooked cured products. Curing refers to the addition of certain additives to preserve the product and stabilize the color, most commonly salt and/or nitrite. The amounts of nitrite and the less commonly used nitrate are restricted by FSIS regulations; thus, they are often referred to as "restricted ingredients." These cure ingredients are sometimes mixed with water to form a curing solution, or "pickle," before adding them to the meat or poultry products. Some examples of uncooked, cured products include corned beef briskets, cured beef tongues, or cured pork tails packed in brine.

46.4.7 By-Products

Establishments may deal with edible by-products in either the slaughter or the raw—not-ground process category. They may be sold as fresh or frozen items, or used to make other processed foods. Here are a few examples of edible by-products:

1. Casings for sausages are sometimes made from sheep, hog, or beef intestines (distal portion of ileum removed as SRM for beef).
2. Blood is used as an ingredient of certain specialty products.
3. Sweetbreads are thymus glands obtained from the ventral side of the neck and inside the chest cavity of young cattle.
4. Hearts, livers, and tongues are sometimes used in the production of processed products.

46.4.8 Mechanically Separated Product

The mechanical separation process is a way to obtain more usable products from bones from which the muscle has been removed. Often, one sees these products referred to as "mechanically separated

product." The species that can be used are lamb, pork, chicken, or turkey. Mechanically separated beef is not allowed.

Bones for this process have usually already had most of the muscle tissues removed by hand boning, or they are bones, like neck bones, which are difficult to process. The bones are ground up, and the resulting mass is forced through a sieve. The softer muscle particles are thus separated from the hard bone particles, which remain behind the sieve. The resulting product has a paste-like consistency.

Great pressure is used to force the product through the sieve, which results in a temperature rise in the product. Therefore, the product must be processed quickly and the temperature immediately reduced to prevent oxidation and microbial degradation of the product. Even with these precautions, this product will deteriorate quickly.

Although mechanically separated products have many of the characteristics of meat and may be used as a meat ingredient in the formulation of quality meat food products, it is not "meat" as defined in the regulations. In particular, the consistency of mechanically separated livestock products and their mineral content are materially different from those of meat. A certain amount of fine bone particles and bone marrow are incorporated as part of the process, increasing the calcium and iron content of the product. There are specific limits on the quantity and size of the bone particles included in the final product.

A similar process is called advanced meat recovery (AMR). This process obtains the meat tissues from the bones without including materials that are not normally expected in meat. The resulting product consists of distinct particles of meat, with the typical color and texture of the species used. There are no special limits on the use of this product. FSIS has established and enforced regulations that prohibit spinal cord from being included in products labeled "meat."

As the vertebral column and skull in cattle 30 months and older are considered inedible (SRM), these cannot be used for AMR.

46.4.9 Packaging

Packaging materials protect the product from damage during refrigerated or frozen storage. Products may be packaged into retail size packages, into larger containers for institutional use, or into bulk containers for sale to other establishments for further processing. Although there are many different combinations of packaging materials in use, plastic films and cardboard boxes are some of the materials commonly used.

46.4.10 Distribution

The final step is distribution, either to other departments in the same plant, or to other plants or retail markets.

46.5 Processing Raw Product: Ground

This processing category includes all raw products that are ground. Beef, pork, veal, lamb, chicken, and turkey can all be ground and sold or used in other products. Some of the common products are ground beef, hamburger, ground beef patties, ground pork, fresh sausage, Italian sausage, and ground poultry products.

Let us use raw pork sausage tenderized and refer to Figures 46.9 through 46.12 to follow up the discussion.

46.5.1 Raw Materials

Raw materials for ground products include boneless meat, trimmings of different fat content, meat from older animals, head meat, cheek meat, diaphragm meat, esophagus, and AMR products. Grinding is a

1. Common name?	Fresh pork sausage
2. How is it to be used?	Cooked and consumed
3. Type of package?	Bulk-packed (e.g., plastic bag, vacuum packed);
4. Length of shelf life, at what temperature?	3–6 Months at 0°f or below; 7 days at 40°f
5. Where will it be sold? Consumers? Intended use?	Retail and hri, wholesale general public, no distribution to schools or hospitals
6. Labeling instructions?	Keep refrigerated; cooking instructions (minimum internal temperature for cooking); safe food handling label
7. Is special distribution control needed?	Keep refrigerated

FIGURE 46.9 Raw pork sausage: product description. (Modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. Used with permission.)

way that establishments can use lower-quality products that would not be saleable to a retail consumer. In addition to beef trimmings, ground beef is also commonly made from flanks, short plates, shank meat, briskets, and chucks. Meat ingredients used may be fresh or frozen, or a combination.

Some of these raw materials have undergone several processing steps already and have the potential to have become contaminated. Grinders are dependent on their suppliers to eliminate or reduce microbial hazards because antimicrobial treatments and interventions are most practical when the product is still intact.

46.5.2 Nonmeat Ingredients

Sometimes ground products contain nonmeat ingredients. Ground products are often seasoned with salt, sugar, spices, or other flavorings. Depending on the product being made, water may be added, and some product formulations include binders and extenders such as soy flour or nonfat dry milk.

Establishments use a formula to create a consistent product batch after batch. The formula lists the weights or percentages of ingredients to be used. Meats and other ingredients are weighed before use to ensure that the proper amount of each is added to the batch.

46.5.3 Reduction of Particle Size

Comminution is the process of reducing the particle size of meats. Several different machines are used, including the flaker, the grinder, and the bowl chopper. Some producers use a combination of several of these in the production of a product.

The flaker is used on large frozen blocks of meat or meat trimmings. The product is pressed against the knife blades, which shave off pieces of the still-frozen meat, enabling it to be used in formulation without thawing.

The grinder consists of a hopper into which the meat chunks are placed. The meat then moves along an auger or screw, through a cylinder, at the end of which is a grinding plate and a knife.

As the meat is pressed up against the plate, the knife turns and cuts off small bits of the meat. The size of meat particle produced is determined by the size of the holes in the grinding plate.

Another method of reducing particle size is the bowl chopper. This machine consists of a metal bowl that revolves and a metal knife that rotates, cutting through the meat pieces in the bowl. The bowl chopper also mixes the product as it chops it.

Sometimes meat ingredients go through several grinding processes. Often, fat and lean meat ingredients are ground separately and then combined.

46.5.4 Mixing

After comminuting, products are mixed thoroughly. The product is often transferred to a separate piece of equipment, called a mixer or blender, in order to mix it. The mixer consists of a chamber

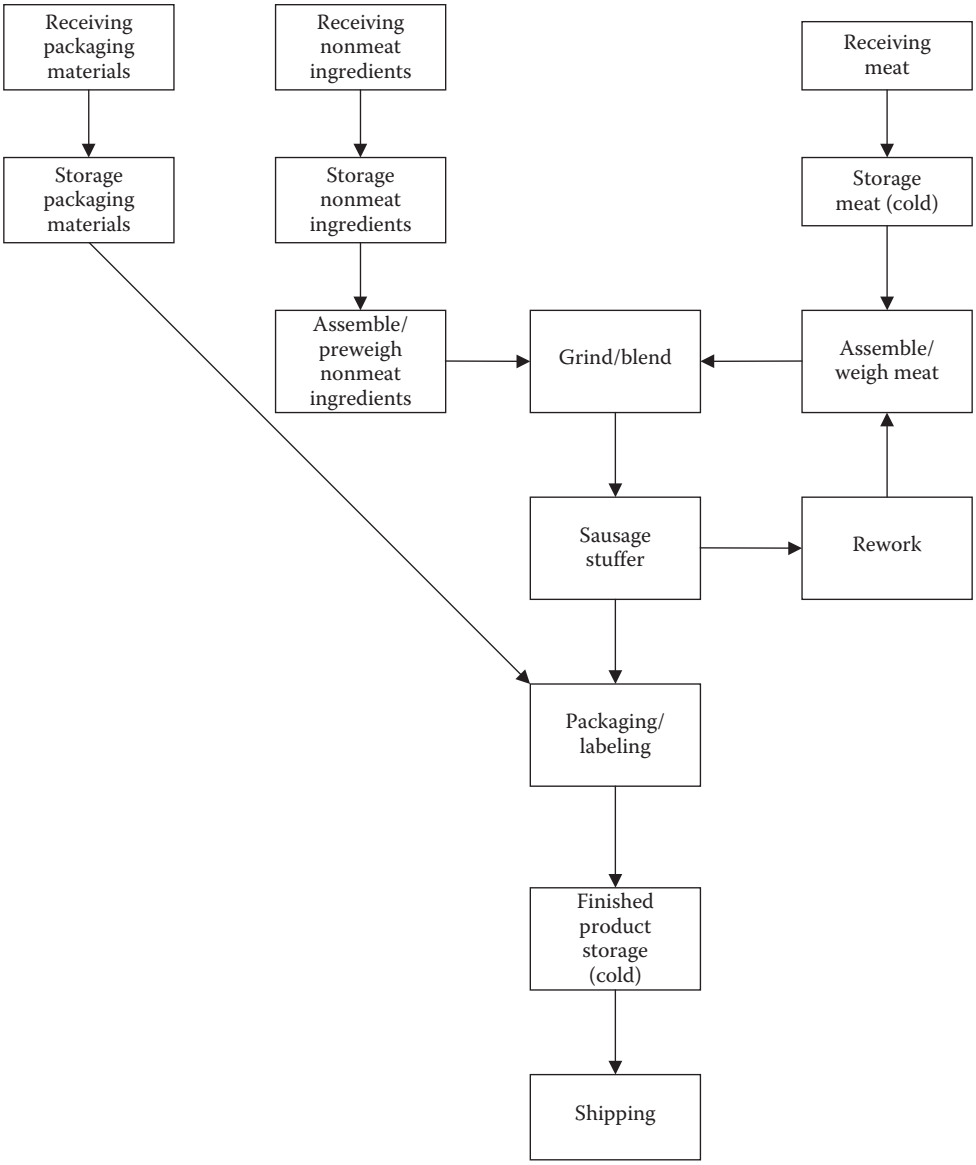

FIGURE 46.10 Raw pork sausage: process flow diagram. (Modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. Used with permission.)

that the ingredients are placed into, and blades or paddles that turn and mix the product, resulting in a uniform distribution of fat and lean particles. Nonmeat ingredients, if used, are added at this stage.

46.5.5 Shaping/Forming

After being comminuted and mixed, the ground meat mixture is often shaped into different forms. Fresh sausage may be extruded into a casing. Hamburger or ground beef is often shaped into patties using a patty machine. After formation, the patties may be frozen.

Process Step	Food Safety Hazard	Reasonably Likely to Occur?	Basis	If Yes in Column 3, What Measures Could Be Applied to Prevent, Eliminate, or Reduce the Hazard to an Acceptable Level?	Critical Control Point
Receiving—Meat	Biological: Pathogens—Microbial (*Salmonella, Escherichia coli 0157:H7)*	Yes	Either pathogen may be present on incoming raw product.	Letters of guaranty that supplier meets base line criteria or in process control of room temperature or storage temperature to prevent growth	1B
	Chemical—None				
	Physical—Foreign materials	No	Plant records show that there has been no incidence of foreign materials in products received into the plant.		
Receiving—Nonmeat Ingredients/Packaging Materials	Biological—None				
	Chemical—Not acceptable for intended use	No	Letters of guaranty are received from all suppliers of nonmeat ingredients and packaging materials.		
	Physical—Foreign materials	No	Plant records demonstrate that foreign material contamination has not occurred during the past several years.		
Storage (Cold)—Meat	Biological—Pathogens (List those specific to the product)	Yes	Pathogens are reasonably likely to grow in this product if temperature is not maintained at or below a level sufficient to preclude the growth.	Maintain product temperature at or below a level sufficient to preclude pathogen growth.	2B
	Chemical—None				
	Physical—None				
Storage—Nonmeat Ingredients/ Packaging Materials	Biological—None				
	Chemical—None				
	Physical—None				
Assemble/Preweigh Nonmeat Ingredients	Biological—None				

	Chemical—None				
	Physical—None				
Assemble/Weigh Meat	Biological—None				
	Chemical—None				
	Physical—None				
Grind/Blend	Biological—None				
	Chemical—None				
	Physical—Metal contamination	Yes	Plant records show that during the grinding process metal contamination is likely to occur.	In-line magnets are installed on the stuffing lines.	3P
Sausage Stuffer	Biological—None				
	Chemical—None				
	Physical—None				
Rework	Biological—Pathogens	Yes	Rework can be a source of continuing inoculation with pathogens.	Rework is condemned or used in a cooked product at the plant. If it will not be used that day or is coded and not mixed so that the identity and total time in plant or process can be determined.	4B
	Chemical—None				
	Physical—None				
Packaging/Labeling	Biological: Pathogens—parasitic (Trichina)	Yes	Trichina has historically occurred in raw pork products.	Labels that clearly indicate this is a raw product, along with cooking instructions, and the safe food handling statement.	5B
	Chemical—None				
	Physical—Metal contamination	Yes	Metal contamination that may have come into the establishment or is contaminated during the grinding process must be removed.	Functional metal detector is on-line in the packaging/labeling area to remove metal contamination.	6P

FIGURE 46.11 Raw pork sausage: hazard analysis. (Modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. Used with permission.)

Process Step	Food Safety Hazard	Reasonably Likely to Occur?	Basis	If Yes in Column 3, What Measures Could Be Applied to Prevent, Eliminate, or Reduce the Hazard to an Acceptable Level?	Critical Control Point
Finished Product Storage (Cold)	Biological—Pathogens	Yes	Pathogens are reasonably likely to grow in this product if temperature is not maintained at or below a level sufficient to preclude their growth.	Maintain product temperature at or below a level sufficient to preclude pathogen growth.	7B
	Chemical—None				
	Physical—None				
Shipping	Biological—None				
	Chemical—None				
	Physical—None				

FIGURE 46.11 Continued.

CCP # and Location	Critical Limits	Monitoring Procedures and Frequency	HACCP Records	Verification Procedures and Frequency	Corrective Actions
1B Receiving —Meat *Salmonella* and other pathogens in raw pork trimming.	Supplier certification that product meets FSIS performance standard for *Salmonella* must accompany shipment.	Receiving personnel will check each shipment for *Salmonella* certification.	Receiving Log	Every 2 months QA will request FSIS *Salmonella* data results from the company for at least 2 suppliers.	Will not receive product unaccompanied by *Salmonella* certification. If product does not have certification, it is rejected or returned. Assure that procedures for only guaranteed supplier list is kept current and guaranty on file in shipping/receiving log. If supplier does not meet FSIS performance standards product will not be purchased from them until they can maintain bacterial levels meeting performance standard.
2B Storage —(Cold) Meat	Raw product storage area shall not exceed 40°F.	Maintenance personnel will record raw product storage area temperature every 2 h, initial/sign and date log.	Room Temperature Log	Maintenance supervisor will verify accuracy of the Room Temperature Log once per shift and observe plant employee performing monitoring.	QA will reject or hold meat until time/temperature deviation and its implications are reviewed. Product disposition will depend on this expert review.
			Corrective Action Log		
			Thermometer Calibration Log	QA will check all thermometers used for monitoring devices for their accuracy and verify to within 2°F on a daily basis.	QA will identify the cause of the deviation and devise measures to prevent reoccurrence. Maintenance will adjust scheduled cooler upkeep and review if necessary or repair any malfunctioning equipment.

FIGURE 46.12 Raw pork sausage: HACCP plan. (Modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. Used with permission.)

CCP # and Location	Critical Limits	Monitoring Procedures and Frequency	HACCP Records	Verification Procedures and Frequency	Corrective Actions
3B Packaging/ Labeling (*Trichina*)	Product must clearly be labeled as raw—Cook before eating	Packaging line supervisor will select two packages of product hourly and ensure labeling requirements are met.	Corrective Action Log	QA will observe packaging line supervisor perform monitoring activity once per shift.	QA will segregate and hold all affected product.
	Cooking instructions that state "cook to 145°F" must be on package.		Labeling Log	QA will select three labels intended for use from label storage area twice weekly to ensure label accuracy.	QA will ensure that proper labeling is applied to all affected product prior to shipment.
	Safe food-handling statement must be part of label.			QA will check labels once a day on packaged product to ensure accurate labels are placed on packaged product.	QA will determine cause of deviation and institute preventive action.
4P Packaging/ Labeling	No metal particles to exceed 1/32 in.	Packaging line supervisor will check the metal detector using a seeded sample every 2 h to determine limits are not exceeded.	Metal Detection Control Log	QA, outside the packaging unit, will verify that the metal detector is functioning as intended by running the seeded sample through the metal detector twice per shift (once AM, once PM).	Packaging supervisor will control and segregate affected product.
	All contaminated product is removed from system by functioning kick out mechanism. All kick out product will be visually examined and any metal removed.		Corrective Action Log		Maintenance personnel will identify and eliminate any problems with the metal detector or kick out mechanism. Preventive maintenance program will be implemented. QA will run seeded sample through detector after repair. All potentially contaminated product will be run through functional metal detector prior to shipment. All product rejected by detector will be reworked.

5B Finished Product Storage (Cold)	Finished product storage area shall not exceed 40°F.	Maintenance personnel will check finished product storage area temperature every 2 h.	Room Temperature Log	Maintenance supervisor will verify the accuracy of the room temperature log once per shift.	If a deviation from a critical limit occurs, the following corrective actions will be taken:
			Corrective Action Log	QA will check all thermometers used for monitoring and verification activities for accuracy daily and calibrate to within 2°F accuracy as necessary.	1. Product which may not have met CL will be identified and held.
			Thermometer Calibration Log	QA will observe maintenance personnel check finished product storage area once per shift.	2. The cause of the temperature exceeding 40°F will be identified and eliminated.
					3. The CCP will be monitored hourly after the corrective action is taken to ensure that it is under control.
					4. When the cause of the deviation is identified, measures will be taken to prevent it from recurring e.g., if the cause is equipment failure, preventive maintenance program will be reviewed and revised, if necessary.
					5. If room temperature exceeds the critical limit, the processing authority will evaluate the product temperature to ensure the temperature is sufficient to preclude pathogen growth prior to shipment. If temperature is not sufficient to preclude pathogen growth, product will be cooked in the establishment to ensure destruction of pathogens.

FIGURE 46.12 Continued.

46.5.6 Metal Detection

Because of the moving parts and high mechanical forces common in these operations, there is a possibility of metal chipping or breaking. Proper maintenance of equipment is essential to reduce this possibility. Some establishments use a metal detector to identify the product that may be contaminated with metal fragments.

46.5.7 Trace Back and Trace Forward

Although the grinding establishment may not have access to records of the farm sources of their raw material, or records maintained by the plants that slaughter, dress, and bone their raw materials, they should purchase raw materials from suppliers that maintain such records. Establishments should also maintain records of distribution of products. These records can facilitate trace back and trace forward in the case of a recall or of an outbreak of foodborne illness.

Some establishments have developed a production-coding system for tracking purposes. These systems enable the establishment to track the product from the raw material source up to the finished product. Some establishments use the period of time between cleanups as a production lot. This is because all products produced between cleanups would be implicated in a recall.

46.5.8 Rework

Rework is sound finished product that is reincorporated into a batch of fresh ingredients prepared to make similar finished products. Establishments also sometimes choose to develop a rework tracking system to reduce the amount of product that would be implicated in a recall. Some establishments include all rework at the end of the production day, or divert it to cooked product-processing departments.

There have been instances where a product recall was greatly affected by the establishment's ability to track the use of rework. In one example, an establishment recalled a large amount of product due to the presence of *E. coli* O157:H7, found during the investigation of an outbreak of foodborne illness. Review of the establishment's production practices revealed that some of the production lot that was recalled had been used as rework in subsequent days' production. As a result, the recall was expanded to include the entire amount of production that may have included the rework. This recall eventually involved over 25 million pounds of products.

46.6 Antimicrobial Interventions for Raw Products

46.6.1 Acidified Sodium Chlorite

Acidified sodium chlorite (Sanova) is an antimicrobial agent, effective against most of the pathogens of concern. Acidified sodium chlorite solutions are typically applied at ambient temperature as either sprays or immersion dips, directly to the surfaces to be treated, at 500–1200 ppm. Although it is most commonly used in slaughter operations, it is permitted for use on parts or trimmings postchill with no requirements for treated product labeling. Products treated with acidified sodium chlorite that retain water would need to reflect this on the label. Use of acidified sodium chlorite results in significant reduction of all microbial species, for example, 2-log10 reduction for *E. coli* and 1-log10 reduction for total aerobics.

46.6.2 Milk-Derived Lactoferrin

Milk-derived lactoferrin is permitted for use as an antimicrobial spray that contains up to 2% lactoferrin, applied to uncooked beef parts. It must be declared on the label as "treated with lactoferrin from milk."

46.6.3 Ozone

Ozone may be used in contact with food as a gas or liquid as an antimicrobial in meat and poultry products, including ground meats.

46.6.4 Irradiation

Food irradiation is the process of exposing food to radiant energy to reduce or eliminate bacteria. Ionizing radiation will reduce, and in some circumstances eliminate, pathogenic microorganisms in or on meat and poultry. FSIS has included ionizing radiation as an approved additive in pork carcasses and fresh, or previously frozen, cuts of pork that have not been cured or heat processed for the control of *Trichinella spiralis*, which causes trichinosis. Ionizing irradiation is also recognized as an approved additive in fresh or frozen, uncooked, packaged meat or poultry products for the purpose of reducing pathogenic microorganisms and extending shelf life.

Radiation is broadly defined as energy moving through space in invisible waves. Radiant energy has differing wavelengths and degrees of power. Forms of radiant energy include microwave and infrared radiation, which heat food during cooking; visible light or ultraviolet light, which are used to dry food or kill surface microorganisms; and ionizing radiation, which penetrates deeply into food, killing microorganisms without raising the temperature of the food significantly.

Treating products with irradiation could result in the significant reduction or even the elimination of certain pathogens. Ionizing radiation has been shown to be effective at eliminating *Salmonella*, *E. coli* O157:H7, *Clostridium perfringens, Staphylococcus aureus, Listeria monocytogenes*, and *Campylobacter jejuni*.

Irradiation dose is measured in kiloGray (kGy); the maximum dose for use on meat products is 4.5 kGy. The radiation dose necessary to reduce the initial population of many of the bacterial pathogens by 90% (the *D*-value, or 1-$\log_{10}$) ranges from 0.1 kGy to just below 1 kGy. Higher radiation doses are needed to accomplish the same antimicrobial effect in a frozen food versus a nonfrozen food.

Irradiation does not significantly increase the temperature or change the physical, sensory, or nutritional characteristics of foods. As irradiation does not raise product temperature, the product is still raw and requires refrigeration.

The irradiation process requires a source of energy. The two types are radioisotopes (radioactive materials such as cobalt or cesium) or machines that produce high-energy beams.

The Food and Drug Administration (FDA) regulates all aspects of irradiation: what products it can be used on, what dose can be used, and how those products are labeled. The United States Department of Agriculture (USDA) is responsible for the inspection and monitoring of irradiated meat and poultry products and for the enforcement of FDA regulations concerning those products.

The "radura" is an internationally recognized symbol identifying irradiated food. The FDA requires that both this logo and a statement ("Treated with irradiation" or "Treated by irradiation") must appear prominently on the label of packaged foods, and on bulk containers of unpackaged foods.

46.7 Concluding Remark

This chapter provides a brief description of processing raw meat and meat products covering the following:

- The HACCP system of identifying hazards and control measures.
- The common methods in preventing pathogens.

The next chapter will provide more details on processing raw ground beef.

47

*Ground Meat Processing and Safety**

Y. H. Hui

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47.1 Hazard Analysis of the Raw Ground Process

As far as the consumers are concerned, ground beef is probably the most common meat product that has caused them injuries, from minor to death, in the last few years. Chances are good that this trend will continue though the government is doing everything it can to stop this trend.

This chapter addresses the production of safe raw ground products and the thought process an establishment follows in its hazard analysis. We will use raw ground beef as our model. Figure 47.1 shows product description and basic process flow diagram for raw ground beef patties. Figure 47.2 shows safety recommendations according to processing stages. The recommendations are food safety hazards that the

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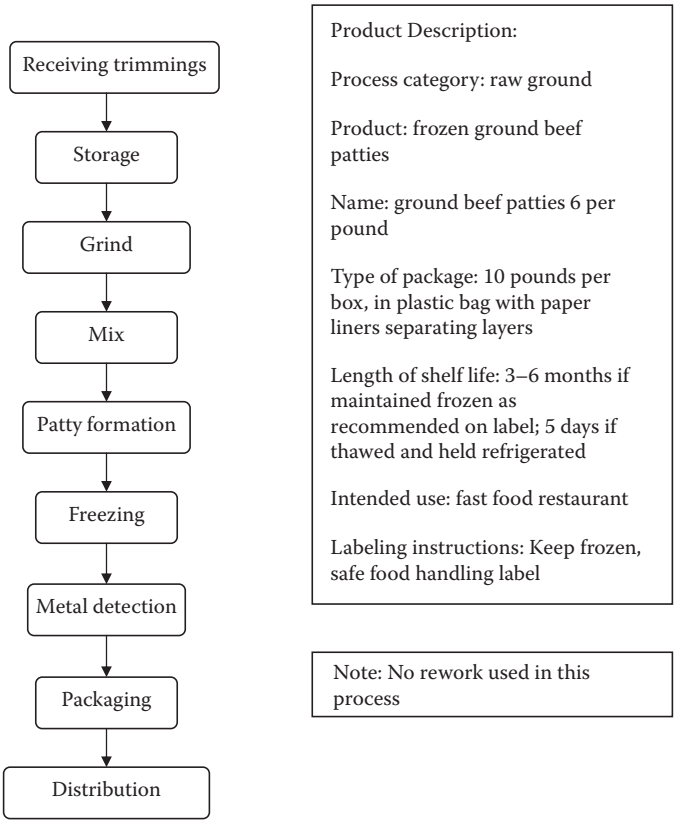

FIGURE 47.1 Raw ground beef patties: Product description and process flow diagram. (Modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. Used with permission.)

establishment could judge reasonably likely to occur. Keep in mind that processes, hazards, and other criteria will vary among establishments.

Let us take a look at specific steps in the raw ground process and the food safety considerations the establishment could use in performing a thorough hazard analysis. Keep in mind that for every hazard identified in the previous flow chart, the establishment must determine if the hazard is reasonably likely to occur in its operation. If a hazard is identified as likely to occur in the operation, there must be a step somewhere in the process to address the hazard. This does not have to be at the location at which the hazard is identified.

In the United States, the Food Safety Inspection Service (FSIS), a unit of the Department of Agriculture (USDA), is responsible for the safety of meat and meat produces. In accordance with the following, please note that, for this chapter:

1. The text and tables have been collated from multiple USDA public documents (legal, regulatory, and training), distributed in USDA'S website, http://www.fsis.usda.gov.
2. The figures were originally adapted from a series of USDA continuing educational documents, *USDA HACCP Generic Models on Raw Ground and Not Ground Meat Products*. They are available at USDA's website, http://www.fsis.usda.gov.

It must be emphasized that, in this chapter:

1. All information have been designed by the USDA for training purposes only and is not meant to detail the actual manufacturing processes.

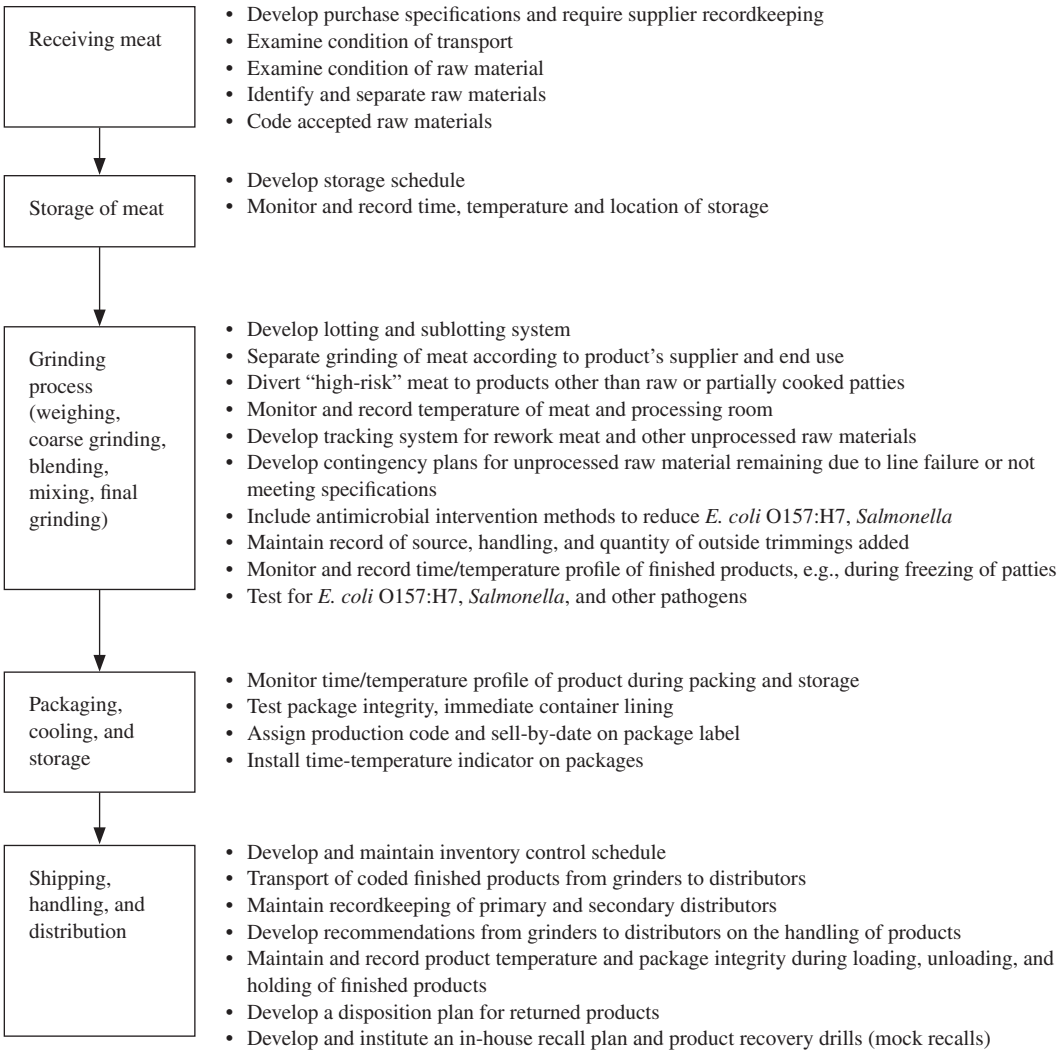

FIGURE 47.2 Raw ground beef patties: Processing stages and safety recommendations. (Modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. Used with permission.)

2. All abbreviations are standard ones used in law, science, and technology as issued by FSIS.
3. All terms with legal implications apply only in the United States.

The discussion in the following sections refers to Figure 47.1 through Figure 47.5.

Figure 47.3 that outlines food safety hazards for each major stages during the processing of raw ground beef. Figure 47.4 presents in detail the identification, determination, and controls for safety hazards during the processing of raw ground beef and Figure 47.5 shows Hazards Analysis Critical Control Points (HACCP) plan for processing raw ground beef patties.

47.1.1 Receiving Beef

When meat for grinding arrives at the establishment, it will carry a certain amount of bacteria, both spoilage and pathogenic organisms. The amount and types of bacteria present will vary, depending on the conditions at the slaughter or processing plant from which the meat is received. Grinders are

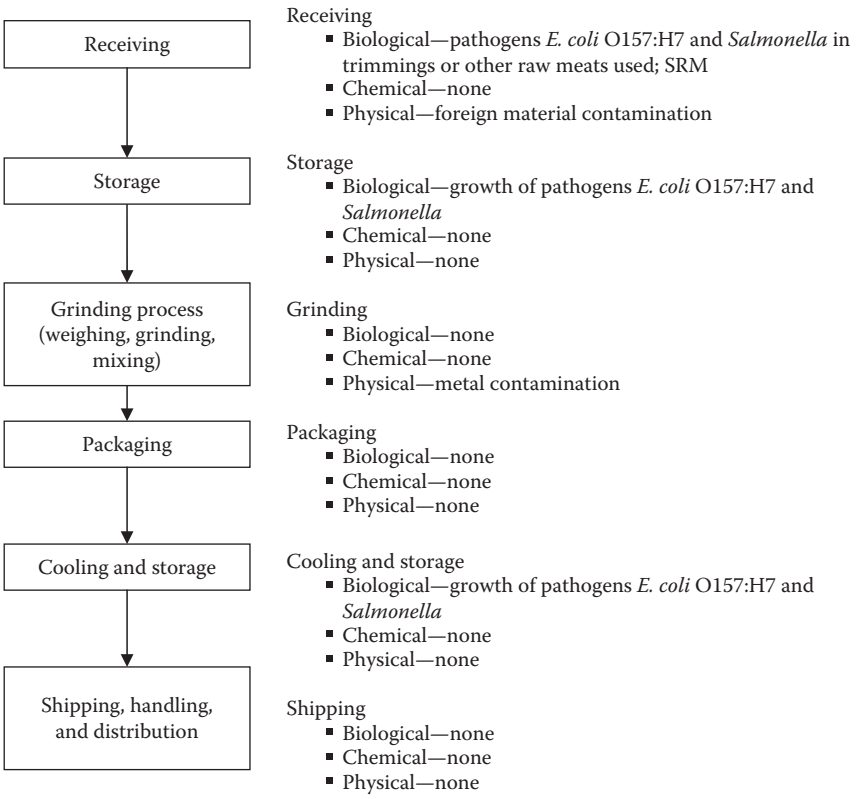

FIGURE 47.3 Raw ground beef patties: Processing stages and outline of food safety hazards. (Modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. Used with permission.)

dependent on their suppliers to eliminate or reduce any contamination. Therefore, raw meat may pose a biological hazard at this point.

The pathogen *Escherichia coli* O157:H7 is of particular concern to grinding operations because it is considered an adulterant in ground beef. There is recent evidence that the prevalence of *E. coli* O157:H7 in cattle is higher than previously assumed. The presence of *Salmonella*, although not considered an adulterant, is also a public health risk. Establishments must demonstrate that they are able to control the occurrence of *Salmonella* in their product by producing products that meet the FSIS *Salmonella* Performance Standards. Raw ground products, by their very nature, do not have a lethality step to eliminate pathogens (except when processors choose to apply a lethal dose of irradiation). Decontamination interventions can significantly reduce the levels of pathogens when the control measures are effectively implemented and validated. These interventions, such as steam pasteurization, organic acid spray, hot-water treatment, and steam vacuuming, are applied to intact carcasses at the slaughter establishment. Microbiological testing, when properly designed, can be used to verify that control measures are working. Microbiological testing should be used in combination with strict process controls that include intervention methods, in order to reduce the likelihood that the pathogen is present. Any ground beef found to be contaminated with *E. coli* O157:H7 must be treated to inactivate the pathogen: for example, by diverting it to a cooking operation.

Establishments should consider specific risk materials (SRM) for raw beef products.

The establishment must address this hazard. In order to deal with the risk of pathogens entering the system with the starting materials, many establishments develop purchase specifications programs. Purchase specifications may specify that raw materials come from slaughter establishments that apply one or more intervention or antimicrobial treatment, validated to reduce or eliminate microorganisms on carcasses.

Process Step	Food Safety Hazards	Is Hazard Likely to Occur?	Justification for Decision	What Control Measures Can Be Applied to Prevent the Significant Hazards?	Is Step a Critical Control Point?
Receiving trimmings	Biological: Pathogens *E. coli* O157:H7 and *Salmonella*	Yes	*E. coli* O157:H7 or *Salmonella* may be present on trimmings received	Purchase specifications for certification from all suppliers that trimmings are from carcasses that received validated interventions effective to eliminate or reduce *E. coli* O157:H7 to an undetectable level and negative microbiological test results for *E. coli* O157:H7 required from suppliers	Yes
	Chemical: none				
	Physical: foreign material	No	Plant records show that there has been no incidence of foreign material in products in past several years		No
Storage	Biological: Growth of pathogens	Yes	*E. coli* O157:H7 or *Salmonella* may grow if not maintained at proper refrigeration temperatures	Maintain product temperature at or below a level sufficient to prevent growth	Yes
	Chemical: none				
	Physical: none				
Grind	Biological: none				
	Chemical: none				
	Physical: metal contamination	Yes	Past history indicates that metal contamination has occurred during grinding	Proper maintenance of equipment, routine examination during cleaning, metal detector later in process	No
Mix	Biological: none				
	Chemical: none				
	Physical: none				
Patty formation	Biological: none				
	Chemical: none				
	Physical: metal contamination	Yes	Past history indicates that metal contamination has occurred during patty formation	Proper maintenance of equipment, routine examination during cleaning, metal detector later in process	No
Freezing	Biological: none				
	Chemical: none				
	Physical: none				
Metal detection	Biological: none		Past history indicates that metal contamination has occurred in previous process steps	Functioning metal detection equipment to identify and reject contaminated product	Yes
	Chemical: none				
	Physical: none				
Packaging	Biological: none				
	Chemical: none				
	Physical: none				

FIGURE 47.4 Raw ground beef patties: Hazards analysis. (Modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. Used with permission.)

CCP	Critical Limits	Monitoring Procedures and Frequencies	HACCP Records	Verification Procedures and Frequencies	Corrective Actions
1. Receiving	Product not received without certification of interventions and microtesting	Receipt of certification letter for valid antimicrobial intervention and COA for *E. coli* O157:H7 for each load of product received	Receiving log Corrective Action Log	Every 2 months QA will request FSIS *Salmonella* data results and *E. coli* O157:H7 intervention validation results from at least two suppliers	Corrective actions shall meet all requirements of Part 417.3 (a)
2. Temperature control at storage	Product temperature ≤44	QC personnel will record temperature of product exiting grinder every hour	Product temperature log Corrective action log	HACCP coordinator will verify accuracy of the product temperature log once per shift and observe QC personnel performing monitoring	Corrective actions shall meet all requirements of Part 417.3 (a)
			Thermometer calibration log	HACCP coordinator will verify temperature of raw materials cooler and freezer daily. QC will check all thermometers used for monitoring devices for accuracy by immersion in slush ice, and will verify to within 2 daily All thermometers found to be inaccurate will be calibrated using immersion in slush ice and re-evaluated	
3. Metal detection	Functional metal detector	Packaging line supervisor will check the metal detector using a seeded sample every 2 h to determine limits are not exceeded	Metal detection log	QC personnel will verify that the metal detector is functioning as intended by running the seeded sample (2 mm) through the metal detector twice per shift. Functioning metal detector must identify and remove the seeded sample.	Corrective actions shall meet all requirements of Part 417.3 (a)
			Corrective action log	HACCP coordinator will verify accuracy of the metal detection log and observe packaging line supervisor performing monitoring once per shift. Maintenance personnel will perform calibration procedure once per shift.	

FIGURE 47.5 Raw ground beef patties: HACCP plan. (Modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. Used with permission.)

The grinding plant may have purchase specifications for the controls that the slaughter plant has in place to ensure that no SRM are in the products.

Purchase specifications may also include microbial testing specifications. Purchase specification programs for raw materials may be incorporated as part of an HACCP plan. Establishments can also incorporate their purchase specifications in prerequisite programs.

The grinding establishment may implement an examination step before accepting ingredients. The reduction of microorganisms resulting from the intervention method must be maintained in subsequent processing steps by control of temperature and cross-contamination. As a means of verifying this, the plant may establish a maximum product-receiving temperature, or observation of other receiving condition. Other controls that may be utilized at receiving may include microbial testing as a verification procedure, to confirm results provided by the supplier. The establishment may also choose to verify records that facilitate trace-back and trace-forward, which are essential in case of an outbreak of foodborne illness. Grinding operators may establish recordkeeping requirements for their suppliers to trace products back to the farm of origin.

The establishment may determine that the potential for chemical hazards is negligible and if so may not address them.

There is also a risk that some foreign material has contaminated the product that is being received, thus posing a physical hazard.

Establishments may determine that there is a risk that starting materials may be received with foreign material. If so, they may establish a step at receiving to detect contamination, such as visual inspection or use of detection technology such as an electronic metal detector or imaging system. Establishments may also choose to address this hazard with a step later in the process, for example, by using a metal detector on the finished product.

47.1.2 Storage

Continuous refrigeration is essential to prevent microbial growth. Inadequate holding temperatures can allow the bacteria present to multiply, which increases the risk from pathogens such as *E. coli* O157:H7 and *Salmonella*. Therefore, raw meat may pose a biological hazard at this point.

If the establishment judges this hazard is likely to occur, it must be addressed in the HACCP plan. Establishments commonly establish room-temperature critical limits. It is advisable to control and monitor the product temperature as well as the room temperature. The establishment may have supporting documentation that demonstrates that control of room temperature correlates to control of product temperature. Establishments may also have a time or inventory control to ensure that product does not remain in storage for a time period that would allow bacterial growth at storage temperatures, either in the HACCP plan or addressed in good manufacturing practice and standard operating procedures.

The establishment may determine that the potential for chemical hazards and physical hazards is negligible at this step. If not, the hazards must be addressed at a step in the HACCP plan.

47.1.3 Grinding Process

The establishment may determine that the potential for biological hazards and chemical hazards is negligible at this step.

The grinding process involves reducing the particle size of the raw meat ingredients, and mixing to distribute fat, lean, and any ingredients added. As the equipment used produces high mechanical forces, and has moving metal-against-metal parts, there is a possibility of metal chipping or breaking. There is also the possibility that any foreign material in the product, such as lead shot, would be broken up and distributed throughout the product. Because of the risk that foreign material has contaminated the product received, or that the grinding/mixing process added metal to the product, the establishment may determine that this step constitutes a physical hazard.

Some establishments use a metal detector to identify product that may be contaminated with metal fragments. The metal detector may be here or located further in the process, such as at packaging.

47.1.4 Packaging

The establishment may determine that the potential for biological, chemical, and physical hazards is negligible at this step. If not, the hazards must be addressed at a later stage in the HACCP plan.

47.1.5 Cooling and Storage

Establishments often freeze finished ground product, although some distribute the product refrigerated. Pathogens will grow in raw ground beef if temperature is not maintained at or below a level sufficient to prevent their growth. Since the product is now in a ground form, the small particles of meat provide a greatly increased surface area, which is ideal for bacterial growth. Any bacteria present on the meat pieces used, or present on the equipment surfaces, have been distributed throughout the product. Because of the risk of *E. coli* O157:H7 or *Salmonella* growth in the raw finished product, the establishment may address this as a biological hazard.

The establishment must address the hazard with a control somewhere in the process. Continuous temperature control will prevent growth of bacteria that may have escaped the other control steps. Plants may establish one or a combination of control methods, such as monitoring the time–temperature profile during freezing, testing finished product for pathogens as a means of verifying process control, monitoring the temperature of finished product during storage, or monitoring the temperature of the refrigerator/freezer.

The establishment may determine that the potential for chemical and physical hazards is negligible at this step, and if so, may not address them.

47.1.6 Shipping, Handling, and Distribution

The establishment may determine that the potential for biological, chemical, and physical hazards is negligible at this step. If not, the hazards must be addressed at a location in the HACCP plan.

47.1.7 Reassessment Requirement for *E. coli* O157:H7

The reassessment requirement for *E. coli* O157:H7 that we discussed in beef slaughter also applies to raw—ground and raw—not-ground operations. These establishments must reassess their plans to determine whether *E. coli* O157:H7 is a hazard reasonably likely to occur in their production processes. If reassessment results in a determination that *E. coli* O157:H7 is a food safety hazard reasonably likely to occur, the establishment must address it in the HACCP plan through one or more critical control point (CCPs) designed to control the pathogen. These CCPs may include purchase specifications for microbiological testing and supplier certification of validated interventions in the slaughter process. Additional CCPs may be established to prevent growth or contamination after product receipt. Establishments that determine that *E. coli* O157:H7 is not a hazard reasonably likely to occur may address this pathogen in their Sanitation Standard Operating Procedures (SSOP's) or through a prerequisite program; in this case FSIS would expect the establishment to maintain documents setting out the procedures of the prerequisite program and related records.

The information we just covered gave you an introduction to the thought process the establishment uses in its hazard analysis. Keep in mind that this hazard analysis is only a simplified training example and it is not meant to represent what the hazard analysis in any particular establishment will look like. The process in the establishment to which you are assigned might look different depending on whether the plant adds nonmeat ingredients, uses rework, stuffs product into casings, etc. The same thought process would apply to operations grinding poultry, pork, or other species. Processors would probably focus on the pathogens most prevalent for each species of meat processed: for example, *Salmonella* in pork, and *Salmonella* and *Camplyobacter* in poultry.

47.2 Intervention Treatments for Meat Parts and Comminuted Meat

The following section describes studies on the use of antimicrobial decontamination methods as interventions to reduce microbial load. Establishments should determine the level of effectiveness of their

antimicrobial decontamination methods with regard to reduction of specific pathogens and indicator organisms. Establishments should validate these methods according to the processes they use. The following antimicrobial treatments have been approved for reduction of pathogens in meat parts and comminuted meat: acidified sodium chlorite (ASC), lactoferrin, ozone, and irradiation.

47.2.1 Acidified Sodium Chlorite

ASC was approved as an antimicrobial agent on processed, comminuted, or formed meat food products prior to packaging of the food for commercial purposes, in accordance with current industry standards of good manufacturing practices. ASC may be applied as a spray or dip at levels that result in sodium chlorite concentrations of 500–1200 ppm, in combination with any generally recognized as safe (GRAS) acid at levels sufficient to achieve a pH of 2.5–2.9. When used as such, ASC is considered a secondary direct additive and does not have to be declared on the label of the treated product. Any meat treated with ASC and found to retain water will need to disclose that fact in the labeling in accordance with the Final rule on "Retained Water in Raw Meat and Poultry Products; Chilling Requirements."

A study showed the effectiveness of ASC in reducing the counts of *E. coli* O157:H7 and *Salmonella typhimurium* when inoculated onto various hot-boned individual beef carcass surface regions: inside round, outside round, brisket, flank, and clod. Reduction of *E. coli* O157:H7 and *S. typhimurium* on beef carcass surfaces using ASC. The pathogens were reduced by 3.8–3.9 log cycles by water wash followed by phosphoric acid-activated ASC spray, and by 4.5–4.6 log cycles by water wash followed by citric acid-activated ASC spray. The sprays consisted of applying 140 mL of the appropriate sanitizing solution for 10 s at 69 kPa. Use of water wash alone resulted in 2.3 log reduction.

47.2.2 Milk-Derived Lactoferrin

Milk-derived lactoferrin was approved for use as a component of an antimicrobial spray that contains up to 2% lactoferrin applied to uncooked beef. This antimicrobial was approved for use on carcasses and meat parts only, not for comminuted meats. The maximum concentration to be used is 3.26 mL spray/kg beef (or 6.2 mg lactoferrin/kg of beef). Also, derived lactoferrin be declared on the labels of meat to which it was added. The use of lactoferrin on beef carcasses and cuts (as well as products made from these lactoferrin-treated materials) should be labeled with a statement, for example, "treated with lactoferrin from milk."

A review of the microbial activity of activated lactoferrin has shown that it can extend the retail display life of treated steaks by 1.7–2.5 days for case-ready packaged steaks compared to nontreated steaks in conventional packages. This review also cited a study where 1% activated lactoferrin was used in combination with a series of antimicrobial treatments (cold water, lactic acid, hot water). The study has shown that there was a 99.99% detachment of *E. coli* O157:H7 per gram of beef tissue when activated lactoferrin was added to the series of antimicrobial treatments, and 72.2% detachment when activated lactoferrin was not added. The acid rinses and hot water washes reduced *E. coli* O157:H7 to a significant level. The addition of activated lactoferrin effectively sanitized the contaminated beef surface by removing debris and residual bacteria.

47.2.3 Ozone

Ozone was approved food additive that can be safely used in the treatment, storage, and processing of foods, including meat and poultry. Ozone may be used in contact with food in the gaseous or aqueous phase in accordance with current industry standards of good manufacturing practice. It may be used as an antimicrobial in meat and poultry products, including ground meats.

47.2.4 Irradiation

The Food and Drug Administration rule provides for the use of ionizing radiation at dosages of up to 4.5 kilogray (kGy) for treating refrigerated, and up to 7.0 kGy for treating frozen uncooked meat, meat

by-products, and certain other meat food products to reduce levels of foodborne pathogens and extend product shelf-life. The rule provided maximum dosage that can effectively reduce the levels of pathogens including *E. coli* O157:H7 and *Salmonella*. Meat grinding and boning establishments can now irradiate meat for grinding and ground beef in bulk or in retail packages to reduce or eliminate *Salmonella* and eliminate adulteration with *E. coli* O157:H7. It also appears that resistance of foodborne microorganisms may occur exposed to irradiation at certain temperatures and atmospheric environment.

47.3 Guidance for Minimizing Impact Associated with a Food Safety Hazard in Raw Ground Meat

47.3.1 Background

There are sporadic cases, outbreaks, and product recalls involving *E. coli* O157:H7. After a careful study, FSIS has concluded that guidance can help grinders develop and implement procedures that better protect public health. This guide is intended to illustrate how grinders can avail themselves of opportunities to minimize the food safety hazards associated with their products. It does not prescribe regulatory requirements under the Federal Meat Inspection Act (FMIA).

The guidance provided in this document is premised on three main points:

1. Grinders should structure their operations in a manner that takes into account the safety of their raw materials and their potential product safety responsibilities through to the end user.
2. Grinders should realize that they are in an excellent position to implement process and distribution controls that reduce public health concerns associated with ground beef contaminated with *E. coli* O157:H7.
3. There must be an emphasis throughout the production and distribution chain on maintaining the records that are necessary to identify, trace, and retrieve from commerce any ground beef products that may pose a threat to public health.

Grinding operations (which traditionally buy raw materials from one or more sources and sell the processed products to others) have a primary responsibility and unique opportunity to specify purchase requirements related to incoming raw materials, to process raw materials under processing and recordkeeping controls designed to ensure the safety and traceability of their products, and to distribute products to destinations in a manner such that products can be effectively recalled if food safety hazards are identified. Putting aside any legal considerations, it is essential that grinding operators assume that they are responsible for their products until the products' end use. This is especially true for grinding operators who produce products in retail-ready packages. This guidance material, through several guiding principles and associated detailed explanations and recommendations, is intended to identify how grinding operations can reduce the public health risk.

Processing operations are currently required to have SSOP's and a few are already required to have functional HACCP systems. This guidance material is specifically designed to augment these activities, especially the development and operation of an HACCP plan. Grinding operations not already required to have HACCP plans are encouraged to develop and implement their HACCP plans earlier than required as soon as possible. Although this guidance material highlights issues associated with ground beef, the guidance can be applied to most raw products. This guide is not intended to be prescriptive, in a regulatory sense, but rather offers examples of opportunities to improve food safety through purchase requirements, increased process control, and recordkeeping.

Records that facilitate trace-back and trace-forward are essential whenever there is an outbreak of foodborne illness. Although grinding operators may not have access to records of the farm sources of their raw material, or records maintained by the plants that slaughter, dress, and bone their raw materials, they are advised to purchase raw materials from suppliers that maintain such records. In addition, they are advised to keep records regarding the disposition of their products to enable tracing their products forward to consumers and back to suppliers. In instances where grinders do not control their products through

distribution and retail sale, the chain of records necessary to trace products that raise a public health concern should not be broken. Intermediate handlers, such as distributors and wholesalers, should assume responsibility for keeping adequate records regarding the disposition of ground beef products that pass through their hands. FMIA requires that every person, firm, or corporation engaged in buying or selling of meat food products must maintain records that fully and correctly disclose all transactions in its business subject to the FMIA. The recordkeeping requirements are provided by FMIA. Grinders are advised to impress upon all intermediate handlers of their products the importance of records that will facilitate the efficient retrieval from consumers of ground beef products that are a public health concern.

The pathogen *E. coli* O157:H7 is of particular concern to grinding operations because it is considered an adulterant in ground beef, and because it produces severe and sometimes fatal consequences at a very low infectious dose. Buchanan and Doyle emphasized that "HACCP plans that do not include a lethal step that kills pathogens are more complex, since the focus is on risk reduction instead of risk elimination." At present, applying a lethal step such as heat processing or integrated lethality using fermentation or pH is the only approved method of making food harboring *E. coli* O157:H7 safe for consumption. Results from microbiological testing can provide only a limited measure of assurance that this pathogen is not present. Total reliance on sampling is inadequate because *E. coli* O157:H7, if present, is present sporadically and at extremely low levels. Therefore, microbiological testing should be used in combination with strict process controls to reduce, as much as possible, the likelihood that the pathogen is present in the finished product.

47.3.2 Guiding Principles

These guiding principles are

A. Grinders should determine the specifications for microbial safety that are necessary to ensure that their products will be safe and should only accept starting materials and ingredients that meet their specifications. In developing such specifications, grinders should consider the intended use for the starting materials and ingredients and what, if any, pathogen reduction actions by their suppliers would be beneficial.
B. Grinders should keep abreast of new technologies and interventions that could be introduced into their processes to help prevent adulterated products or to identify product that is adulterated before it enters commerce.
C. Grinders should develop and implement processes and packaging procedures to maintain or improve the microbial integrity of their starting materials to ensure that they produce safe consumer products.
D. Grinders should implement controls to identify and segregate for special handling, product that pose a greater risk of being adulterated. Alternate outlets, such as diversion to FSIS inspected or state-inspected processors that employ a bacterial kill-step (such as cooking), should be acquired for such products.
E. Grinders should develop and implement rework, carry-over, and lot designation procedures that reflect an acceptable degree of product exposure (i.e., economic risk) in the event that a health risk is identified that results in recalling product that is suspected of presenting a potential hazard to the public.
F. In cooperation with their customers, grinders should develop and implement handling and distribution procedures that will not compromise the safety of their ground products once those products leave their establishments.
G. Grinders should develop a system of records, which fits into a farm-to-table continuum, which will facilitate trace-back to the suppliers and trace-forward to the distributors in the event that a public health risk is identified.
H. Grinders should consider both the intended use of their product (hotel, restaurant, institution, or home setting) and the most vulnerable potential user; and should provide information and education aimed at minimizing the potential for foodborne illnesses at the level of the ultimate consumer. Moreover, such information and education can help assure consumers that

product found to have *E. coli* O157:H7 can be made safe by thorough cooking. Steps are available to ensure that product presumed or known to have *E. coli* O157:H7 is made safe as opposed to destroying it.

47.3.3 Suggested Procedures for the Guiding Principles

47.3.3.1 Receiving Meat (Guiding Principles A and B)

I. Develop purchase specifications to ensure receipt of safe and wholesome incoming raw materials. Purchase specifications should take the end use of the product into consideration. For example, purchase specifications for raw materials for processing products with potentially high-risk end use, such as raw patties, should include either:
   1. Microbial specifications, and testing by supplier or grinder, or
   2. Supplier operation under HACCP plans with CCP's that address pathogen intervention or antimicrobial programs, such as hot-water rinses, acid spray, steam pasteurization, or irradiation and any approved methodology.

II. Require suppliers to maintain records of farm source or slaughter plant to facilitate traceback.

III. Examine condition of transport for sanitation-related and other product-handling concerns, including:
   1. Sanitation of the carrier or truck.
   2. Presence of cracks, debris, foreign material, or off-odor.
   3. Condition of insulation and door seals.
   4. Temperature inside transport vehicles and of meat.
   5. Length of time of transport.

IV. Examine and record condition of raw material:
   1. Note and document species identity, origin, age, and temperature of both refrigerated and frozen materials, supply source, boning date/slaughter date.
   2. Conduct organoleptic examination (appearance, smell, any defects or abnormalities).
   3. Check integrity of immediate container, protective covering, or other packaging materials used.
   4. Document type of raw materials (e.g., trimmings, cheek meat, finely textured product, and product resulting from advanced meat recovery systems, and other comminuted products which have undergone additional handling and processing).
   5. Verify that all units are appropriately marked or coded for trace-back purposes.

V. Identify and separate incoming material according to the potential risk of the product's end use. Small-mass products such as raw beef patties are "higher risk" because they are thin, are cooked for a short time, are preferred not fully cooked by some consumers, and the internal temperature is not easily obtained. Large-mass products such as meat loaf or chili are "lower risk" because they are denser and are cooked evenly for a longer time. Ready-to-eat (RTE) products such as cooked beef patties are "lower risk" because these are subjected to a processing step lethal to pathogens.

VI. Affix grinding operation's code after acceptance of raw materials for tracking purposes.

47.3.3.2 Storage of Raw Material (Guiding Principle C)

I. Develop storage schedule for incoming materials to facilitate product identification and inventory control and to maintain package/pallet integrity. Raw materials can be rotated using a First-In-First-Out (FIFO) system or a plant specified product rotation/inventory control schedule.

II. Determine which units will be refrigerated or frozen, and for how long.

III. Record specific locations, times, dates, and temperature of storage.

IV. Provide ample spaces between boxes or pallets to allow for air circulation.
V. Monitor and record temperature of meat during storage.
VI. If material is to be thawed, monitor, and record time, and temperature of thawing.

47.3.3.3 Grinding Process Including Weighing, Coarse Grinding, Blending, Mixing, Final Grinding, and Forming (Guiding Principles C, D, E)

I. Observe and record preoperational and operational SSOP conformance, including those for equipment, floor, ceilings, walls, and employees. Adopt testing (e.g., microbial, ATP bioluminescence) to measure effectiveness of SSOP.
II. Develop a lotting or sublotting system for coding and tracking purposes. Assign lot numbers that will enable tracking lot to the raw material source up to the finished products. Lotting can be based on a full day's production or production from cleanup to cleanup. All lots produced between cleanups would be implicated in any public health-based action (e.g., recall) unless based on the specific circumstances, the problem can be restricted to a subset of the plant's production between cleanups.
III. Separate processing of meat into lower-risk and higher-risk categories. This is to prevent possible cross-contamination among products with different handling history. Separation can be by processing lines, lots, shifts, or production day. Large processing plants may be able to use different processing lines for different categories. In small plants, the categories can be separated by shifts or lots, in which case, processing of "lower-risk" raw materials should always precede "higher-risk" raw materials.
IV. Divert "higher-risk" meat to:
    1. RTE product such as fully cooked beef patties. RTE processing incorporates a kill-step such as heat processing, or cooking to eliminate pathogens including *E. coli* O157:H7.
    2. Large-mass products such as meat for meat loaf or chili. This diversion will help ensure adequate heat processing of the product before consumption, because these large-mass products are more fully and evenly cooked than thin meat patties.

    The regulation states:

    Diverting "higher-risk" meat to RTE or large-mass product processing is an in-house method to reduce the risk of foodborne illness because these products are in general, adequately cooked before consumption. In diversifying, grinders that have no facilities for processing RTE products need outlets for their "higher-risk" products. Grinders should obtain a list of federally and state-inspected establishments that can process ground beef RTE products.
V. Monitor and record temperature of the meat and the processing room during the whole operation.
VI. Develop a rework tracking system.
    1. Estimate the amount of meat for the production shift or day, so that the amount of carry-over or rework (excess raw materials at the end of the production period that are not in final product form) is minimal, or there is no rework at all.
    2. If rework is unavoidable, use only rework meat from the immediately preceding lot or shift, and not more than the last 24 h. Controls should be instituted to prevent this practice from incriminating a whole week's or month's production if a food safety hazard is identified.
    3. Include all rework with "higher-risk" meat and process at the last shift or the end of the production day; or divert to RTE product processing.
    4. Develop a recording system for rework that includes the time, quantity, area, and processing step it was collected from, the original lot or batch number/code, and the code of the lot or batch it was added to or included in.

I. Develop contingency plans or strategies to address unprocessed raw materials remaining due to line failure or not meeting specifications. Maintain adequate records of the origin, handling, and disposition of these raw materials.
II. Maintain a record of source, handling, and amount of outside trimmings added (i.e., trimmings not from the same batch or lot as the rest of the raw materials).
III. Monitor the time and temperature of finished products, for example, freezing of beef patties.
IV. Test for *E. coli* O157:H7 at any point during the grinding operation. To reduce the risk of a recall, hold the product until the test results confirm that none has been detected. FSIS Directive 10,101.1 on Microbiological Testing Program for *E. coli* O157:H7 in Raw Ground Beef sets out instructions for sampling ground beef that take into account industry action to reduce the presence of *E. coli* O157:H7. FSIS has instructed inspection program personnel that (unless otherwise directed) they are not to collect samples at an inspected establishment which makes only raw ground products that are tested daily for *E. coli* O157:H7, which are made from boneless beef lot which is certified to have tested negative for *E. coli* O157:H7, or that are made from carcasses which are subjected to a valid pathogen reduction intervention, the effectiveness of which is routinely verified and confirmed by testing, as long as no sample has tested positive for *E. coli* O157:H7, in the last 6 months.
V. Testing may help determine whether to divert to other products. Because of the low incidence of *E. coli* O157:H7 in meat, testing is no guarantee of the absence of the pathogen, and the amount and frequency of sampling from different volumes of meat being processed will vary. The point of sampling, whether raw material or the finished product, will also vary, depending on the condition of the raw material, whether there was previous testing, the system of controls in the plant, and the type of finished product.

47.3.3.4 Packaging, Cooling, and Storage (Guiding Principle C)

I. Monitor the temperature or frozen condition of the finished product during the packing operation.
II. Use only clean food grade immediate container liners.
III. Monitor finished product package integrity (seal, durability).
IV. Monitor and record the temperature of the refrigerator/freezer and the product during storage.
V. Include production code and sell-by-date on package label, in addition to the required handling statement and safe-handling instructions.
VI. Install a time–temperature indicator on the package to indicate adequate temperature of storage, distribution, and display (in grocery and other retail establishments).

47.3.3.5 Shipping, Handling, and Distribution (Guiding Principle F)

I. Develop and maintain an inventory control schedule for products in storage. The FIFO inventory control schedule could also be used at this point.
II. Transport coded products in clean, sanitized, temperature-controlled, and well-maintained carriers to distributors.
III. Maintain records of primary and secondary distributors.
IV. Develop recommendations for distributors concerning the safe handling, distribution, and coding of the finished products.
V. Maintain and record product temperature and package integrity during loading, unloading, and holding of finished products.
VI. Separate and divert to “higher-risk” any product that was returned after having left the plant and been out of the control of the plant.
VII. Develop an in-house recall plan to test the efficiency of the plant’s recording or coding system. Conducting product recovery drills (mock recalls) regularly can determine and assess the

ongoing effectiveness of the recall plan. A practical and effective recall plan that is understood by all employees should contain the following elements:

1. Step-by-step procedures to follow in the event of product recall.
2. List of people who will take part in any recall activities, including their assignments, business, and home phone numbers.
3. Measures to retrieve documentation identifying the product-coding system and product designation.
4. Measures to retrieve product distribution records.
5. Means of coordinating recall with regulatory authority or authorities.
6. Means of notifying distributors, wholesalers, retailers, and customers.
7. Measures for assuring the speedy return of recalled product.
8. Methods for disposition of recalled product.

The following information should be given to regulatory authorities: product name, product brand name, product codes, reason for the recall, areas of distribution, contact person within the company.

47.3.3.6 Recordkeeping System (Guiding Principle G)

I. Develop a recording or coding system so that each shipping container or a retail-ready package of ground beef has trace-back and trace-forward codes.
   1. A coding system could be as simple as indicating the shift, date, and production line. For example, a code of 1/020898/2 would mean produced on 'first shift of February 8, 1998, line.
   2. Corresponding records of all incoming products used on February 8, by shift and line, would enable full trace-back to sources. These codes should facilitate tracking or trace-back to the farm source, slaughter plant, and boning plant; a determination whether the meat was reconditioned, had intervention treatment, or had rework meat added to it; and a determination of the dates of slaughter and fabrication, lot number, storage, and transport records.

II. Encourage primary and secondary distributors to maintain a record of the companies to which they supply finished products. This will ensure effective trace-forward of all products, if the need arises.

Thorough recordkeeping, including tracing back and forward, will facilitate recall efforts. This will make possible rapid identification of sources of microbial contamination leading to containment of any product that could result in foodborne illness and public health implications. This will minimize the economic impact of recalls on affected plants, by narrowing down implicated products to a certain lot or production code.

47.3.3.7 Education (Guiding Principle H)

I. Grinding plants that have developed programs to educate their employees, distributors, food handlers, and consumers of the risks of foodborne illness associated with the production of ground beef products should continually monitor and update their programs. If needed, training in the language understood by food handlers and other employees should be provided.

II. Grinding plants without such programs should develop training and educational programs for employees, distributors, food handlers, and consumers on the microbiological risks that may be associated with ground beef products, and on measures to prevent foodborne illness. Training in the language understood by the people concerned should be provided, if necessary.

III. Safe handling instructions are currently required on the labels of not-ready-to-eat products when distributed to consumers, hotels, restaurants, or similar institutions. Grinding plants should include handling information on products distributed between establishments.

IV. Grinding plants should include cooking instructions that are targeted to the specific purchaser (e.g., product distributed to institutions with elderly, young, or immune-compromised populations should include more rigorous cooking and handling instructions than those provided for the general population).

V. Raw meat products with altered appearances due to additives or processing should be conspicuously identified as such, for example, "raw meat balls" or "raw-spiced beef."

48

Guidance on Meat Establishment Facilities and Equipment[*]

Y. H. Hui

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48.1 Establishment Facilities and Equipment

The information has been collated from public documents (legal, regulatory, and training) provided on the website of Food Safety Inspection Service (FSIS) of the United States Department of Agriculture (USDA) at http://www.fsis.usda.gov/. It is intended for use by meat and poultry establishments in considering decisions about design and construction of their facilities, as well as the selection of equipment to be used in their operations. The material is drawn principally from technical knowledge and experiences used by the FSIS in its regulatory role about the acceptability of facilities and equipment. An acceptable design is aimed at one major goal, that is, the production of a wholesome product for public consumption.

The technical considerations will be of special interest to establishments in the future.

48.2 Location

Selecting the location for your establishment is an important factor in providing a sanitary environment for producing meat and poultry products. When selecting a location, you need to consider the physical environment of the site, accessibility, separation of your premises from other businesses, common areas shared by you and other establishments, and whether or not you will conduct uninspected businesses such as retail stores or custom slaughter on or near your premises. You may wish to consider these guidelines when selecting a location for your establishment.

48.2.1 Site

The size of the site should allow for all buildings, parking lots, access roads, and future expansion. The site should be large enough to accommodate a potable water supply for your processing needs, and a

sewage system that can efficiently handle liquid waste and process water created by your establishment. In addition, potential building locations should be evaluated for sanitation hazards. In determining that possibility, consider the following guidelines:

1. To the extent possible, establishments should be located in areas free of industries that attract vermin such as sanitary landfills and junk yards.
2. To the extent possible, establishments should be located in areas free of odors and airborne particulate matter that may be produced by neighboring industries or other outside sources, such as oil refineries, trash dumps, chemical plants, sewage disposal plants, dyeworks, and paper pulpmills.
3. The prevailing winds are an important factor in site determination because substances emanating from more distant sources may be a problem if the winds carry them to the establishment site.

48.2.2 Separation of Official and Nonofficial Establishments

Sometimes an establishment is located next to or in the same building as other businesses that are not under FSIS inspection. In those circumstances you should take great care to keep product from becoming contaminated from the operation of the adjoining business.

48.3 Layout

One of the most important decisions you make in building or modifying an establishment is how you plan the layout of your building, including the placement of rooms and equipment, product flow, and people traffic patterns. Not only does a poorly designed establishment affect your productivity, but it may result in congested operations that can lead to unsanitary conditions. This section provides guidelines that you may wish to consider in planning any modifications to your existing establishment or in building a new one.

48.3.1 Flow of Operations

The direction in and means by which product moves or flows within a plant is an important but often neglected consideration that can have enormous influence on sanitation and the safety of finished products. From a product flow standpoint, all raw meat and poultry products ought to be considered as potentially microbiologically contaminated and handled accordingly. Products being processed should flow progressively from highest potential exposure to contamination to the least potential exposure to contamination, with intervening processes designed to remove or otherwise reduce the contaminants whenever possible. The flow of air and people should be just the opposite, moving from the cleanest areas progressively toward less clean areas.

When designing product flow, consider the following:

1. Moving products from raw to final cooked product areas to systematically reduce the risks of contamination along the way.
2. Locating trash dumpsters and receptacles so that they do not create a risk of product contamination.
3. Selecting rooms large enough to permit the installation of all necessary equipment with space for establishment operations and inspection.
4. Locating people passageways to provide maximum clearance to products, work areas, and production equipment.
5. Keeping truckways unobstructed.

48.3.2 People Traffic Flow

Inadequate control of the flow of people through product operational areas is one of the most serious risks for production contamination. People can act as carriers and bring from the outside contaminants such as dirt, debris, and vermin that are ideal vectors for microbiological growth and which can both directly and indirectly contaminate product. Ways in which you can reduce and control the flow of people include the following:

1. Establishment design should not require personnel not routinely assigned to specific work areas to be routed through those work areas. For example, personnel working in the live animal areas should not be required to travel through cooked product areas to use welfare rooms.
2. Welfare rooms, such as toilet rooms, dressing (locker) rooms, and cafeterias, should be designed to minimize the contamination because of the traffic patterns of the people.

48.3.3 Separation of Raw and Ready-to-Eat Product

Cross-contamination of ready-to-eat product by raw products may occur if the layout does not provide for separation of these products. To prevent cross-contamination in the preparation of products, the following are guidelines for you to consider:

1. Exposed cooked product areas should be physically separated from other areas of the establishment. Nonpedestrian passage openings may be present for the transfer of product or supplies.
2. A ventilation system should be used to direct air flow away from exposed cooked product areas.
3. Environmental control equipment such as fans and evaporator condensation pans should not be located above the product.
4. Welfare rooms, dry storage, maintenance, box/carton makeup, packaging, and palletizing areas should be separate, but adjacent to, the exposed cooked product rooms.
5. Cooked products should be covered in rigid containers to protect them from contamination while in storage.
6. Separate coolers and/or freezers should be available to use for exposed cooked products.
7. All cooking apparatuses for exposed products should have separate entry and exit portals.
8. No cooked product wash or reconditioning sinks should be used.

48.3.4 Perishable Product Rooms

Special care should be taken in perishable product rooms to inhibit growth of microorganisms in operations that could contaminate product. In addition, care should be taken to prevent contamination from other operations such as where raw ingredients are prepared. Nonmeat or nonpoultry ingredients should be prepared in a room or rooms separate from meat or poultry-processing rooms. For example, preparation of raw vegetables for use in products should be performed in a room separate from meat or poultry-processing rooms.

48.3.5 Edible and Inedible Products Rooms and Areas

Edible products can be easily contaminated by contact with inedible products, grease, or sewage from inedible product areas. In order to prevent this contamination from occurring, consider the following in the placement of these rooms:

1. The flow of inedible and condemned products should be designed so that it does not come into contact with edible products.
2. An inedible products department should be separate and distinct from the areas used for edible products. Inedible product rooms, grease interceptors, and sewage treatment equipment must be located away from edible product rooms.

3. Hooded, closed chutes that lead directly from the slaughter room to the inedible handling room are designed to prevent objectionable odors from inedible and condemned products from entering edible products rooms.
4. If rendering facilities are not available at the establishment, watertight storage facilities should be provided to hold these products before their removal to rendering plant. These storage facilities should be separate from edible products rooms, and constructed to prevent unsanitary conditions including attraction or harborage for vermin.
5. Areas for inedible trucks should be paved and enclosed for the ease of cleaning and to control odors and vermin.
6. Where necessary, the boiler room should be a separate room to prevent dirt and objectionable odors entering from it into rooms where meat products are processed or handled.

48.3.6 By-Products for Use in Animal, Pet, or Fish Food

Establishments that process by-products into animal, pet, or fish food should provide rooms for decharacterizing, chilling, packaging, or otherwise preparing the by-products. Consider the following guidelines when designing and constructing these rooms. By-products to be used as animal, pet, or fish food should be stored separately to prevent cross-contamination and commingling with edible products.

48.3.7 Coolers and Freezers

Coolers and freezers need to have enough space to refrigerate and store products. Products should be stored in a manner that will preclude conditions that may lead to contamination of products. The following guidelines will assist you in preventing conditions that could lead to contamination of your products:

1. Coolers and freezers, including doors, should be constructed of materials that can be readily and thoroughly cleaned, and durable, rigid, impervious to moisture, nontoxic, and noncorrosive. Freezer doors should be constructed and installed to prevent accumulation of frost.
2. Coolers and freezers should be equipped with floor racks, pallets, or other means to ensure protection of products from contamination from the floor.

48.3.8 Dry Storage

Packaging materials and ingredients should be stored to preclude conditions that may lead to contamination of products. The following are guidelines that may assist you in the planning of your dry storage area:

1. Dry storage materials should be stored in a room dedicated to dry storage only.
2. The dry storage area should be constructed so that racks can be spaced away from the walls and passageways maintained between rows. This facilitates cleaning of the area. In addition, the construction should allow for all meat or poultry ingredients and/or packaging materials to be stored in closed containers on racks or pallets.

48.3.9 Incubation Room for Canned Products

A room or incubator for incubating samples of fully processed canned meat or poultry must be provided in all establishments conducting regular canning operations. Consider the following guidelines when building this room:

1. An accurate time/temperature recorder must be provided. To prevent temperature variations, a means for air circulation should be provided.
2. Shelves should be provided to hold canned product. The shelves should be made of expanded metal or heavy gauge wire mesh and be removable for cleaning.

3. The floor in the room should be pitched to a floor drain equipped with a removable screw-plug.
4. The door of the room should be equipped for sealing by the inspector, if necessary.

48.3.10 Vehicular Areas Outside the Building

Special care should be given in the design of vehicular areas outside your building, not only to provide room for trucks and other vehicles to operate without damaging your building, but also to prevent unsanitary conditions that might contaminate product in your establishment. You should consider the following in designing your vehicular areas:

1. Areas outside the building where vehicles are loaded or unloaded should be paved with concrete or a similar hard surface. Hard-surface areas allow these areas to be kept clean and eliminate the potential for water puddles or dust.
2. Areas outside the building where vehicles are loaded or unloaded should be drained. Drainage from the loading docks should be confined to the immediate area of the dock.
3. The vehicular areas should be large enough to accommodate the turning radius of the largest trucks or shipping vehicles used by the establishment.
4. The vehicular areas adjacent to the establishment should have hose connections for cleaning.

48.4 Welfare Facilities for Establishment Employees

One source of potential contamination of products is cross-contamination from employee welfare facilities. In designing and locating employee facilities, great care should be given to preventing overcrowding and congestion and to providing enough handwash sinks and toilets for your employees. This section provides additional guidelines that you may wish to consider in making any modifications to or building any welfare facilities for your employees.

48.4.1 Dressing (Locker) Rooms

Dressing rooms must be provided for employees. In addition to privacy considerations, these dressing rooms should be located where they will not be a potential source of cross-contamination of products. Consider the following guidelines for these dressing rooms:

1. Dressing rooms should be separate from rooms or compartments where products are prepared, stored, or handled.
2. Dressing rooms should be separated from the toilet area.
3. Separate dressing rooms should be provided for each sex if both sexes are employed by the establishment.
4. Dressing rooms should have abundant, well-distributed light of good quality.
5. Separate dressing rooms for raw products and other product department employees will help in preventing cross-contamination of products.
6. Receptacles for soiled clothing should be provided adjacent to employees' dressing rooms.

48.4.2 Lockers

Lockers should be provided for employees clothing and personal items. To prevent insanitary conditions, consider the following guidelines when choosing the type of lockers and the arrangement and locations for them:

1. To prevent the potential for cross-contamination, the location of lockers should be separate from rooms or compartments where products are prepared, stored, or handled.

2. Lockers should be large enough to store a change of clothing and other personal items.
3. For the ease of cleaning, lockers should be constructed of materials that are rigid, durable, noncorrosive, easily cleaned and inspected, impervious to moisture, a light, solid color, with a smooth or easily cleaned texture, and have sloping tops.
4. Lockers should either be installed so that there is enough room under them that they can be easily cleaned and inspected, or they should be sealed to the floor.

48.4.3 Drinking Fountains

Sanitary drinking water fountains should be provided. Consider the following guidelines when installing drinking water fountains:

1. Drinking water fountains should be provided at convenient locations throughout the establishment to minimize the distance that employees need to travel to reach a fountain. This is especially important in preventing cross-contamination from employees working in raw or inedible areas and traveling to processing or ready-to-eat areas to use a fountain. Consider the following locations for placing drinking fountains:
   a. Welfare areas including cafeterias, dressing (locker) rooms, and toilet rooms
   b. Inspectors' offices
   c. Edible product areas including kill floor, deboning, and cut-up areas
   d. Inedible product areas
   e. Immediately outside freezers and coolers
   f. Storage areas
2. Drinking water fountains should be connected to the potable water supply and either directly connected to the underfloor drainage system or should discharge through an air gap to a hub drain.
3. Drinking water fountains should be other than hand operated, and if placed as part of handwash sink, should be located high enough to avoid splash from the sink.

48.4.4 Toilet Rooms

Toilet rooms can easily become a source of potential contamination of products. Care should be taken in the design of these rooms from their location in the establishment's layout to the number of toilets provided. Consider the following guidelines:

1. Toilet rooms need to be separated from the rooms and compartments in which products are prepared, stored, or handled.
2. Toilet rooms that open directly into rooms where meat products are exposed should have self-closing doors and should be ventilated to the outside of the building.
3. Toilet rooms should be arranged so that they are entered through an intervening dressing room or vestibule and not directly from a production or storage room.

48.4.5 Eating Rooms and Areas

To prevent employees from contaminating products or contaminating their food with microorganisms from the raw products or from their working environment, one method is to provide separate eating rooms or areas for employees.

48.4.6 Handwash Sinks

One of the most important steps you can take to prevent cross-contamination of products by your employees is to provide conveniently located handwash sinks. Handwash sinks are needed in toilet rooms,

dressing (locker) rooms, and production rooms. Consider the following guidelines when making decisions as to where you need a handwash sink:

1. Handwash sinks are needed near toilet rooms and dressing (locker) rooms. They should be other than hand operated. There should be hot and cold running water, soap, and towels. Single-use towels should be kept.
2. Handwash sinks in welfare rooms and areas should have a combination mixing faucet delivering both hot and cold water high enough above the rim of the bowl to enable the washing of arms as well as hands.

48.4.7 Ventilation

In designing your welfare rooms, such as toilet and dressing rooms, care should be taken to make sure that they are ventilated to prevent odors from entering production areas. Consider the following guidelines:

1. Welfare rooms that are not air-conditioned should be mechanically ventilated through an exhaust fan taking air to the outside. Airflow from welfare rooms should be released outside the establishment.
2. Toilet and dressing rooms that are located where no natural ventilation is available should be equipped with an exhaust fan (activated by a common switch with the lighting in the area) and a duct leading to the outside. Doors to dressing and toilet rooms ventilated in this manner should have a louvered section about 12 in. × 12 in. minimum in the lower panel to facilitate airflow.

48.4.8 Employees Working in Inedible Product Areas

Association of employees working in inedible product areas with other employees through common welfare rooms increases the risk of cross-contamination of products. To minimize this risk to products, consider the following. Separate welfare rooms for employees working in areas such as hide cellars, condemned or inedible product rooms, or live animal holding areas, from welfare rooms of other employees working with raw or heat processed, exposed, edible products.

48.5 Construction

A frequently overlooked area of construction design is the selection of appropriate construction materials for the establishment. This section provides guidelines for construction and the selection of construction materials that you may wish to consider when making modifications to your current establishment or building a new one.

48.5.1 Building Construction Materials for Rooms (Finished Surfaces)

Production and storage areas need to be constructed with materials that are readily and thoroughly cleaned. Products in production and storage areas are at risk of contamination from indirect contact with materials used for construction of the building. In order to be readily and thoroughly cleaned, building construction materials in production and storage areas must be:

1. Rigid and durable.
2. Nontoxic and noncorrosive.
3. Impervious to moisture.
4. A light, solid color such as white.
5. Smooth or textured with an easily cleaned, open pattern, for example, a pattern where the veins and depressed areas are continuous or have an outlet and are not enclosed.

In addition, consider the following guidelines for selecting construction materials:

1. In nonproduction and nonstorage areas, building construction materials should be easy to clean thoroughly.
2. Special consideration should be given before using wood as a construction material:
   a. Wood is absorbent and can absorb not only water, but also other substances including chemicals that create a risk of contamination of meat or poultry products.
   b. Wood is easily damaged and may create wood particles (splinters) that can be a source of contamination for meat products.
   c. If wood is used as a construction material in exposed product areas of the official establishment, it is recommended that the wood be milled smooth and completely sealed with a coating to prevent the wood from adulterating meat or poultry product. The coating should be able to be readily and thoroughly cleaned durable, rigid, impervious to moisture, nontoxic, and noncorrosive.
   d. The use of hot linseed oil to treat or coat wood in exposed product areas is not recommended because it promotes the growth of molds and fungi.

48.5.2 Floors

In addition to any obvious debris on a floor, products can become contaminated by the flooring or microorganisms living in debris in tiny crevices in the floor. In order to avoid these sources of contamination, consider the following guidelines when selecting and installing flooring in your establishment:

1. Floors in areas where products are handled or stored should be constructed of durable, easily cleanable materials, and be impervious to moisture. Commonly used materials are concrete, quarry tile, brick, and synthetic material.
2. Floors should be installed and maintained to reduce the likelihood of cracks, depressions, or other low areas that would accumulate moisture.
3. Floors where operations are conducted should have a slip-resistant surface. Good results are obtained by using brick or concrete floors with abrasive particles embedded in the surface. Concrete floors should have a rough finish.
4. Floors should be sloped to avoid puddles or depressions within the slope where water will stand.

48.5.3 Coving/Curbs

Coving is used at the wall-floor juncture, column (post)–floor juncture, and equipment support-floor juncture to provide a smooth transition for ease of cleaning and inspection. Consider the following guidelines when using coving or curbs:

1. Coving in production and storage areas should include the following criteria:
2. All seams should be tight-fitting and sealed to eliminate all cracks and crevices that may shelter insects, vermin, and microorganisms.
3. The coving should eliminate any sharp angles that allow the accumulation of materials.

Curbs should be provided to protect walls and wall finishes. Curbs should be high enough to protect the walls from pallets, trucks, or containers used in the establishment. Coving should be provided at the base of the curb.

48.5.4 Stairs

In selecting stairs, consider the following:

1. Stairs should have solid treads and closed risers and should have side curbs of similar material.

48.5.5 Catwalks and Access Platforms

When installing catwalks and access platforms, consider the following guidelines:

1. Catwalks and access platforms in edible product handling departments should be constructed of materials that meet the same guidelines as flooring.
2. Open grating should not be used for the flooring of catwalks and access platforms inside the establishment, particularly in production areas. Dirt and other debris from shoe soles can be scraped off by the grating and contaminate products, packaging material, and equipment.
3. Catwalks and access platforms should not be installed over production lines and processing equipment.

48.5.6 Interior Walls Including Posts and Partitions

To prevent products from becoming contaminated by contact with interior walls, care needs to be taken with regard to selection of materials for the finished surface of walls. Consider the following when selecting a finish:

1. Interior walls, in areas where products are stored or handled, should be finished with materials that will make them susceptible to being readily and thoroughly cleaned and impervious to moisture. Examples of such materials are glazed brick, glazed tile, smooth concrete, and fiberglass-reinforced plastic (FRP).
2. Walls should have a smooth texture, not one that is rough or uneven.
3. Fasteners for wall covering material should be solid, smooth headed, and not have recesses that allow the collection of foreign material.

48.5.7 Ceilings

Ceilings, in areas where the product is stored or handled, should be constructed to prevent the collection of dirt or dust that might sift through from the areas above or fall from overhead collecting surfaces onto equipment or exposed products. Therefore, it is recommended that ceilings and overhead structures be maintained free of sealing paint or plaster, dust, condensate, leaks, and other materials or defects. In addition, ceilings in areas where the product is stored or handled should be constructed and finished with materials that can be thoroughly cleaned and are moisture resistant. Examples of such materials are smooth concrete and FRP.

48.5.8 Windows and Skylights

Windows (and skylights) can be a potential source of contamination of products by dirt, water, debris, or broken glass. Consider the following when selecting and installing windows:

1. All outside windows, except for those in receiving and feed rooms, should have protection to exclude insects, birds, and other vermin.
2. Window ledges should be sloped about 45° to prevent the accumulation of dirt, water, or debris.
3. To avoid damage to window glass from the impact of hand trucks and similar equipment, the sills should be at least 3 ft above the floor.
4. Windows that are installed in walls in exposed product rooms should have panes of acrylic or polycarbonate plastic or other shatter-proof material.

48.5.9 Doorways and Doors (General)

Doors are not only barriers that allow the movement of products and people, but also present a barrier to contamination such as dirt, insects, and other vermin as well as the microbiological hazards that they

carry. The door type, construction material, and room in which the door is located are all important considerations when doors are installed in the establishment. Doors are important in maintaining sanitary conditions especially in production and storage areas. In production and storage, consider the following guidelines for doors:

The most effective doors have the following characteristics:

1. They are impervious to moisture.
2. They are tight fitting to minimize air exchange and to prevent the entry of insects and vermin into the establishments.
3. They are self-closing and used throughout the establishment, especially in areas where toilet rooms open directly into rooms where meat and poultry are exposed, to prevent contamination of products with odors and their associated contaminants.
4. They are high and wide enough to allow the movement of the exposed product through the doorways without it coming into contact with the door or jamb.
5. They are rigid and durable, and the junctions at jambs, walls, and floors are sealed to eliminate all cracks and crevices for debris, insects, and dirt to collect.
6. Doors that open directly to the outside of the building from production rooms should have an intervening closed space, such as a vestibule or enclosed lock, to prevent the direct access of contaminants and microbial organisms to areas inside the establishment.

48.5.10 Types of Doors

In selecting a type of door for your establishment, you need to consider the location of the door and whether or not product will be traveling through it. The following guidelines for different types of doors may be useful to you when selecting a door:

1. The horizontal double-swinging, impact door is a biparting, inflexible panel door with plastic windows (vision panels) that swings only in the horizontal plane. If you select this door, consider the following:
   a. This door may be useful in rooms with dimensions that would not permit the use of a roll-up, vertical sliding, or horizontal sliding door.
   b. Because this door must be manually opened, the door can be damaged creating sanitation and maintenance problems.
2. The horizontal sliding door (manual and automatic) is a single or biparting, inflexible door that moves only in the horizontal plane. If you select this door, consider the following:
   a. This door may be useful in rooms with dimensions that would not permit the use of a roll-up or vertical sliding door.
   b. The automatic opening option is recommended not only for sanitation reasons, but it also prevents damage.
3. The vertical sliding door (manual or automatic) is a single, inflexible panel door that moves only in the vertical plane. If you select this door, consider the following:
   a. This door may be useful in rooms with dimensions that would not permit the use of a roll-up or horizontal sliding door.
   b. The automatic opening option is recommended not only for sanitation reasons, but it also prevents damage.
4. The overhead garage-type door (manual or automatic) is a hinged, multipaneled door that moves from the vertical to the horizontal plane. If you select this door, consider the following:
   a. This door may be an excellent choice for sheds or buildings used to store equipment, such as a lawn mower, which is used for the outside maintenance of the establishment's property.

   b. It is recommended to avoid these types of doors in exposed product areas or areas subject to wet cleanup because these doors have spaces between the panels that allow the collection of product, such as meat and fat, as well as contaminants.
5. The roll-up door (manual or automatic) is a single flexible panel door that moves only in the vertical plane and when open, coils tightly onto a drum assembly. If you select this door, consider the following:
   a. This door can be an excellent alternative especially where space for opening a door is limited.
   b. Several additional features should be installed on this type of door to make it an effective barrier against contamination.
6. The air curtain or air door is a door that uses a layer of air generated by mechanical fans to separate two rooms or areas. If you select this door, consider the following:
   a. This door needs to be carefully selected, installed, and maintained to be effective.
   b. If an air imbalance (pressure imbalance) develops at the door opening, the separation effect may be diminished or eliminated. Air imbalance can occur from air flow changes from any other openings in the rooms especially other doors.
   c. The movement of the air can stir up contaminants, such as dirt and dust, if the area around the door is not kept clean.

48.6 Lighting, Ventilation, Refrigeration, and Equipment

Controlling the manufacturing environment is important in maintaining a sanitary environment in meat and poultry operations. This section provides guidelines concerning lighting, ventilation, refrigeration, and equipment for meat and poultry establishments that you should consider in building or modifying an establishment.

48.6.1 Lighting

Well-distributed, good-quality artificial lighting is needed at all places where natural light is unavailable or insufficient. Lighting is critical to maintaining a sanitary environment for slaughter and processing operations. Without adequate lighting, insanitary conditions are often difficult to see and correct. When selecting and installing lighting systems, consider the following requirements:

1. Light fixtures in rooms where exposed meat or poultry is handled should ensure maximum safety, to preclude contamination of products with broken glass and prevent the collection of dirt, product, and debris on lamp surfaces, including fixture surfaces not easily cleaned or inspected.
2. Lighting must be intense enough to allow both the establishment and inspection personnel to see insanitary conditions and product contamination. The intensity of lighting is measured in foot candles. Consult the USDA website (http://www.fsis.usda.gov/) for more details.

48.6.2 Ventilation

There should be enough ventilation for all areas of the establishment including workrooms, processing, packaging, and welfare rooms to ensure sanitary conditions. A good ventilation system is important to the production of wholesome meat and poultry products. Without controlling the quality of the air coming into the establishment, products may become contaminated with dust, insects, odors, or condensation. When designing your ventilation systems, you should consider the following guidelines:

1. The ventilation system should be designed so that turbulence is avoided. The longer the distance the air has to flow, the greater the resistance the air encounters not only from static air, but also from solid objects such as walls, equipment, people, and products.

2. The ventilation system should be designed with the size of the establishment in mind. The larger the facility, the greater the volume of air that must be moved.
3. The ventilation system should be designed to compensate for changes in outside temperature and humidity that cause condensation problems within the establishment.
4. Screens and filters should be used where needed to screen out dust, odors, and insects brought in from the outside to prevent product contamination.
5. Mechanical ventilation should be used to bring in fresh air to areas where natural ventilation is inadequate.
6. Ventilation should prevent vapor formation, such as steam or fog, which would affect sanitation or interfere with the inspector's ability to perform inspection.
7. When exhaust fans are installed, provision should be made to provide enough outside make up air to prevent air from being drawn into and through docks, coolers, and production areas to the area served by the exhaust fan.

48.6.3 Equipment (General Design and Construction)

Equipment materials should comply with 21 CFR, Parts 170–190 of the FDA regulations for direct food contact.

Equipment and utensils used for handling as preparing edible products or ingredients in any official establishment should be easily cleaned and not be a source of contamination. Consider the following guidelines when selecting equipment.

1. All direct product contact surfaces should be smooth; maintained free of pits, cracks, crevices and scale; corrosion and abrasion resistant; nonabsorbent; shatterproof; nontoxic; and not capable of migrating into food products.
2. Equipment should not be painted on areas in or above the direct product contact area.
3. Construction materials that are sources of contamination include cadmium, antimony, or lead as plating or the plated base material, lead exceeding 5% in an alloy and enamelware and porcelain used for handling and processing products.
4. Equipment should be designed and installed in such a way that foreign materials, such as lubricants, heat exchanger media, condensate, cleaning solutions, sanitizers and other nonfood materials, do not contaminate food products.
5. Equipment is self-draining or designed to be evacuated of water.
6. All product contact surfaces allow contact with cleaning solutions and rinse water.
7. Clean-in-place (CIP) systems should have sanitation procedures that are as complete and effective as those for cleaning and sanitizing disassembled equipment. To remove all organic and inorganic residues, CIP systems should meet the following criteria:
   a. Cleaning and sanitizing solutions and rinse water should contact all interior surfaces of the system.
   b. The system should be self-draining, with no low or sagging areas.
   c. The pipe interiors should be highly polished (120–180 grit) stainless steel for easy inspection.
   d. Easily removable elbows with quick-disconnect mechanisms should be installed at each change of direction. Elbows should be short enough to permit verification that the interior has been cleaned.

48.7 Water Supply

The water supply should be ample, clean, and potable with adequate pressure and facilities for its distribution in the establishment and its protection against contamination and pollution.

48.7.1 Potable Water

An adequate supply of fresh clean water is of primary importance in plant operations. The first requirement is that the water supply to the plant be potable or safe for human consumption or food processing. The plant water supply must meet the potability standards in the National Primary Drinking Water Regulations issued by the Environmental Protection Agency (EPA).

48.7.2 Backflow

Public health officials have long been concerned about cross-connections that may permit backflow in potable water supply distribution systems. Cross-connections may appear in many forms and in unsuspected places. Reversal of pressure and flow in the water system may be unpredictable. Plumbing cross-connections between a potable and nonpotable water supply may constitute a serious public health hazard. There are numerous cases where cross-connections have been responsible for the contamination of potable water and have resulted in the spread of disease. These concerns, as they relate to meat and poultry plants, deserve special attention. The problem is continual as potable water and piping systems are installed, repaired, replaced, or extended.

Two basic types of hazard may be created in piping systems: the solid pipe with valved connections and the submerged inlet. The solid pipe connection is often installed to supply an auxiliary piping system from the potable source. It is a direct connection of one pipe to another pipe or receptacle. Solid pipe connections may be made accidentally to waste disposal lines when it is incorrectly assumed that the flow will always be in one direction. An example would be connecting a line carrying used, nonpotable cooking water from a water jacket or condenser directly to a waste line without an air gap (see below). "Backflow" will occur with a submerged inlet if the pressure differential is reversed without an air gap. Submerged inlets are created when the outflow end of a potable water line is covered with water or other liquids. The other liquids may not be potable. Submerged inlets could be created by a hose lying in a pool or puddle of water on the floor.

Once a cross-connection exists, any situation that causes a pressure differential with the potable line having the lower pressure can result in contamination of the entire water distribution system and potable water supply. This is called backflow and can be produced under a variety of circumstances as illustrated below:

1. Backsiphonage is one form of backflow. It is caused by negative pressure in the delivery pipes of a potable water supply and results in fluid flow in the reverse direction. It may also be caused by atmospheric pressure exerted on a pollutant liquid source that forces the pollutant into a potable water supply system that is under vacuum. The action in this case is the common siphon phenomenon. The negative pressure differential that will begin the siphoning action is a potential occurrence in any supply line.
2. Differential pressure backflow refers to a reversed flow because of backpressure other than siphonic action. Any interconnected fluid systems in which the pressure in one exceeds the pressure of the other may cause flow from one to the other because of the differential. This type of backflow is of concern in buildings where two or more piping systems are maintained. The potable water supply is usually under pressure from the city water main. Occasionally, a booster pump is used. The auxiliary system often is pressurized by a centrifugal pump, although backpressure may be caused by gas or steam pressure from a boiler. A reversal in differential pressure may occur when pressure in the potable system drops below that in the system to which the potable water is connected. The best method of preventing this type of backflow is the complete separation of the two systems and/or an air gap. Other safety methods involve the installation of mechanical backflow prevention devices. All methods require regular scheduled inspection and maintenance to ensure ongoing effectiveness of installed devices.

Some areas that you should consider providing some form of protection from backflow and back siphonage include the following:

1. Water supply to pens for wash down or livestock watering.

2. Water supply to compressor cooling systems, cooling towers, and boiler rooms.
3. Water supply to cleanup systems, CIP systems, etc.
4. Water supply to hose connections.

Various mechanical ant-backflow devices are available to prevent backflow into a potable water supply system. Generally, the selection of the type and number of fail-safe devices should be based on the degree of hazard from contamination. Additional considerations include piping size, location, and the need to test periodically the backflow devices to ensure proper operation.

There are seven basic types of devices that can be used to correct cross-connections:

1. Air gap
2. Barometric loops
3. Vacuum breakers—both atmospheric and pressure type
4. Double-check valves with intermediate atmosphere vent
5. Double-check valve assemblies
6. Reduced pressure principal backflow preventers
7. Specific requirements concerning backflow can be found in local building and board of health codes.

48.8 General Plumbing Facilities

One of the most important factors to consider in the design and modification of establishments is the plumbing system. If the plumbing system is not properly installed, contamination of products can occur from flooding, back siphonage, stoppages, and cross-connections with the potable water system. This section provides guidelines concerning the plumbing facilities, in meat and poultry establishments. For additional information on the design and modification of plumbing facilities, refer to the National Plumbing Code.

48.8.1 Hose Connections and Hoses

There should be enough conveniently located hose connections with steam and water-mixing valves or hot-water connections provided throughout the establishment for cleaning purposes. Hose connections are important in promoting routine cleaning of the establishment. Consider the following guidelines when determining how many hose connections, location of hose connections, and storage of hoses:

1. The number of hose connections depends on the number of drains.
2. If a shut-off nozzle is provided on the hose after the hot- and cold-water-mixing valve, the vacuum breaker at the hose connection to the mixing valve will not work. Vacuum breakers should be installed on the hot- and cold-water supplies prior to the mixing valve to prevent such problems.
3. Hose connections should be provided with vacuum breakers to prevent back siphonage.

48.8.2 Establishment Drainage System

There need to be efficient drainage and plumbing systems for the prompt removal of liquid and suspended solid wastes from the processing environment. Consider the following guidelines when designing or modifying your drainage system:

1. All plumbing should be sized, installed, and maintained in accordance with applicable state and local plumbing codes, ordinances, and regulations.

2. Drainage lines should be located so that if leakage occurs, it will not affect product or equipment.

48.8.3 Floor Drains

All parts of floors where operations are conducted should be well drained. There are two basic types of drains: point drains and trench drains. Point drains, the most commonly used drain in most areas, are located in strategic points in the room with the floor sloped toward the drain. The waste water flows over the surface of the floor until it reaches and is carried away by the drain. Trench drains involve a trough or trench that collects the waste from a larger area and directs the flow to a drain opening. The flooring is sloped toward the trench.

In a typical plant, one 4-in. (10.16 cm) drainage inlet is provided for each 400 sq. ft (37.16 sq. m) of floor space. A slope of about one-quarter inch per foot (2.08 cm per meter) to drainage inlets is generally adequate to ensure proper flow with no puddling. In dry production areas, where only a limited amount of water is discharged on to the floor, an adequate slope may be about one-eighth inch per foot (1.04 cm per m). It is important that floors slope uniformly to drains with no low spots to collect liquid.

1. The location of floor drains depends on many factors such as the type of task conducted in the space, the geometric shape of the area drained, truck traffic patterns, and equipment locations.
2. There are special drainage considerations in areas where there is a high volume of water usage. The water in trench drains should flow in the opposite direction of the product flow, for example, from the poultry evisceration to the picking areas.
3. All parts of floors where wet operations or where floors are to be frequently hosed down should be pitched to floor or trench drains.
4. Floor drains should not be located under equipment because it makes them inaccessible cleaning.
5. Rooms without floor drains such as dry storage, large finished product coolers, and distribution warehouses may prefer to use mechanical cleaning machines instead of installing drains. Examples of such cleaning devices are floor scrubbers and dry/wet vacuum machines.

48.8.4 Trap Seals

Each floor drain should be equipped with a deep seal trap and vented properly to the outside. The purpose of such traps is to seal off the drainage system so that foul odors (sewer gases) cannot enter the plant. Effectiveness of the trap depends on enough water remaining to constitute a seal. As water flows through the trap and down the drainpipe, suction is created that will pull the water out of the trap and break the seal unless the suction is broken by venting the drainpipe on the effluent side of the trap to the outside air. The seal can also be broken by evaporation of trapped water. This is not a problem in frequently used drains, but does occur where drains are seldom used.

48.8.5 Drainage Lines

All drainage lines must comply with local code requirements. They should be installed and maintained to be leakproof. To prevent drainage lines from becoming entrances into the plant for pests, including rats and mice, all lines must be equipped with effective rodent screens. Secure drain covers, in addition to keeping out pests, also serve to prevent blockage of the traps and drainage lines with product scraps or other material too large to flow freely.

48.8.6 Cleanouts

Cleanouts should be installed in the drainage system to prevent sewer blockages. Consider the following guidelines when installing cleanouts:

1. Cleanouts should be located so that they are readily accessible, and can be used without constituting a threat of contamination to edible products.
2. To help avoid water puddling, cleanouts should be located on the "high lines" of floor slopes and away from traffic patterns.

48.9 Establishment Sewage Treatment

The design and construction of sewage treatment facilities must comply with local code requirements. An improperly designed sewage system can contaminate the ground and water supply. This section provides guidelines concerning sewage treatment at meat and poultry establishments that you may wish to consider in the installation of a sewage treatment facility.

48.9.1 Establishment Sewage Facilities

Sewage, one the most dangerous sources of human pathogens, should never be allowed to come into contact with products, equipment, utensils, or any food contact surfaces. When installing an establishment sewage treatment facility, consider the following guidelines:

1. The system should be large enough to handle the amount of sewage that the establishment produces and accommodate future increases.
2. If a private septic tank, pretreatment, or treatment system is used, it should be designed and operated to prevent contamination of products.
3. The sewage facility should be located away from product operations and ingredient and packaging storage areas.
4. An area for cleaning solid waste containers with hot water, drains, and curbing should be located near any solid waste disposal facility.

48.9.2 Grease Catch Basins or Interceptors

Grease catch basins can be a source of contamination of products if not properly designed and located. Consider the following guidelines when constructing a grease catch basin:

Catch basins or interceptors for recovering grease should not be located in or near edible product departments or areas where edible products are shipped or received.

When a catch basin is located inside an establishment, it should be sealed with a gastite cover and located in a ventilated room.

Grease catch basins should be constructed so that they can be completely emptied of their contents for cleaning.

The area surrounding an outside catch basin should be paved with impervious material, such as concrete, and drained.

48.10 Meat Slaughter Establishments: General Facilities Guidelines

Although the flesh of healthy livestock is practically sterile, when the animal is killed many factors can contribute to contamination of the carcass including improperly designed and constructed slaughter

facilities. This section provides guidelines for meat slaughter facilities to consider in building or modifying slaughter facilities. As different species of livestock need different slaughter facilities, this section is organized in the following way:

The following guidelines apply to all establishments that slaughter cattle, calves, sheep, goats, hogs, and equines. If you are building or modifying an establishment that slaughters these species, consider these facilities guidelines to prevent contamination of carcasses during slaughter operations. Additional guides are available for the establishing slaughtering a specific group (cattle, calves, etc.) at the website http://www.fsis.usda.gov/.

48.10.1 Livestock Pens

In addition to preventing contamination of the slaughter department and minimizing contaminates on the hides of the animals, proper design and construction of livestock pens prevent injury to the animals. Consider the following facilities guidelines when designing and constructing livestock pens:

1. Livestock pens should be located outside the slaughter department to prevent contamination of products from dust, odors, and other contaminates. If possible, the livestock pens should be separated from the department by fullheight partitions of impervious material.
2. Livestock pens, driveways, and ramps should be free from sharp or protruding objects which could cause injury or pain to the animals.
3. Floors of the pens, ramps, unloading chutes, and runways should be constructed to provide good footing for livestock. Waffled floor surfaces and cleated ramps are effective construction designs.
4. Floors of the pens, ramps, unloading chutes, and runways should be sloped for drainage and cleaning.
5. Pen enclosures (except gateways) should be high and sturdy enough to prevent livestock from escaping.
6. Gates, fences, and chutes should have smooth surfaces that are easily cleaned.
7. Man gates or, if the walls are concrete, toe holds formed in the walls should be present to allow people to escape from pen enclosures in an emergency.
8. To help prevent livestock from slipping and falling on floors covered with excess water, thereby further contaminating their hides, water troughs should be provided with overflows located above or adjacent to pen floor drains.
9. Hose connections should be provided for cleanups.
10. Covered pens should be provided to protect crippled or downer animals from adverse climatic conditions. If held overnight, the pens should be large enough to allow the animals to lie down and have facilities for feed and water. Pens and driveways should be arranged so that sharp corners and direction reversals of driven animals are minimized.
11. A “U.S. suspect” or “U.S. condemned” pen should be available at all times and designed to allow for complete separation, including the drainage system, from other livestock.

48.10.2 Antemortem Inspection Areas

Antemortem inspection areas should be designed and constructed to facilitate inspection and to prevent animals from being injured. Consider the following guidelines in designing and constructing these areas:

1. To avoid delays in slaughter operations, pens for antemortem inspection should have the capacity for holding the maximum number of animals of the various species that will be slaughtered in a single day.

2. To facilitate the antemortem inspection of animals, a separate suspect pen with a squeeze chute should be provided, where the temperature of the animals may be taken.
3. At least 50% of the livestock pen, including the area where the suspect pen and squeeze chute are located, should be under a weather-tight roof to provide an area for proper antemortem inspection in inclement weather.
4. Special consideration should be given to designing antemortem inspection facilities to allow for humane transporting of crippled or downer animals into the slaughtering department. As crippled and downer animals have difficulty moving, special doorways and hoists to transport them to the stunning area should be provided.

48.10.3 Slaughter Area

The slaughter area is one of the most difficult areas to keep sanitary because of the nature of slaughter operations. Consider the following guidelines in designing and constructing slaughter areas to minimize the contamination of carcasses:

1. The slaughter area should be separated from the outside by a fullheight partition or wall made of impervious material.
2. Any doors to the outside of the slaughter area should be self-closing to minimize the risk of contamination, including contamination by vermin.
3. Slaughter areas should have floor space arranged to facilitate the sanitary conduct of operations and efficient inspection. For example, to prevent contamination of carcasses, truckways through which products are conveyed from the slaughter area to rooms such as the offal cooler should be located so that the material is not trucked beneath rails from which dressed carcasses and products are suspended. For the same reason, personnel traffic should not move through lines of carcasses.

48.10.4 Stunning Areas Including Chutes and Alleys

Stunning areas, chutes, and alleys should be designed to prevent congestion, injury to animals, and minimize the contamination of hides that can lead to contamination of the carcasses. Consider the following guidelines when designing these facilities:

1. All pathways, chutes, and alleys leading to stunning areas, and the stunning areas, should be large enough for the species being slaughtered.
2. All pathways, chutes, and alleys leading to stunning areas, and the stunning areas, should be free from pain-producing restraining devices, sharp projections such as loose boards, exposed bolt ends, splintered or broken planking, protruding metal, and exposed wheels or gears.
3. All pathways, chutes, and alleys leading to stunning areas, and the stunning areas, should be free of unnecessary holes and openings where the animals' feet or legs may be injured.
4. Overhead gates should be covered at the bottom edge to prevent, injury to the animals.
5. Flooring should be constructed of roughened or cleated cement to reduce the falls.
6. Stunning areas should be provided for confining animals for stunning before bleeding.
7. If ritualistic slaughter operations are conducted in the stunning area, shackles to confine the animals also should be provided.
8. When captive bolt stunners are used, the stunning areas should be designed and constructed to limit the free movements of animals so that the operator can locate the stunning blow with a high degree of accuracy.

9. When electrical stunning is used, the stunning area should be constructed so that any power activated gates will not cause injury to the animals.

48.10.5 Rail Arrangement and Truckways

To prevent contamination of carcasses, rails should be arranged to provide enough room for carcasses to move without touching equipment, walls, columns, other fixed parts of the building, and other carcasses. Consider the following guidelines when arranging rails in your establishment:

1. Consideration should be given to the type of rail and the rail speed when determining how rails are to be arranged.
2. Trim rails should be arranged so that carcasses pass the final carcass inspection position after the final trim.
3. To prevent the carcass from becoming contaminated by debris on the floor and from splashes during cleanups, the cooler rails should provide for clearance from the lowest part of the carcass to the highest point of the floor.
4. A room or area for washing gambrels, hooks, and trolleys should be provided. The room or area should have an exhaust fan in an outside wall to dispense steam.

48.10.6 Viscera Separation and Edible By-Products' Refrigeration

As edible organs and parts (offal) are handled at temperatures conducive to bacterial growth, care must be taken in providing facilities for separation of viscera and for refrigeration of edible by-products to prevent them from becoming contaminated. Consider the following guidelines for holding edible by-products:

1. Facilities, such as viscera trucks or pans, should be provided for separating and handling viscera of the various species of animals to prevent commingling.
2. To prevent cross-contamination, a separate cooler or a separately drained part of a carcass cooler should be provided for holding edible organs and parts (offal) under refrigeration.
3. To convey the edible by-products to a cooler, a truck with removable metal drip pans should be provided.
4. To prevent cross-contamination, establishment and inspection personnel from the slaughter department should be able to access the edible by-product cooler without passing through a line of carcasses or through a congested carcass cooler.

48.10.7 Carcass Washing

Special facilities for washing inspected carcasses are needed to remove bone dust and other accidental contamination from the carcass. Consider the following guidelines when designing and constructing this area:

1. A separately drained area or an area that is sloped to a floor drain should be provided where inspected carcasses are washed.
2. If the carcasses are washed manually by establishment personnel, a platform should be provided to allow establishment personnel to be able to reach all parts of the carcass.

48.10.8 Retain Room/Compartment

1. A retain room, cage, compartment, or receptacle may be required by inspection. Depending on the needs of inspection, consider the following guidelines for designing and constructing this room:

2. The retain room or compartment must be equipped for locking or sealing.
3. The room or compartment needs to be marked conspicuously "U.S. Retained."
4. If the retain compartment is located in the cooler, the compartment should be separated from the remainder of the cooler to prevent cross-contamination of inspected and passed carcasses. The separation can be accomplished by creating a compartment constructed of partitions of corrosion-resistant wire screen or flat-expanded metal.

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*Sanitation of Food-Processing Equipment**

Y. H. Hui

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49.1 General Considerations

The information in this chapter has been collated from multiple public documents (legal, regulatory, and training) from the following sources:

1. Food and Drug Administration (FDA) documents: Code of Federal Regulations, current good manufacturing practices (CGMPs), the Food Code, hazard analysis, and critical control point (HACCP) programs.
2. U.S. Department of Agriculture (USDA) documents: Code of Federal Regulations, HACCP programs, inspection manuals, directives, etc.

The information is easily accessed via the respective websites of the agencies.

Food-processing equipment used in all food plants (meat and nonmeat) is "predetermined" under usual circumstances:

1. Mandatory specifications. Most of those used in processing low-acid (acidified, etc.) foods in hermetically sealed containers (thermal processing) and meat and poultry products are directly regulated by regulations promulgated by the FDA and USDA in relation to safety and sanitation.
2. New establishments. Newly constructed establishments should take into consideration all aspects of good manufacturing practices (GMPs) before their construction or equipment purchases.

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3. Most specialty equipment (for bakery, dairy, pasta, oil, etc.) is under voluntary/mandatory requirements established and distributed by trade associations such as the American Institute of Baking, Dairy and Food Industries Supply Association.
4. With built-in designs to comply with FDA/USDA GMPs, this equipment saves food processors time and money in looking for the "right" equipment.
5. Custom-made equipment. Plant personnel may build their own equipment or have an outside contractor fabricate equipment for them. Even though it is custom made and not intended for resale, such equipment should be built to comply with GMPs. The same standards are applicable to custom-made equipment as are applicable to commercially available equipment.
6. Many food-processing plants require some equipment that is custom made for particular operational requirements. It may not be possible to comply with certain GMPs in the same way as other conventional equipment. If so, it is always advisable to inform the appropriate state and federal regulators of the circumstances.
7. All other food-processing equipment that is commercially available.

The bottom line is that all food-processing equipment, no matter how they are predetermined, must comply with FDA. USDA, and state GMP guidelines.

Some equipment manufacturers or brokers are sometimes not interested in complying with FDA/USDA and GMP. In such events, equipment is considered the same as custom made, and food establishment operators should be aware of this responsibility before they purchase any equipment.

All food processors using a variety of equipment, especially custom-made equipment, should focus on correcting problems during the initial development of equipment instead of resolving problems which may result when improperly designed or constructed equipment is put into widespread use. This preventive anode of action benefits equipment manufacturers, food processors, state and federal regulators, and American consumers.

49.2 Materials for Construction and Repair

Materials that are used in the construction of utensils and food-contact surfaces of equipment may not allow the migration of deleterious substances or impart colors, odors, or tastes to food, and under normal use conditions these should be

1. Safe
2. Durable, corrosion resistant, and nonabsorbent
3. Sufficient in weight and thickness to withstand repeated warewashing finished to have a smooth, easily cleanable surface
4. Resistant to pitting, chipping, crazing, scratching, scoring, distortion, and decomposition

Multiuse equipment is subject to deterioration because of its nature, that is, intended use over an extended period of time. Certain materials allow harmful chemicals to be transferred to the food being prepared, which could lead to foodborne illness. In addition, sonic materials can affect the taste of the food being prepared. Surfaces that are unable to be routinely cleaned and sanitized because the materials used could harbor foodbome pathogens. Deterioration of the surfaces of equipment, such as pitting, may inhibit adequate cleaning of the surfaces of equipment, so that food prepared on or in the equipment becomes contaminated. Inability to effectively wash, rinse, and sanitize the surfaces of food equipment may cad to the buildup of pathogenic organisms transmissible through food. Studies regarding the rigor required to remove biofilms from smooth surfaces highlight the need for materials of optimal quality in multiuse equipment.

It must be emphasized that each food-processing operation is unique, which applies to the equipment used. The acceptability and nonacceptability of any food-processing equipment or its component materials occasionally depends on the operation itself. This must be taken into consideration when evaluating an item of equipment in relation to the GMP.

Equipment should be constructed of materials that will not deteriorate from normal use under the anticipated environment. For example, equipment must be constructed of materials that will withstand one category of environment, for example, generally humid-operating environment and high pressure, hot water cleaning with strong chemical cleaning agents. Of course, there are other categories of food-processing environments. In addition, all equipment surfaces should be smooth, corrosion and abrasion resistant, shatterproof. nontoxic. nonabsorbent, and not capable of migrating into food product (staining).

Surface coatings and platings may be used if the base material is nontoxic and rendered noncorrosive and the plating material is USDA/FDA acceptable. Chrome, nickel, tin, and zinc (galvanization) platings will generally be acceptable for most appropriate applications. Clearance of other plating materials and processes can be obtained by receiving a favorable opinion (or the intended use from the FDA, Office of Premarket Approval. Surface coatings, and platings must remain intact. If a surface coating or plating begins to peel or crack, the FDA/USDA inspection will request correction from the management and may even disallow the use of the equipment.

Hardwod maybe used for dry curing, In addition, solid (unlaminated) pieces of hardwood are acceptable as removable cutting boards, provided the wood is maintained in a smooth, sound condition and is free from cracks.

Nonfood-contact surfaces of equipment that are exposed to splash, spillage, or other food soiling or that require frequent cleaning should be constructed of a corrosion-resistant, nonabsorbent, and smooth material to facilitate cleaning. Equipment that is easily cleaned minimizes the presence of pathogenic organisms, moisture, and debris and deters the attraction of rodents and insects.

49.3 Design and Construction

Equipment and utensils should be designed and constructed to be durable and to retain their characteristic qualities under normal use conditions.

Equipment should be designed so that all product contact surfaces can be readily and thoroughly cleaned with high-temperature/high-pressure water and caustic soap solution. Components such as electric motors, electric components, etc., which cannot be cleaned in this manner should be completely enclosed and sealed.

Equipment and utensils must be designed and constructed to be durable and capable of retaining their original characteristics so that such items can continue to fulfill their intended purpose for the duration of their life expectancy and to maintain their easy cleanability. If they cannot maintain their original characteristics, they may become difficult to clean, allowing for the harborage of pathogenic microorganisms, insects, and rodents. Equipment and utensils must be designed and constructed so that pans do not break and end up in food as foreign objects or present injury hazards to consumers. A common example of presenting an injury hazard is the tendency for tines of poorly designed single service forks to break during use.

Food temperature-measuring devices may not have sensors or stems constructed of glass, except that thermometers with glass sensors or stems that are encased in a shatterproof coating such as candy thermometers may be used.

Food temperature-measuring devices that have glass sensors or stems present a likelihood that glass will end up in food as a foreign object and create an injury hazard to the consumer. In addition, the contents of the temperature measuring device, for example, mercury, may contaminate food or utensils.

49.4 Cleanability

Multiuse food-contact surfaces should be

1. Smooth.
2. Free or breaks, open scams, rocks, chips, pits, and similar imperfections. Free of sharp internal angles, corners, and crevices. Finished to have smooth welds and joints.

3. Accessible for cleaning and inspection by one of the following methods, without being disassembled.
4. By disassembling without the use of tools.
5. By easy disassembling with the use of only simple tools such as mallets, screwdriver, or wrenches that are kept near the equipment and are accessible for use.

The purpose of the requirements for multiuse food-contact surfaces is to assure that such surfaces are capable of being easily cleaned and accessible for cleaning. Food-contact surfaces that do not meet these requirements provide a potential harbor for foodborne pathogenic organisms. Surfaces that have imperfections such as cracks, chips, or pits allow microorganisms to attach and form biofilms. Once established, these biofilms can release pathogens to food. Biofilms are highly resistant to cleaning and sanitizing efforts. The requirement for easy disassembly recognizes the reluctance of food employees to disassemble and clean equipment if the task is difficult or requires the use of special, complicated tools.

A clean-in-place (CIP) system is defined as follows:

1. CIP means cleaned-in-place by the circulation or flowing by mechanical means through a piping system of a detergent solution, water rinse, and sanitizing solution onto or over equipment surfaces that require cleaning, such as the method used, in part, to clean and sanitize a frozen dessert machine.
2. CIP does not include the cleaning of equipment such as band saws, slices, or mixers that are subjected to in-place manual cleaning without the use of a CIP system.

Sanitation procedures for CIP systems must be as effective as those for cleaning and sanitizing disassembled equipment. Only equipment that meets the following criteria may be cleaned-in-place. Any equipment or portions of equipment not meeting these requirements should be disassembled for daily cleaning and inspection.

1. CIP equipment should meet the characteristics as specified.
2. Cleaning solutions, sanitizing solutions, and rinse water should circulate throughout a fixed system and contact all interior surfaces of the system.
3. All internal surfaces should be either designed for self-draining (of cleaning and sanitizing solutions) or physically disassembled for draining after rinsing. CIP equipment that is not designed to be disassembled for cleaning should be designed with inspection access points to assure that all interior food-contact surfaces throughout the fixed system are being effectively cleaned.
4. Pipe interiors should be highly polished (120–180 grit abrasive) stainless steel or some other acceptable, smooth-surfaced material which is easy to inspect.
5. Easily removable elbows with quick-disconnect mechanisms should be located at each change of direction.
6. All sections of the system should be capable of being completely disassembled for periodic inspection of all internal surfaces.
7. All sections should be available for inspection without posing any safety hazard to the inspector.

Certain types of equipment are designed to be cleaned-in-place where it is difficult or impractical to disassemble the equipment for cleaning. Because of the closed nature of the system, CIP cleaning must be monitored via access points to assure that cleaning has been effective throughout the system.

The CIP design must assure that all food-contact surfaces of the equipment are contacted by the circulating cleaning and sanitizing solutions. Dead spots in the system, that is, areas that are not contacted by the cleaning and sanitizing solutions, could result in the buildup of food debris and growth of pathogenic microorganisms. There is equal concern that cleaning and sanitizing solutions might be retained

in the system, which may result in the inadvertent adulteration of food. Therefore, the CIP system must be self-draining.

The cleanability of the following equipment and surfaces require special attention to comply with GMP.

1. V-type threads
2. Hot oil-filtering equipment
3. Can openers
4. Nonfood-contact surfaces
5. Kick plates

At least two types of temperature-measuring devices are used in food processing: (a) for food, and (b) for ambience. USDA, FDA, and state regulations must be consulted to make sure that the devices are acceptable for accuracy and sanitation.

Equipment offering special functions requires special attention to sanitation:

1. Exhaust ventilation hood systems in food preparation and warewashing areas including components such as hoods, fans, guards, and ducting should be designed to prevent grease or condensation from draining or dripping onto food, equipment, utensils, linens, and single-service and single-use articles. The dripping of grease or condensation onto food constitutes adulteration and may involve contamination of the food with pathogenic organisms. Equipment, utensils, linens, and single-service and single-use articles that are subjected to such drippage are no longer clean,
2. Equipment openings and covers must be designed to protect stored or prepared food from contaminants and foreign matter that may fall into the food. The requirement for an opening to be flanged upward and for the cover to overlap the opening and be sloped to drain prevents contaminants, especially liquids, from entering the food-contact area.
3. Some equipment may have parts that extend into the food-contact areas. If these parts are not provided with a watertight joint at the point of entry into the food-contact area, liquids may contaminate the food by adhering to shafts or other pans and running or dripping into the food. An apron on pats extending into the food-contact area is an acceptable alternative to the watertight seal. If the apron is not properly designed and installed, condensation, drips, and dust may gain access to the food.
4. Equipment containing bearings and gears that require lubricants should be designed and constructed so that the lubricant cannot leak, drip, or be forced into food or onto food-contact surfaces. It is not unusual for food equipment to contain bearings and gears. Lubricants necessary for the operation of these types of equipment could contaminate food or food-contact surfaces if the equipment is not properly designed and constructed.
5. If a condenser unit is an integral competent of equipment, the condenser unit should be separated from the food and food storage space by a dust-proof barrier. A dust-proof barrier between a condenser and food storage areas of equipment protects food and food-contact areas from contamination by dust that is accumulated and blown about as a result of the condensess operation.
6. The proper use and location of temperature-measuring devices are extremely important. Otherwise, the food being refrigerated or frozen may allow the growth of harmful microorganisms. The placement of the temperature-measuring device is important. For example, if the device is placed in the coldest location in the storage unit, it may not be representative of the temperature of the unit. Food could be stored in areas of the unit that exceed requirements. Therefore, the temperature-measuring device must be placed in a location that is representative of the actual storage temperature of the unit to assure that all potentially hazardous foods are stored at least at the minimum temperature required for the specified food. The importance of maintaining potentially hazardous foods at the specified temperatures requires that

temperature-measuring devices be easily readable. The inability to accurately read a thermometer could result in food being held at unsafe temperatures.

7. Equipment such as dollies, pallets, racks, and skids used to store and transport large quantities of packaged foods received from a supplier in a cased or overwrapped lot should be designed to be moved by hand or by conveniently available equipment such as hand trucks.

49.5 Water Usage

Water-wasting equipment should be installed so that waste water is delivered into the drainage system through an interrupted connection without flowing over the floor or is discharged into a properly drained curbed area. Waste water from cooking tanks, soaking tanks, chilling tanks, and other large vessels may be discharged for short distances across the floor to a drain after operations have ceased and all products have been removed from the area,

An air gap should be provided between the highest possible level of liquids in equipment and a directly connected water supply line(s). The air gap must be at least twice the diameter of the supply side orifice. If submerged lines are unavoidable due to design considerations, then the equipment must include a functional vacuum breaker which will, without fail, break the connection in the event of water pressure loss.

Equipment that recirculates water as part of its intended function should be equipped with sanitary-recirculating components if the water directly or indirectly contacts the food product or the product-contact surfaces. For example, recirculating pumps should be accepted for direct product contact and piping must be easily demountable with quick disconnect mechanisms at each change of direction.

In addition, establishment operators using equipment or systems that reuse water may be required to have written approval of a water reuse procedure. However, the requirement is mandatory for meat and poultry processors by the USDA. Although the FDA does not require a written approval at this stage, its GMP regulations make it clear that there must be built-in safeguards in the reuse of water in a food plant.

49.6 Specialized Equipment

Piping systems used to convey edible products (including pickle solutions) should be readily disassembled for cleaning and inspection. Pumps, valves, and other such components should comply with the sanitary requirements of GMPs promulgated by USDA/FDA. Piping systems must be designed so that product flow will be smooth and continuous, that is, no traps or dead ends.

Clear demountable rigid plastic piping may be used for a two-way flow, provided it is chemically and functionally acceptable. Opaque plastic piping may be used for one-way purposes only.

The cited requirements apply to systems for conveying raw fat and to recirculate cooking and frying oils. Black iron pipes with threaded or welded joints are acceptable for conveying completely finished, rendered fats. Continuous rendering is not considered complete until after the final centrifuge.

Pipeline-conveying systems for aseptic processing and packaging should comply with the requirements promulgated by the FDA and USDA in the U.S. Code of Federal Regulations.

The extensive exposure of some products to metal equipment such as grinders, choppers, mixers, shovels, etc., causes the possibility of metal contamination. Magnetic traps have been found effective in removing iron particles from chopped or semiliquid products. However, these magnetic traps are not useful for removing nonmagnetic metals such as stainless steel or aluminum. Therefore, the use of electronic metal detectors is highly recommended for sausage emulsions, can filling lines (especially baby foods), etc. Metal detectors are usually installed, so an alarm (either a bell or light or both) is activated when a metal fragment is in the detection zone. The production line should stop automatically when the detector is activated. Alternatively, some systems are arranged so that the portion of the product containing the metal contaminants is automatically removed from the production line.

The FDA and USDA currently regulate the use of metal detectors for certain food-processing operations but not all. The agencies do encourage food plant operators to voluntarily use metal detectors whenever possible. The agencies review and evaluate metal detectors using the same sanitary standards applied to other types of equipment.

Conveyor belts used in direct contact with food products must be moisture resistant and nonabsorbent. Conveyor belts should have the edges scaled with the same material as is used for the foal-contact surface. In addition, belting material must be chemically acceptable and approved by the FDA/USDA. Conveyors with trough-like sides and bed should have a quick belt tension release device to allow cleaning under the belt.

Equipment used for cleaning jars or cans should have safety devices to indicate malfunction of either jet or vacuum elements. If necessary, vents to the outside should be provided to control exhaust currents and to prevent dust and/or paper particles from teeing blown hack into cleaned containers.

Hoses used for product contact should comply with recommendations of trade associations or be accepted by both the FDA/USDA. The hose material must be installed in a manner that allows for inspection of the interior surface. Sanitary connectors can be installed at appropriate intervals to allow breakdown for visual inspection or use of inspection devices such as horoscopes.

Hoses without sanitary connectors are acceptable for steam and water lines where breakdown for cleaning and inspection is not necessary. However, hoses used for recircuting water into and out of product contact areas must satisfy the requirement for product-contact hoses.

Pickle lines should be either stainless steel or some other USDA acceptable material. If recirculated, pickle brine should be filtered and recirculated through a system that can be disassembled to the extent necessary for thorough cleaning and inspection.

Smokehouses and ovens must be designed for easy cleaning and inspection of all inner and outer surfaces. Ducts should be designed to be easily disassembled to the extent necessary for thorough cleaning and inspection. Spray heads for dispensing liquid smoke must be mounted below the level of the rails and trolleys. If liquid smoke is to be recirculated, the pump and pipelines must be of sanitary-type construction. Liquid smoke cannot be recirculated if the product is on rack trucks.

Screens and straining devices should be readily removable for cleaning and inspection and should be designed to prevent incorrect installation. Permanent screens should be constructed of noncorrosive metals. Synthetic filter materials should have clearance from trade associations. The same applies to filters intended for direct product contact. Filter paper should be single service. Filter cloths should be washable. Asbestos is not acceptable for use as filtering material or for any other purpose.

Vent stacks from covered cooking vats or hoods over cook tanks and CO_2 equipment should be arranged or constructed so as to prevent drainage of condensate back into the product zone.

Heat exchangers may be used to heat or cool a product. Head exchangers may also be used to heat or cool gases or liquids that directly contact the product. However, extreme caution should be exercised to prevent contamination. Inspectors and plant personnel should be alert to the following conditions and requirements:

1. Only heat exchangers media authorized by trade associations, FDA, USDA, and other standardization bodies in the United States can be used for applications involving food products. Common materials such as brine or ammonia need not be submitted for review. Under no circumstances can toxic materials be used.
2. Heat exchangers should be routinely pressure tested to ensure that pinholes, hairline cracks, loose fittings, or other similar defects are not present. Presence of off-color, off-odor, and/or off-flavor may indicate leakage. Frequent depletion of heat exchange media may also indicate leakage.
3. Pressure on the product side should be higher than that on the media side.

Trucks used to transport products within the plant should be constructed of stainless steel. However, galvanized metal is acceptable, provided it is maintained in a good state of repair and is regalvanized when necessary. Trucks should be free of cracks and rough seams. Metal wheels should be avoided as they cause deterioration of the floor surfaces. All trucks should have some means of affixing a tag.

Compressed air may be used to directly contact the product and/or product-contact surfaces, provided the air is filtered before entering a compressor and it is clean and free of moisture, oil, or other foreign materials when contacting the product or product-contact surfaces. Lubricants and coolants directly contacting air should be authorized by trade associations, FDA, USDA, and other standardization bodies in the United States.

Compressed air storage tanks should have a drain. Water and oil traps must be located between storage tanks and the point of use. Spent air must be exhausted in a manner to prevent product contamination.

Air directly contacting products or product-contact surfaces should be filtered as near the air outlet as-feasible. Filters should be readily removable for cleaning or replacement and should be capable of filtering out 50 μm particles (measured in the longest dimension). Air intake on votators should also be filtered.

Product-reconditioning equipment also requires special considerations. The product that is accidentally soiled may be cleaned on a separate, conveniently located wash table or sink. This wash station should be properly equipped with sprays and a removable, perforated plate to hold the product off the bottom. The station should be identified as a "product wash station" and cannot be used for hand or implement washing.

Accepting the use of electric cords should be based on both sanitary and safety considerations. Drop cords suspended from the ceiling may be retractable and used to connect portable equipment on an as-needed basis if the cords are properly wired to the power source. Electric cords should not be strung across the floor even on a temporary basis.

Electric insect traps may be used in edible product handling and storage areas provided the following conditions are met

1. The equipment should be made of acceptable noncorrosive materials.
2. The traps must not be placed above the uncovered product or above the uncovered product traffic ways.
3. The electrified components are either apparent or properly identified, insulated from nonelectrificd components, and covered with a protective grille to prevent electric shock hazard.
4. The equipment should have a removable shelf or drawer that collects all trapped insects.
5. The equipment is designed and constructed so that all dead insects are trapped in the removable shelf or drawer. (Insects must not collect on the protective grill.)

Removable drawers or shelves should be emptied as often as necessary. If the drawer or shelf becomes full of dead insects, then the fourth requirement above cannot be met, so the equipment should be rejected for use. Dead insects must be removed from the unit before they create an odor problem. They cannot be left in the unit as bait.

Containers for handling and transporting inedible products should be watertight, maintained in a good state of repair (no rust or corrosion), and clearly marked with an appropriate identification. All inedible product containers in the plant should be uniformly identified. Inedible product containers should be cleaned before being moved into an edible products department.

Metal barrels, tanks, or trucks may be used as a lot for holding inedible poultry products in specially designated inedible product rooms. Alternatively, the containers may be stored outside the building, provided the storage area is paved, drained, and conveniently located. These storage areas should also be equipped with nearby hose connections for cleanup.

Filters used on the compressed air line should be readily removable for cleaning or replacement and should be capable of filtering out 50 μm panicles. The air pressure must be measured and recorded with appropriate devices. The blow air must be confined so that it is captured by a water curtain or by an exhaust system with standard suction capacity at the point of exhaust.

Equipment that produces ozone may be used only in coolers designated for certain types of products, for example, aging meat. The ozone concentration in the air must be measured and recorded with appropriate devices. Ozone-generating equipment should be turned off and the ozone permitted to dissipate before any in-house or external inspection is performed.

Equipment used to ozonize and recycle water should be constructed from noncorrosive material with safe and easy access for cleanup and sanitary inspection of all component pans. Pumps and piping should be of acceptable sanitary type, demountable, and have quick-disconnect mechanisms at each change of direction. Tanks, funnels, ozone generators, filter housings, and filter media should have FDA/USDA acceptance and approval and they should be easily demountable for cleanup and sanitary inspection. A written approval of a wafer reuse procedure may be needed by state and federal regulators. There should also be written procedures for measuring and recording the total bacteria count and the total organic carbon level in the ozone-treated water returning to the source of use.

A test kit or other devices that accurately measure the concentration in milligrams per liter of sanitizing solutions should be provided. Testing devices to measure the concentration of sanitizing solutions are required for two reasons:

1. Use of chemical sanitizers requires minimum concentrations of the sanitizer during the final rinse step to assure sanitization.
2. Use of more sanitizer in the final rinse water could be toxic.

49.7 Equipment Location and Installation

A unit of equipment that is fixed because it is not easily movable should be installed so that it is

1. Spaced to allow access for cleaning along the sides, behind, and above the unit.
2. Spaced from the adjoining equipment, walls, and ceilings: a distance of not more than 1 mm or 1/32 in.
3. Scaled to adjoining equipment or walls, if the unit is exposed to spillage or seepage.

Table-mounted equipment that is not easily movable should be installed to allow cleaning of the equipment and areas underneath and around the equipment by being (1) sealed to the table, or (2) elevated on legs as specified.

When the weight of the equipment exceeds 14 kg (30 lb), it is no longer considered by the definition to be easily movable. Consequently, the following guide is noted:

1. Allow accessibility for cleaning on all sides, above, and underneath the units or minimize the need for cleaning due to closely abutted surfaces.
2. Assure that equipment that is subject to moisture is settled.
3. Prevent the harborage of insects and rodents.
4. Provide accessibility for the monitoring of pests.

Floor-mounted equipment that is not easily movable should be sealed to the floor or elevated on legs that provide a proper clearance between the floor and the equipment. Table-mounted equipment that is not easily movable should be elevated on legs that provide a proper clearance between the table and the equipment.

In sum:

1. Stationary equipment or equipment not easily movable (i.e., no casters) should be installed far away from walls and support columns to allow thorough cleaning and inspection. In addition, there must be ample clearance between the floor and the ceiling. If these clearances are not possible, then equipment should be scaled watertight to the surfaces. All wall-mounted cabinets, electric connections, and electronic components should be at least 1 in. from the wall or sealed watertight to the wall.
2. The inability to adequately or effectively clean areas under equipment could create a situation that may attract insects and rodents and accumulate pathogenic microorganisms that are transmissible through food.

3. The effectiveness of cleaning is directly affected by the ability to access all areas to clean fixed equipment. It may be necessary to elevate the equipment. When elevating equipment is not feasible or is prohibitively expensive, sealing to prevent contamination is required.
4. The economic impact of the requirement to elevate display units in retail food stores, coupled with the fact that the design, weight, and size of such units are not conducive to casters or legs, led to the exception for certain units located in consumer-shopping areas, provided the floor under the units is kept clean. This exception for retail food store display equipment, including shelving, refrigeration, and freezer units in the consumer shopping areas, requires a rigorous cleaning schedule.

49.8 Maintenance and Operation

Equipment should be in good repair and proper adjustment such that

1. Equipment should be maintained in a state of repair and condition that meets the requirements specified in this chapter.
2. Equipment components such as doors, seals, hinges, fasteners, and kick plates should be kept intact, tight, and adjusted in accordance with manufacturers' specifications.
3. Culling or piercing parts of can openers should be kept sharp to minimize the creation of metal fragments that can contaminate food when the container is opened.

Proper maintenance of equipment to manufacturer specifications helps assure that it will continue to operate as designed. Failure to properly maintain equipment could lead to violations of the associated requirements that place the health of the consumer at risk. For example, refrigeration units in disrepair may no longer be capable of properly cooling or holding potentially hazardous foods at safe temperatures.

The cutting or piercing parts of can openers may accumulate metal fragments that could lead to food containing foreign objects and, possibly, result in consumer injury.

Adequate cleaning and sanitization of dishes and utensils using a warewashing machine is directly dependent on the exposure time during the wash, rinse, and sanitizing cycles. Failure to meet manufacturer and stated requirements for cycle times could result in failure to clean and sanitize. For example, high-temperature machines depend on the buildup of heat on the surface of dishes to accomplish sanitization. If the exposure time during any of the cycles is not met, the surface of the items may not reach the time–temperature parameter required for sanitization. Exposure time is also important in warewashing machines that use a chemical sanitizer since the sanitizer must contact the items long enough for sanitization to occur. In addition, a chemical sanitizer will not sanitize a dirty dish; therefore, the cycle limes during the wash and rinse phase % are critical to sanitization.

Culling surfaces such as cutting boards and blocks that become scratched and scored may be difficult to clean and sanitize. As a result, pathogenic microorganisms transmissible through food may accumulate. These microorganisms may be transferred to foods that are prepared on such surfaces.

Surfaces such as cutting blocks and boards that are subject to scratching and scoring should be resurfaced if they can no longer be effectively cleaned and sanitized, or discarded if they are not capable of being resurfaced.

49.9 Cleaning of Equipment and Utensils

Equipment food-contact surfaces and utensils should be clean to sight and touch. The food-contact surfaces of cooking equipment and pans should be kept free of encrusted grease deposits and other soil accumulations. Nonfood-contact surfaces of equipment should be kept free of an accumulation of dust, dirt, food residue, and other debris. The objective of cleaning focuses on the need to remove organic

matter from food-contact surfaces so that sanitization can occur and to remove soil from nonfood-contact surfaces so that pathogenic microorganisms will not be allowed to accumulate and insects and rodents will not be attracted.

Nonfood-contact surfaces of equipment should be cleaned at its frequency necessary to preclude accumulation of soil residues. The presence of food debris or dirt on nonfood-contact surfaces may provide a suitable environment for the growth of microorganisms that employees may inadvertently transfer to food. If these areas are not kept clean, they may also provide harborage for roaches, flies, mice, and other pests.

If used, dry cleaning methods such as brushing, scraping, and vacuuming should contact only surfaces that are soiled with dry food residues that are not potentially hazardous. Cleaning equipment used in dry-cleaning food-contact surfaces may not be used for any other purpose. Dry-cleaning methods are indicated in only a few operations, which are limited to dry foods that are not potentially hazardous. Under sonic circumstances, attempts at wet cleaning stay create microbiological concerns.

Food debris on equipment and utensils should be scrapped over a waste disposal unit, scupper, or garbage receptacle or should be removed in a warewashing machine with a prewash cycle. If necessary for effective cleaning, utensils and equipment should be preflushed, scrubbed with abrasives, or presoaked.

Precleaning of utensils, dishes, and food equipment allows for the removal of grease and food debris to facilitate the cleaning action of the detergent. Depending on the condition of the surface to be cleaned, detergent alone may not be sufficient to loosen soil for cleaning. Heavily soiled surfaces may need to be presoaked or scrubbed with an abrasive.

Food-contact surfaces and utensils should be effectively washed to remove or completely loosen soils by using the manual or mechanical means necessary, such as the application of detergents containing wetting agents and emulsifiers: acid, alkaline, or abrasive cleaners; hot water; brushes: scouring pads; or high-pressure sprays. The washing procedures selected should be based on the type and purpose of the equipment or utensil, and on the type of soil to be removed.

Because of the variety of cleaning agents available and the many different types of soil to be removed, it is not possible to recommend one cleaning agent to fit all situations. Each of the different types of cleaners works best under different conditions (e.g., some work best on grease; some work best in wane water; others work best in hot water). The specific chemical selected should be compatible with any other chemicals to be used in the operation such as a sanitizer or drying agent.

49.10 Sanitization of Equipment and Utensils

Equipment food-contact surfaces and utensils should be sanitized. Effective sanitization procedures destroy those organisms of public health importance. They may be present on wiping cloths, food equipment, or utensils after cleaning, or which have been introduced into the rinse solution. It is important that surfaces be clean before being sanitized to allow the sanitizer to achieve its maximum benefit.

Utensils and fool-contact surfaces of equipment should be sanitized before use and after cleaning. Sanitization is accomplished after the warewashing steps of cleaning and rinsing so that utensils and food-contact surfaces are sanitized before their contact with food and before use.

After being cleaned, equipment, food-contact surfaces, and utensils should be sanitized by hot water manual operations by immersion for at least 30 s as specified in standard manuals or hot water mechanical operations by being cycled through equipment that is set up as specified elsewhere achieving a utensil surface temperature of 71°C (160°F) as measured by an irreversible-registering temperature indicator or through the use of chemical means.

Chemical manual or mechanical operations, including the application of sanitizing chemicals by immersion, manual swabbing, brushing, or pressure-spraying methods, use a solution with standardized concentration and time requirement. According to FDA/USDA regulations or trade recommendations, the following guide applies:

1. An exposure time of at least 10 s for chlorine solution.
2. An exposure time of at least 30 s for other chemical sanitizer solutions.

3. An exposure time used in relationship with a combination of temperature, concentration, and pH that, when evaluated for efficacy, yields sanitization as defined elsewhere.

Efficacious sanitization is dependent on warewashing being conducted within certain parameters. Time is a parameter applicable to both chemical and hot water sanitization. The time that hot water or chemicals contact utensils or food-contact surfaces must be sufficient to destroy pathogens that may remain on surfaces after cleaning. Other parameters, such as temperature or chemical concentration, are used in combination with time to deliver effective sanitization.

49.11 Protection of Clean Items

For equipment and utensils, air-drying requirements include:

1. After cleaning and sanitizing, equipment and utensils may not be cloth dried.
2. Equipment and utensils may be air dried or used after adequate draining as specified in FDA regulations before their contact with food.
3. Utensils that have been air dried may be polished with cloths that are maintained clean and dry.
4. Items must be allowed to drain and to air dry before being stacked or stored. Stacking wet items such as pans prevent them from drying and may allow an environment where microorganisms can begin to grow. Cloth drying of equipment and utensils is prohibited to prevent the possible transfer of microorganisms to equipment or utensils.

Lubricants should be applied to food-contact surfaces that require lubrication in a manner so that food-contact surfaces are not contaminated.

Equipment should be reassembled so that food-contact surfaces are not contaminated. Cleaned equipment and utensils should be stored:

1. In a clean, dry location.
2. Where they are not exposed to splash, dust, or other contamination.
3. At least 15 cm (6 in.) above the floor.

Further, equipment and utensils should be stored in a self-draining position and covered or inverted.

Clean equipment and multiuse utensils that have been cleaned and sanitized can become contaminated before their intended use in a variety of ways, such as through water leakage, pest infection, or other insanitary conditions.

Cleaned and sanitized equipment and utensils may not be stored:

1. In locker rooms.
2. In toilet rooms.
3. In mechanical rooms.
4. Under sewer lines that are not shielded to intercept potential drips.
5. Under leaking water lines including leaking automatic fire sprinkler heads or under lines on which water has condensed.
6. Under open stairwells.
7. Under titer sources of contamination.

The improper storage of clean and sanitized equipment and utensils may allow contamination before their intended use. Contamination can be caused by moisture from adsorption, flooding, drippage, or splash. It can also be caused by food debris, toxic materials, litter, dust, and other materials. The

contamination is often related to unhygienic employee practices, unacceptable high-risk storage locations, or improper construction of storage facilities.

49.12 Conclusion

For any food processor, the sanitation of equipment is a permanent problem. Contamination of food or food ingredients by unclean equipment has been the cause of foodborne diseases in human population throughout the history of modern civilization. It is important that a food processor complies with GMPs to reduce the potential of equipment being a source of human or poisonous chemicals.

50

Enforcement Tools for Meat in Commerce in the United States[*]

Y. H. Hui

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[*] The information in this chapter has been modified from *Meat and Poultry Safety Manual* published by Science Technology System (STS) of West Sacramento, California. Copyrighted 2011©. With permission.

50.1 Background Information

The information in this chapter is provided by the United States Department of Agriculture's (USDA) website (http://www.usda.gov/) and is only applicable in this country.

USDA's Food Safety and Inspection Service (FSIS) is charged with ensuring that meat, poultry, and egg products are safe, wholesome, and properly labeled. FSIS, in cooperation with state counterparts, inspects, monitors, and verifies the proper processing, handling, and labeling of meat and poultry products from the delivery of animals to the slaughterhouse to when the products reach consumers. FSIS, in cooperation with Food and Drug Administration (FDA) and the states, provides similar coverage for egg products—the processed whole egg ingredients used in manufacturing other foods. This regulatory oversight generally reflects compliance by the large majority of businesses. However, if FSIS detects problems at any step along the way, it can use a number of product control and enforcement measures to protect consumers.

USDA has traditionally focused much of its effort on the plants that slaughter food animals and process products. USDA ensures that products at these establishments are produced in a sanitary environment in which inspectors or plant employees identify and eliminate potential food safety hazards. These establishments must apply for a grant of inspection from FSIS and demonstrate the ability to meet certain requirements for producing safe, wholesome, and accurately labeled food products. Requirements include meeting sanitation, facility, and operational standards and, through new requirements now being implemented, having preventive systems in place to ensure the production of safe and unadulterated food. Products from official establishments are labeled with the mark of inspection, indicating that they have been inspected and passed by USDA and can be sold in interstate commerce.

FSIS uses Compliance Officers or Consumer Safety Inspector (CSI) throughout the chain of distribution to detect and detain potentially hazardous foods in commerce to prevent their consumption and to investigate violations of law. Even if products are produced under conditions that are safe and sanitary, abuse on the way to the consumer, for example, if transported in trucks that are too warm or if exposed to contamination, can result in product that can cause illness or injury. FSIS has recognized a need to spend increasing amounts of its energy on activities to promote safe transporting, warehousing, and retailing of meat, poultry, and egg products, and is moving forward on these efforts.

FSIS also works closely with USDA's Office of Inspector General (OIG), which assists FSIS in pursuing complex criminal cases. In addition, many state and local jurisdictions have enforcement authorities that apply to USDA-regulated products. FSIS cooperates with these other jurisdictions in investigations and case presentations. FSIS also participates with OIG and the U.S. Department of Justice in monitoring conditions of probation orders and pretrial diversion agreements developed to resolve cases.

In January 1997, FSIS began implementing new requirements in plants that produce meat and poultry. New regulations, entitled "Pathogen Reduction; Hazard Analysis and Critical Control Point (HACCP) Systems," require that federally inspected meat and poultry plants: (1) develop and implement a preventive HACCP plan; (2) develop and implement Sanitation Standard Operating Procedures (SSOP's); (3) collect and analyze samples for the presence of generic *Escherichia coli*, and record results; and (4) meet *Salmonella* performance standard requirements. These new requirements are designed to help target and reduce foodborne pathogens.

This report provides a discussion of the regulatory and enforcement actions, including actions that address the Pathogen Reduction/HACCP regulatory requirements. FSIS has taken to ensure that products that reach consumers are safe, wholesome, and properly labeled.

50.2 Establishment Inspection

The FSIS CSI conducts scheduled establishment inspections (EI), following standard agency requirements and procedures. There are many types of EI depending on what types of meat and meat products the establishment processes, for example, premortem, postmortem, shelf stable, not shelf stable, etc.

During an EI, the CSI check if the establishment complies with all requirements of processing. The reports from the CSI will determine further actions from FSIS if any. EI was originally the major tool of enforcement. Of course, the last 20 years have seen many changes in the method of EI. Currently, the result of an EI may trigger one of the enforcement actions discussed below.

50.3 Noncompliance Records and Appeals

FSIS inspection program personnel perform thousands of inspection procedures each day in federally inspected establishments to determine whether or not inspected plants are in compliance with regulatory requirements. Each time inspection program personnel make a noncompliance determination they complete a Noncompliance Record (NR). An NR is a written report that documents noncompliance with FSIS regulations. An NR notifies the establishment of the noncompliance and that it should take action to remedy the situation and prevent its recurrence. Noncompliance reported on NRs varies from nonfood safety issues to serious breakdowns in food safety controls. When noncompliance occurs repeatedly, or when a plant fails to prevent adulterated product from being produced or shipped, FSIS takes action to control products and may take enforcement action, such as to suspend inspection.

50.4 Port-of-Entry Reinspection

FSIS conducts port-of-entry reinspections of imported meat, poultry, and egg products. This activity is a reinspection of products that have already been inspected and passed by an equivalent foreign inspection system. Thus, imported product reinspection is a monitoring activity for verifying on an ongoing basis the equivalence of a foreign country's inspection system.

Port-of-entry reinspection is directed by the Automated Import Information System (AIIS), a centralized computer database that generates reinspection assignments and stores results. After clearing the Department of Homeland Security, Customs and Border Protection, and the Animal and Plant Health Inspection Service, every imported meat, poultry, or egg product shipment must be presented to FSIS. When a meat or poultry shipment is presented for reinspection, the AIIS verifies that the product is from an eligible country and certified establishment. Shipments are refused entry if the foreign country, or the foreign establishment that produced the product, is not eligible to export to the United States.

All imported product shipments are reinspected for general condition, labeling, proper certification, and accurate count. In addition, other types of inspection (TOI) may be generated by the AIIS. These could include a physical examination of the product for visible defects, collection of samples for microbiological analysis, sampling for food chemistry analyses, and samples to be analyzed for drug and chemical residues. Shipments are randomly selected for reinspection using a statistical sampling plan that allocates samples by HACCP process categories. The level of sampling is based on the volume imported from the country within each category. Products that fail reinspection are rejected and must be reexported, converted to nonhuman food, or destroyed. Product rejections cause the AIIS to automatically generate an increased rate of reinspection for future shipments of like product from the same establishment.

50.5 Product Control Actions

FSIS takes product control actions to gain physical control over products when there is reason to believe that they are adulterated, misbranded, or otherwise in violation of the Federal Meat Inspection Act (FMIA), Poultry Products Inspection Act (PPIA), or Egg Products Inspection Act (EPIA). These actions are designed to ensure that those products do not enter commerce or, if they are already in commerce, that they do not reach consumers.

50.5.1 Retentions and Condemnations

In official establishments, FSIS inspection program personnel may retain products that are adulterated or mislabeled when there are insanitary conditions, for inhumane slaughter or handling, when conditions preclude FSIS from determining that the product is not adulterated or misbranded, or for other reasons authorized by the statutes. FSIS inspection program personnel condemn animals for disease, contamination, or other reasons to prevent their use as human food.

50.5.2 Detentions

FSIS Investigators, Enforcement, Investigation and Analysis Officers, import officers, and other designated FSIS inspection program personnel will detain products that may be adulterated, misbranded, or otherwise in violation of the law when found in commerce. Most detentions result in voluntary action by the product owner or custodian, such as voluntary disposal of the product. If detained product cannot be disposed of within 20 days, then FSIS may request, through Office of the General Counsel (OGC) and the U.S. Attorney, that a U.S. District Court enter an order to seize the product as provided for in the FMIA, PPIA, and EPIA.

50.6 Food Recalls

This section will

1. Explain why products are recalled.
2. Identify the classes of product recalls.
3. The recall process.

A recall is a firm's voluntary removal of product from trade or consumer channels (e.g., by manufacturers, distributors, or importers) to protect the public from consuming adulterated (injurious to health or unfit for human consumption) or misbranded (false or misleading labeling and/or packaging) products. If a company refuses to recall its product, then FSIS has the legal authority to detain and/or seize meat and poultry product(s) in commerce when there is a reason to believe that they are hazardous to public health or if other consumer protection requirements are not met. Although recalls are voluntary, FSIS oversees all recall activities by official meat and poultry establishments, and coordinates any FSIS actions with the recall taken by the firm. For recalls conducted by state-inspected firms or retail establishments, the appropriate state agency oversees the recall in most cases. FSIS will provide the state agencies any needed assistance and information.

FSIS assesses the public health concern or hazard presented by a product being recalled, or considered for recall, whether firm initiated or requested by FSIS, and classifies the recall based on the relative health risk as follows:

1. Class I: Reasonable probability of serious, adverse health problem or death.
2. Class II: Remote probability of adverse health problem.
3. Class III: No adverse health consequences.

Class I and Class II are therefore public health related. Let us look at each of these in more detail.

Class I. This is a health hazard situation where there is a reasonable probability that the use of the product will cause serious, adverse health consequences or death. For example, the presence of pathogens in ready-to-eat product or the presence of *E. coli* O157:H7 in ground beef.

Class II. This is a health hazard situation where there is a remote probability of adverse health consequences from the use of the product, for example, the presence of undeclared allergens such as very small amounts of potential allergenic substances (milk or soy) or small-sized nonsharp-edged foreign materials (plastic).

Class III. This is a health hazard situation where the use of the product will not cause adverse health consequences. For example, the presence of undeclared generally recognized as safe nonallergen substances, such as excess water.

The process of recalling a product begins with problem identification. A problem with a product can be identified through various sources such as the firm, the Agency, or sources outside of the Agency. The most common sources are

1. A positive result from FSIS sampling programs (microbiological, physical, chemical, misbranding).
2. The company that manufactured or distributed the food product informs FSIS of the potential hazard (e.g., positive microbiological test results, consumer complaint, formulation records).
3. Information from in-plant inspectors and program investigators in the course of their routine duties.
4. Information from outbreak investigations, epidemiological or laboratory data submitted by State or local public health department, or other Federal Agencies.
5. Consumer complaints reported to FSIS (reported in the FSIS Consumer Complaint Monitoring System).

The recall process can start with the FSIS or the affected companies. In cases where the company conducts a voluntary recall, the FSIS then issues a press release, since the type of hazard in this scenario is classified as Class I. This press release is posted on the FSIS website.

Effectiveness checks constitute a process by which FSIS program personnel verify that the recalling firm has been diligent and successful in notifying and advising the consignees of the need to retrieve and control recalled product, and that the consignees have responded accordingly. Subsequent consignees are then expected to notify their customers of the recall. FSIS will conduct these effectiveness checks throughout the distribution chain.

The effectiveness checks are conducted based on the risk to public health. Risk is measured by combining the hazard, as defined by the class of recall and any available epidemiological data, and potential exposure to the product measured by the number of the consignees or exposure. By means of this effectiveness checks FSIS program personnel ensure that the firm makes all reasonable efforts to retrieve the recalled meat, poultry, or egg product. A sufficient number of effectiveness checks are made to verify that the recall is conducted in an effective manner, and that the firm locating, retrieving, controlling, and disposition of the product is acting according to regulatory requirements.

After FSIS has determined that the recalling firm has made all reasonable effort to retrieve and appropriately dispose of the recalled food product, the firm is officially notified by letter that the recall is completed and no further action is expected.

Some examples of complaints associated with consumption of a meat, poultry, or egg product are as follows:

1. Product caused an illness or injury.
2 Product contained a foreign object/material.
3. Product caused an allergic reaction.
4. Ready to Eat product has been under processed.
5. Prohibited Specific Risk Materials (SRM).
6. Product is misbranded/economic adulteration.
7. Product is of inferior quality.

A food recall is a voluntary action by a manufacturer or distributor to protect the public from products that may cause health problems or possible death. A recall is intended to remove food products from commerce when there is reason to believe the products may be adulterated, misbranded, or otherwise in

violation of the FMIA, PPIA, or EPIA. Recalls are initiated by the manufacturer or distributor of the meat, poultry, or egg products, sometimes at the request of FSIS. All recalls are voluntary. However, if a company refuses to recall its products, then FSIS has the legal authority to detain and seize those products in commerce.

50.7 Notices of Prohibited Activity

Firms that recall products are expected to provide notification to their consignees concerning the recalled product and request that customers review inventory records and segregate, hold, or destroy product. Firms that have already shipped or sold recalled products are to retrieve and control the product, prevent further distribution, and contact their consignees and have them retrieve and control product that is part of a recall. FSIS program personnel conduct "effectiveness checks" to ensure that the recalling firm and firms that received or distributed product subject to a recall take effective action to notify all consignees of the recalled product that there is a need to control and remove the recalled product from commerce.

In situations where FSIS determines that a federally inspected establishment or a firm operating in commerce has not taken responsibility to remove or control adulterated, misbranded, or other unsafe product in commerce or to advise its consignees of product that is subject to recall, it may issue prohibited activity warning notices to the firm. FSIS issues prohibited activity notices for the following:

1. Failure of a recalling firm to notify its consignees of recalled products
2. Failure of a consignee to notify its customers of recalled products
3. Recalling firm or consignee found offering recalled products for sale

50.8 Notices of Warning

A Notice of Warning (NOW) provides notice of violations to firms and responsible individuals. FSIS issues an NOW to firms and individuals to notify them of prohibited acts or other conduct that violates FSIS statutes or regulations. Generally, FSIS issues an NOW for minor violations of law that are not referred to a U.S. Attorney for prosecution or other action. FSIS may also issue an NOW when a U.S. Attorney declines to prosecute a case or bring action against a specific business or individual. An NOW identifies the violative conduct, condition, practice, or product, and the statutory or regulatory provisions violated. It advises the firm or individual that the Agency will not pursue further action for the violation and warns that FSIS may seek criminal prosecution or other action for continued or future violations. Notices of Warning may be issued to any individual or business, including Federal plants, wholesalers, distributors, restaurants, retail stores, and other entities that process, store, or distribute meat, poultry, and egg products.

50.9 Administrative Actions

The FSIS Rules of Practice, which are set out in 9 CFR Part 500, define the type of administrative enforcement actions that FSIS takes, the conditions under which these actions are appropriate, and the procedures FSIS will follow in taking these actions. These regulations provide notice to establishments of FSIS enforcement actions, criteria, and processes, and ensure that all establishments are afforded due process.

Administrative actions in the Rules of Practice include regulatory control action, withholding action, and suspension. These actions are defined in 9 CFR 500.1. FSIS takes these actions to prevent preparation

and shipment of adulterated products, when products are produced under insanitary conditions, and for other reasons described in the Rules of Practice. When there is an imminent threat to public health or safety, such as the shipment of adulterated product, FSIS takes immediate enforcement action. In other situations, FSIS provides the establishment prior notification of intended enforcement action and the opportunity to demonstrate or achieve compliance. This is called a Notice of Intended Enforcement Action. In appropriate situations, FSIS may defer an enforcement decision based on corrections submitted by the establishment. FSIS also may place a suspension action, if taken, in abeyance if an establishment presents and puts into effect corrective and preventive actions. FSIS Office of Field Operations, District Offices monitor and verify an establishment's implementation of corrective and preventive actions, and take follow-up action if needed to protect public health.

50.9.1 Formal Adjudicatory Actions for Food Safety

In some situations, it is necessary to withdraw inspection from an establishment based on the failure of a recipient of inspection service to meet critical sanitation and food safety regulatory requirements (e.g., SSOP's or HACCP system regulations) necessary to protect the public health. In these cases, FSIS files an administrative complaint with the USDA Hearing Clerk. The plant may request a hearing before a USDA Administrative Law Judge. If the action is based on insanitation, or other imminent threats to public health or safety, the plant may remain closed while proceedings go forward. In other cases that do not involve a threat to public health, operations may continue. These actions may be resolved by FSIS and the plant entering into a consent decision, which allows the plant to operate under certain specified conditions. If inspection service is withdrawn, a closed plant must reapply to receive federal inspection. FSIS also may take enforcement action, by filing an administrative complaint, to deny federal inspection service to an applicant. These actions are taken in accordance with the FSIS Rules of Practice (9 CFR 500) and Department regulations governing formal adjudicatory proceedings (7 CFR 1).

50.9.2 Withdrawal for Unfitness

FSIS can move to withdraw or deny inspection, after opportunity for a hearing, based on the unfitness of recipient or applicant for inspection because of a felony conviction, more than one violation involving food, or certain other violations set out in the statutes. Actions pending or taken (other than outstanding consent decisions) are reported.

50.9.3 Removing Exempt Privilege

The meat and poultry laws exempt certain custom, retail, or other operations from inspection, such as facilities that slaughter animals or poultry, or process meat or poultry, for owners of the animals. When insanitary conditions create health hazards at these businesses, FSIS may remove custom or other exempt privileges through issuance of a Notice of Ineligibility, and require the business to cease operations until sanitary conditions are restored. FSIS can also take action when custom or other exempt facilities fail to properly label product as "Not for Sale." These exempt businesses have the opportunity to correct violations before or after such actions, contest the basis for such actions at hearings, and to enter settlement agreements to resolve actions.

50.10 Criminal Actions

If evidence is found that a person or business has engaged in violations of the FMIA, PPIA, or Egg Products Inspection Act (EPIA), USDA may refer the case, through the OGC or OIG, to the appropriate U.S. Attorney to pursue criminal prosecution. Conviction for a criminal offense can result in a fine, imprisonment, or both. In certain situations, U.S. Attorneys may enter into Pre-Trial Diversion (PTD)

agreements with alleged violators in lieu of actual prosecution. Under these agreements, the government agrees not to proceed with criminal prosecution if the alleged violator meets certain terms and conditions. The terms and conditions of a PTD may be tailored to each individual case. If the divertee successfully completes the program, no criminal charges are filed. If, on the other hand, the divertee does not successfully complete the program, criminal charges may be reinstated. FSIS frequently monitors these agreements so that it can assist the U.S. Attorneys in determining whether the terms have been met or that prosecution should be reinstituted.

50.10.1 Examples of Criminal Actions

In early 2010, FSIS reported the following criminal actions against the Brown Packing Company, Inc., of South Holland, IL.

The corporation and its officials were found criminally responsible as follows:

1. On February 11, 2010, the corporation was sentenced on one felony count for charges related to the illegal use of growth-promoting hormone implants in nonruminant veal calves, mail, and wire fraud. The firm was ordered to pay a $2,000,000 fine.
2. On February 11, 2010, the President of the corporation was sentenced on a single misdemeanor count for violations to USDA and FDA laws and was ordered to pay a $5000 fine.
3. On February 11, 2010, two officials of the corporation were each sentenced on a single misdemeanor count for violations to USDA and FDA laws and were ordered to pay a $5000 fine each.

This legal action is a result of a joint investigation conducted by the USDA, the FSIS, and the FDA.

50.11 Civil Actions

FSIS also has authority to seek a variety of civil actions and case dispositions in Federal Court.

50.11.1 Seizures

When FSIS has reason to believe distributed products are adulterated, misbranded, or otherwise in violation of law, the Agency will, through the USDA OGC and U.S. Attorney, institute a seizure action against the product. The product is held pending an adjudication of its status. If the court finds that the product is adulterated, misbranded, or otherwise in violation of FSIS laws, it will condemn the product. Condemned product cannot be further processed to be used for human food.

50.11.2 Injunctions

FSIS, through the U.S. Attorney, may request a U.S. District Court to enjoin firms or individuals that engage in repetitive violations of the FMIA, PPIA, or EPIA, or their actions pose a threat to public health and safety. An injunction requires an individual or firm to take certain action or to refrain from doing acts that violate the law. Injunctions may be resolved by a consent decree.

50.11.3 False Claims Act and Other Actions

FSIS also works with OGC, OIG, and U.S. Attorneys to obtain other civil case outcomes. The Department of Justice Affirmative Civil Enforcement program is used by U.S. Attorneys to recover damages when a violation of law involves fraud against the Federal government. Case examples where civil action may be

appropriate include cases involving products, not in compliance, sold to the military, to public schools engaged in the school lunch program, or to other Federal institutions.

50.12 Examples of Food Recalls and Outbreaks

50.12.1 Injuries from Pathogens

50.12.1.1 Illness

On August 6, 2010, USDA-FSIS announced a Class I Recall—Valley Meat Company, a Modesto, Calif. establishment, was recalling approximately one million pounds of frozen ground beef patties and bulk ground beef products that could be contaminated with *E. coli* O157:H7.

FSIS became aware of the problem on July 15 when the agency was notified by the California Department of Public Health (CDPH) of a small *E. coli* O157:H7 cluster of illnesses with a rare strain. A total of six patients with illness onset dates between April 8 and June 18, 2010 were reported at that time. After further review, CDPH added another patient from February to the case count, bringing the count to seven. FSIS is continuing to work with the CDPH and the meat company on the investigation. Anyone with signs or symptoms of foodborne illness should contact a health-care provider.

The products subject to recall were distributed to retail outlets and institutional foodservice providers in CA, TX, OR, AZ, and internationally. FSIS and the establishment were concerned that some product may still be frozen and in consumers' freezers. FSIS strongly encouraged consumers to check their freezers and immediately discard any product that was the subject of this recall.

50.12.1.2 Death

According to the USDA-FSIS in November 2009, a New York meat company recalled almost 546,000 pounds of ground beef because, according to health officials, contaminated meat had caused illness and one death. The meat sold by Ashville, NY-based Fairbank Farms was linked to cases of *E. coli* O157:H7-related illness in several states (CT, ME, MA, NH). Illnesses and deaths had been reported. This was a Class I recall.

According to the federal agency, the ground beef was sold at numerous retail stores. FSIS became aware of the problem during the course of an investigation of a cluster of *E. coli* O157:H7 illnesses. Working with the Centers for Disease Control and Prevention and state health and agriculture departments, FSIS determined an association between the fresh ground beef products subject to recall and the illnesses identified in several states.

These products were distributed to retail establishments in MD, MA, NC, NJ, NY, PA, and VA (Maryland, Massachusetts, North Carolina, New Jersey, New York, Pennsylvania, and Virginia) for further processing.

This multistate outbreak of *E. coli* O157:H7 infections was associated with beef from Fairbank Farms. Health officials in several states who were investigating a cluster of *E. coli* O157:H7 illnesses, with isolates that matched by "DNA fingerprinting" analyses, found that most ill persons had consumed ground beef, with several purchasing the same or similar product from a common retail chain. Two samples from opened packages of ground beef recovered from a patient's homes were tested by the Massachusetts and Connecticut Departments of Health and yielded *E. coli* O157:H7 isolates that matched patient isolates by DNA analysis.

The cluster included 26 persons from 8 states infected with matching strains of *E. coli* O157:H7. The number of ill persons identified in each state was as follows: California (1), Connecticut (6), Massachusetts (8), Maryland (1), Maine (4), New Hampshire (4), New York (1), and Vermont (1).

The first reported illness began on September 17, 2009, and the last began on November 6, 2009. Nineteen patients were reported to have been hospitalized and five developed a type of kidney failure called hemolytic uremic syndrome (HUS). Two deaths were reported. Fifty percent of patients were male and 38% were <18 years old (range 1–88 years).

50.12.2 Potential Hazards from Chemical Agents

50.12.2.1 Color Additive

On June 30, 2009, the USDA-FSIS announced a Class II recall:

> Buffalo SAV, Inc., a Buffalo, N.Y. establishment, is recalling ~208,768 pounds of frozen meat and poultry products because they contain an unapproved ingredient or color additive, Amaranth Red #2, as declared on the label. According to the FDA, Amaranth Red #2 is not approved for use in products sold in the United States.
>
> The frozen meat and poultry products were produced from June 1, 2008, through June 5, 2009, and were sent to distributors and retail establishments in California, Illinois, New York, and Pennsylvania.

50.12.3 Potential Hazards from Physical Agents

50.12.3.1 Plastics

On November 27, 2009, the USDA-FSIS announced a Class II recall:

> Advance Food Company, a Enid, Oklahoma establishment, is recalling ~110,730 pounds of frozen beef steak fritter products that may contain foreign materials. The problem was discovered after the company received customer complaints about finding pieces of plastic in the product. FSIS has not received any consumer complaints or reports of injury at this time.

50.12.3.2 Pebbles and Stones

On January 20, 2009, the USDA-FSIS announced a Class I recall:

> Windsor Quality Food Co., Ltd., doing business as Windsor Foods, a Tulsa, Oklahoma establishment, is recalling ~676,560 pounds of frozen chili beef products that may contain foreign materials from an ingredient source. The products were distributed to food service and limited retail establishments nationwide. The problem was discovered after the company received 16 reports of consumer complaints about finding small pebbles or stones in the product and four reports of injury.

50.12.4 Other Hazards

50.12.4.1 Allergens

On August 3, 2010, USDA-FSIS announced a Class I Recall. Ugo. DiLullo & Sons, a Westville, N.J., establishment, is recalling ~147,667 pounds of canned meatball products because they contain an allergen, egg, which is not declared on the label.

The canned meatball products were distributed to institutions, including food banks, in Maryland, New Jersey, and New York. The problem was discovered by FSIS during a routine inspection. FSIS and the company have received no reports of adverse reactions due to consumption of these products.

Most meat products with allergens not declared on the label are Class I Recalls because of the potential serious effects of consumers allergic to products such as egg.

50.12.4.2 Prohibited-Specific Risk Materials

On April 5, 2010, USDA-FSIS announced a Class II recall:

> North American Bison Co-Op, a New Rockford, N.D., establishment is recalling ~25,000 pounds of whole beef heads containing tongues that may not have had the tonsils completely removed,

> which is not compliant with regulations that require the removal of tonsils from cattle of all ages.

According to FSIS:

> Tonsils are considered an SRM and must be removed from cattle of all ages in accordance with FSIS regulations. SRMs are tissues that are known to contain the infective agent in cattle infected with Bovine Spongiform Encephalopathy (BSE), as well as materials that are closely associated with these potentially infective tissues. Therefore, FSIS prohibits SRMs from use as human food to minimize potential human exposure to the BSE agent. The recalled products were shipped to distribution centers in Md., CO, and Minn. for further sale.
>
> The problem was discovered during FSIS inspection activities at the establishment. FSIS routinely conducts recall effectiveness checks to verify recalling firms notify their customers of the recall and that steps are taken to make certain that the product is no longer available to consumers.

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Meat Processing and Workers' Safety[*]

Y. H. Hui

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51.1 Introduction

The information in this chapter has been adapted from official documents distributed by the U.S. Department of Labor, which are available on its website, http://www.dol.gov/

This chapter discusses two important topics:

1. Industrial classification of food manufacturing.
2. Safety of works in the meat industry.

In a modern society, trading and commercial transactions, when reduced to the simplest term, are made by three parties, willingly or unwillingly: government, business or industry, and the consumers. A business manufactures a quality product according to the need and preference of the consumers. The government monitors the activities of the businesses or firms and incorporates such activities into a data base. It uses the information for a variety of reasons such as taxation, safety of the consumers and workers in the workplace, technology transfers, economic profiles of various industries, the country's growth in relation to any particular industry, etc. This approach is practiced in most Western countries in the world. The foundation of this government activity or its data base is to classify accordingly the different manufacturing businesses such as car manufacturers, chemical manufacturers, computer manufacturers, food manufacturers, etc.

For many years and even the present, the Standard Industrial Classification (SIC) is an establishment–industry classification system that is prepared by the U.S. government for use by all federal agencies especially those involved in statistics, labor, consumer safety, etc. Food manufacturing is included in this classification. The SIC is used, among other functions, in the presentation of the state and local area estimates of earnings and employment by industry, and a record of worker injuries, etc. It is used by the federal government for the estimates for the private sector only, although it is designed to cover both public and private economic activities. To understand food manufacturing in the United States, it is important to know the classification of various establishments engaged in food manufacturing in this industry. This is especially important to a food company because state agencies also use the classification system to assist its state officials in monitoring the food manufactured by each company in terms of

- Compliance with state standards
- Product safety for the consumer publics
- Potential economic frauds involving the products

In February 1999, the statistical agencies of Canada, Mexico, and the United States launched a joint multiphase initiative to develop a comprehensive industrial classification, known as the North American Industrial Classification System (NAICS). NAICS is meant to replace SIC. The foundation of the project was basically completed in 2007. However, permanent revision to refine the system will take place every few years.

In basic principles and format NAICS is essentially the same as SIC, with two major differences:

1. It links three countries in trades, Canada, Mexico, and the United States.
2. The code for each industry is different.

However, it must be emphasized that many other details differentiate NAICS from SIC. The Bureau of Labor Statics (BLS), a unit of the U.S. Department of Labor, maintains both systems in their regulations and rules, as confirmed on their website. This practice is encouraged by the following:

1. For all practical purposes NACIS is of less significance for domestic trading.
2. To change from SIC to NACIS within a short time will affect thousands of government documents.

The change will be slow. Eventually, the old system will be replaced by the new one. At present, SIC is preferred by both government and the food industries.

3. For trading between the United States and Canada or Mexico, NACIS will definitely be used.

This chapter does not explore the practices in Canada or Mexico. For ease of reference, we will use the NAICS here. The NAICS is made up of many sectors. Sectors 31, 32, and 33 pertain to manufacturing. Food manufacturing is included under NAICS Industry 311,000. The sections related to meat and meat processing include the following:

- Animal slaughtering and processing
- Animal (except for poultry) slaughtering
- Meat processed from carcasses
- Rendering and meat by-product processing

51.2 Animal Slaughtering and Processing

This industry, Section 31161, comprises establishments primarily engaged in one or more of the following:

1. Slaughtering animals
2. Preparing processed meats and meat by-products
3. Rendering and/or refining animal fat, bones, and meat scraps

This industry includes establishments primarily engaged in assembly cutting and packing of meats (i.e., boxed meats) from purchased carcasses.

51.2.1 Cross-References

Establishments primarily engaged in

- Manufacturing canned meat for baby food—are classified in Industry 31142, Fruit and Vegetable Canning, Pickling, and Drying;
- Manufacturing meat-based animal feeds from carcasses—are classified in Industry 31111, Animal Food Manufacturing;
- Blending purchased animal fats with vegetable fats—are classified in Industry 31122, Starch and Vegetable Fats and Oils Manufacturing;
- Manufacturing canned and frozen specialty foods containing meat, such as nationality foods (e.g., enchiladas, pizza, egg rolls) and frozen dinners—are classified in Industry Group 3114, Fruit and Vegetable Preserving and Specialty Food Manufacturing;
- Cutting meat (except for boxed meat)—are classified in Industry 42447, Meat and Meat Product Merchant Wholesalers.

51.3 Animal (Except for Poultry) Slaughtering

This U.S. industry, Section 311611, comprises establishments primarily engaged in slaughtering animals (except for poultry and small game). Establishments that slaughter and prepare meats are included in this industry.

51.3.1 Cross-References

Establishments primarily engaged in

- Processing meat and meat by-products (except for poultry and small game) from purchased meats are classified in U.S. Industry 311612, Meat Processed from Carcasses;
- Rendering lard and other animal fats and oils, animal fat, bones, and meat scraps are classified in U.S. Industry 311613, Rendering and Meat By-product Processing; and
- Manufacturing canned and frozen specialty foods containing meat, such as nationality foods (e.g., enchiladas, egg rolls, pizza) and frozen dinners are classified in Industry Group 3114, Fruit and Vegetable Preserving and Specialty Food Manufacturing.

Industries under this group include

1. Abattoirs.
2. Animal fats (except for poultry and small game) produced in slaughtering plants.
3. Bacon, slab and sliced, produced in slaughtering plants.
4. Beef carcasses, half carcasses, primal and subprimal cuts, produced in slaughtering plants.
5. Beef produced in slaughtering plants.
6. Boxed beef produced in slaughtering plants.
7. Boxed meats produced in slaughtering plants.
8. Canned meats (except for poultry) produced in slaughtering plants.
9. Cured hides and skins produced in slaughtering plants.
10. Custom slaughtering.
11. Fats, animal (except for poultry, small game), produced in slaughtering plants.
12. Hams (except for poultry) produced in slaughtering plants.
13. Hides and skins produced in slaughtering plants.
14. Horsemeat produced in slaughtering plants.
15. Hotdogs (except for poultry) produced in slaughtering plants.
16. Inedible products (e.g., hides, skins, pulled wool, wool grease) produced in slaughtering plants.
17. Lamb carcasses, half carcasses, primal and subprimal cuts, produced in slaughtering plants.
18. Lard produced in slaughtering plants.
19. Luncheon meat (except for poultry) produced in slaughtering plants.
20. Meat canning (except for poultry) produced in slaughtering plants.
21. Meats fresh, chilled or frozen (except for poultry and small game), produced in slaughtering plants.
22. Meats, cured or smoked, produced in slaughtering plants.
23. Pork carcasses, half carcasses, and primal and subprimal cuts produced in slaughtering plants.
24. Sausage casings, natural, produced in slaughtering plants.
25. Slaughtering, custom.
26. Tallow produced in slaughtering plants.
27. Variety meats, edible organs, made in slaughtering plants.
28. Veal carcasses, half carcasses, primal and subprimal cuts, produced in slaughtering plants.

51.4 Meat Processed from Carcasses

This U.S. industry, Section 311612, comprises establishments primarily engaged in processing or preserving meat and meat by-products (except for poultry and small game) from purchased meats. This

industry includes establishments primarily engaged in assembly cutting and packing of meats (i.e., boxed meats) from purchased meats.

51.4.1 Cross-References

Establishments primarily engaged in

- Slaughtering animals (except for poultry and small game)—are classified in U.S. Industry 311611, Animal (except for poultry) Slaughtering;
- Rendering animal fat, bones, and meat scraps—are classified in U.S. Industry 311613, Rendering and Meat By-product Processing;
- Manufacturing canned meats for baby food—are classified in U.S. Industry 311422, Specialty Canning;
- Manufacturing meat-based animal feeds from carcasses—are classified in Industry 31111, Animal Food Manufacturing;
- Manufacturing canned and frozen specialty foods containing meat, such as nationality foods (e.g., enchiladas, egg rolls, pizza) and frozen dinners—are classified in Industry Group 3114, Fruit and Vegetable Preserving and Specialty Food Manufacturing; and Cutting meat (except for boxed meat)—are classified in U.S. Industry 424470, Meat and Meat Product Merchant Wholesalers.

Industries under this group (311612) include

1. Bacon, slab and sliced, made from purchased carcasses.
2. Beef stew made from purchased carcasses.
3. Beef, primal and subprimal cuts, made from purchased carcasses.
4. Bologna made from purchased carcasses.
5. Boxed beef made from purchased carcasses.
6. Boxed meat produced from purchased carcasses.
7. Collagen sausage casings made from purchased hides.
8. Cooked meats made from purchased carcasses.
9. Corned meats made from purchases carcasses.
10. Cured meats (e.g., brined, dried, and salted) made from purchased carcasses.
11. Dried meats made from purchased carcasses.
12. Frozen meat pies (i.e., tourtires) made from purchased carcasses.
13. Hams, canned, made from purchased carcasses.
14. Hams, preserved (except for poultry), made from purchased carcasses.
15. Hotdogs (except for poultry) made from purchased carcasses.
16. Lamb, primal and subprimal cuts, made from purchased carcasses.
17. Luncheon meat (except for poultry) made from purchased carcasses.
18. Meat canning (except for baby, pet food, poultry), made from purchased carcasses.
19. Meat extracts made from purchased carcasses.
20. Meat products canning (except for baby, pet food, poultry) made from purchased carcasses.
21. Meats (except for poultry), cured or smoked, made from purchased carcasses.
22. Meats, fresh or chilled (except for poultry and small game), frozen, made from purchased carcasses.
23. Pastrami made from purchased carcasses.
24. Pig's feet, cooked and pickled, made from purchased carcasses.

25. Pork, primal and subprimal cuts, made from purchased carcasses.
26. Potted meats made from purchased carcasses.
27. Processed meats manufacturing.
28. Salted meats made from purchased carcasses.
29. Sandwich spreads, meat, made from purchased carcasses.
30. Sausage and similar cased products made from purchased carcasses.
31. Sausage casings, collagen, made from purchased hides.
32. Smoked meats made from purchased carcasses.
33. Variety meats, edible organs, made from purchased meats.
34. Veal, primal and subprimal cuts, made from purchased carcasses.

51.5 Rendering and Meat By-Product Processing

This U.S. industry, Section 311613, comprises establishments primarily engaged in rendering animal fat, bones, and meat scraps.

51.5.1 Cross-References

Establishments primarily engaged in blending purchased animal fats with vegetable fats are classified in U.S. Industry 311225, Fats and Oils Refining and Blending. Industries under this group include

1. Animal fats rendering
2. Animal oil rendering
3. Bones, fat, rendering
4. Fats, animal, rendering
5. Grease rendering
6. Lard made from purchased fat
7. Meat and bone meal and tankage, produced in rendering plant
8. Neatsfoot oil rendering
9. Oil, animal, rendering
10. Rendering animals (carrion) for feed
11. Rendering fats
12. Rendering plants
13. Stearin, animal, rendering
14. Tallow produced in rendering plant.

51.6 Safety and Health Guide for the Meat-Packing Industry

The meat-packing industry, which employs 1–2 million workers, is considered to be one of the most hazardous industries in the United States. According to the BLS, this industry has experienced one of the highest injury rate of any industry in the country with a rate two to three times that of many other manufacturing industries.

The injuries include cut by knives or machinery, sprains, and strains. Younger new workers are at the highest occupational risk and suffer a significant proportion of all injuries.

Workers can be seriously injured by moving animals prior to stunning, and by stunning guns that may prematurely or inadvertently discharge while they try to still the animal. During the hoisting operation, it is possible for a 2000-pound carcass to fall on workers and injure them if faulty chains break or slip off the carcass' hind leg. Workers can suffer from crippling arm, hand, and wrist injuries. For example,

carpal tunnel syndrome, caused by repetitive motion, can literally wear out the nerves running through one section of the wrist. Workers can be cut by their own knives and by other workers' knives during the butchering process. Back injuries can result from loading and unloading meat from trucks and from moving meat, meat racks, or meat trees along overhead rails. Workers can be severely burned by cleaning solvents and burned by heat sealant machines when they wrap meat. It is not uncommon for workers to sever fingers or hands on machines that are improperly locked out or inadequately guarded, or even amputations. Many workers can also injure themselves by falling on treacherously slippery floors and can be exposed to extremes of heat and cold.

This chapter is designed to increase employer and employee awareness of these and other workplace hazards and to highlight the ways in which employer and employees can work together to eliminate them. Employers are encouraged to review and strengthen overall safety and health precautions to guard against workplace accidents, injuries, and illnesses.

51.6.1 Potential Hazards

Machineries such as head splitters, bone splitters snout pullers and jaw pullers, as well as band saws and cleavers, pose potential hazards to workers during the various stages of processing animal carcasses. A wide variety of other occupational safety and health hazards exists in the industry. These hazards are identified and discussed in the following paragraphs. Examples are described in Table 51.1.

51.6.2 Knife Cuts

Knives are the major causes of cuts and abrasions to the hands and the torso. Although modern technology has eliminated a number of hand knife operations, the hand knife remains the most commonly used tool and causes the most frequent and severe accidents. For example, one worker used a knife to pick up

TABLE 51.1

Operational Hazards in the Meatpacking Industry

Operation Performed	Equipment Substances	Accidents/Injuries
Stunning	Knocking gun	Severe shock, body punctures
Skinning/Removing front legs	Pincher device	Amputations, eye injuries, cuts, falls
Splitting animal	Splitter saws	Eye injury, carpal tunnel syndrome, amputations, cuts, falls
Removing brain	Head splitter	Cuts, amputations, eye injury, falls
Transporting products	Screw conveyers, screw auger	Fractures, cuts, amputations, falls
Cutting/trimming/boning	Hand knives, saws—circular saw, band saw	Cuts, eye injuries, carpal tunnel syndrome, falls
Removing jaw bone/snout	Jaw bone, snout puller	Amputations, falls
Preparing Bacon for slicing	Bacon/belly press	Amputations, falls
Tenderizing	Electrical meat tenderizers	Severe shock, amputations, cuts, eye injuries
Cleaning equipment	Lock-out, tag-out	Amputations, cuts
Hoisting/shackling	Chain/dolly assembly	Falls, falling carcasses
Wrapping meat	Sealant machine/PVC, meat	Exposure to toxic substances; severe burns to hands/arms, falls
Lugging meat	Carcasses	Severe back/shoulder injuries, falls
Refrigeration/curing cleaning, wrapping	Ammonia, carbon dioxide, carbon monoxide, PVC	Upper-respiratory irritation and damage

Source: Compiled by the OSHA, based on unpublished data from "The Unique Hazards of Packing," by Jeff Spahn, Area Director, OSHA, Wichita, Kansas, 1976.

a ham prior to boning; the knife slipped out of the ham striking him in the eye and blinding him. Another worker was permanently disfigured when his knife slipped out of a piece of meat and struck his nose, upper lip, and chin.

Workers have also been cut by other workers as they remove their knives from a slab of meat. These "neighbor cuts" are usually the direct result of over-crowded working conditions.

51.6.3 Falls

Falls also represent one of the greatest sources of serious injuries. Because of the nature of the work, floor surfaces throughout the plants tend to be wet and slippery. Animal fat, when allowed to accumulate on floors to dangerous levels, and blood, leaking pipes, and poor drainage are the major contributors to treacherously slippery floors.

51.6.4 Back Injuries

These injuries tend to be more common among workers in the shipping department. These employees, called "luggers," are required to lug or carry on their shoulders carcasses (weighing up to 300 pounds) to trucks or railcars for shipment.

51.6.5 Toxic Substances

Workers are often exposed to ammonia. Ammonia is a gas with a characteristic pungent odor and is used as a refrigerant, and occasionally, as a cleaning compound. Leaks can occur in the refrigeration pipes carrying ammonia to coolers. Contact with anhydrous liquid ammonia or with aqueous solution is intensely irritating to the mucous membranes, eyes, and skin. There may be corrosive burns to the skin or blister formation. Ammonia gas is also irritating to the eyes and to moist skin. Mild-to-moderate exposure to the gas can produce headaches, salivation, burning of the throat, perspiration, nausea, and vomiting. Irritation from ammonia gas to the eyes and nose may be sufficiently intense to compel workers to leave the area. If escape is not possible, there may be severe irritation of the respiratory tract with the production of cough, pulmonary edema, or respiratory arrest. Bronchitis or pneumonia may follow a severe exposure.

On some occasions, employees have been exposed to unsafe levels of carbon dioxide from the dry ice used in the packaging process. When meat is ready to be frozen for packaging, it is put into vats where dry ice is stored. During this process, carbon dioxide gas may escape from these vats and spread throughout the room. Breathing high levels of this gas causes headaches, dizziness, nausea, vomiting, and even death.

Workers are also exposed to carbon monoxide. Carbon monoxide is a colorless, odorless gas that is undetectable by the unaided senses and is often mixed with other gases. Workers are exposed to this gas when smokehouses are improperly ventilated. Overexposed workers may experience headaches, dizziness, drowsiness, nausea, vomiting, and death. Carbon monoxide also aggravates other conditions, particularly heart disease and respiratory problems.

Workers are also exposed to the thermal degradation products of polyvinyl chloride (PVC) food-wrap film. PVC film used for wrapping meat is cut on a hot wire, wrapped around the package of meat, and sealed by the use of a heated pad. When the PVC film is heated, thermal degradation products irritate workers' eyes, nose, and throat or cause more serious problems such as wheezing, chest pains, coughing, difficulty in breathing, nausea, muscle pains, chills, and fever.

51.6.6 Cumulative Trauma Disorders

Cumulative trauma disorders are widespread among workers in the meatpacking industry. Cumulative trauma disorders such as tendonitis (inflammation of a tendon sheath), and carpal tunnel syndrome are very serious diseases that often afflict workers whose jobs require repetitive hand movement and exertion.

Carpal tunnel syndrome is the disorder most commonly reported for this industry and is caused by repeated bending of the wrist combined with gripping, squeezing, and twisting motions. A swelling in the wrist joint causes pressure on a nerve in the wrist. Early symptoms of the disease are tingling

sensations in the thumbs and in the index and middle fingers. Experience has shown that if workers ignore these symptoms, sometimes misdiagnosed as arthritis, they could experience permanent weakness and numbness in the hand coupled with severe pain in the hands, elbows, and shoulders.

51.6.7 Infectious Diseases

Workers are also susceptible to infectious diseases such as brucellosis, erysipeloid, leptospirosis, dermatophytoses, and warts. Brucellosis is caused by a bacterium and is transmitted by the handling of cattle or swine. Persons who suffer from this bacterium experience constant or recurring fever, headaches, weakness, joint pain, night sweats, and loss of appetite.

Erysipeioia and leptospirosis are also caused by bacteria. Erysipeloid is transmitted by infection of skin puncture wounds, scratches and abrasions; it causes redness and irritation around the site of infection and can spread to the blood stream and lymph nodes. Leptospirosis is transmitted through direct contact with infected animals or through water, moist soil, or vegetation contaminated by the urine of infected animals. Muscular aches, eye infections, fever, vomiting, chills, and headaches occur, and kidney and liver damage may develop.

Dermatophytosis, or the other hand, is a fungal disease and is transmitted by contact with the hair and skin of infected persons and animals. Dermatophytosis, also known as ringworm, causes the hair to fall out and small yellowish cuplike crusts to develop on the scalp.

Verruca vulgaris, a wart caused by a virus, can be spread by infectious workers who have contaminated towels, meat, fish knives, work tables, or other objects.

51.6.8 Protective Measures

The unique safety and health hazards found in this industry can be minimized or eliminated with the proper use of control methods. A preferred way of controlling potential hazards is through the use of engineering controls. Engineering controls are methods that prevent harmful worker exposure through proper design of equipment and processes. Various controls are briefly described in the following paragraphs.

51.6.9 Guardrails

The use of guardrails can protect workers from accidental falls. Open-surface dip tanks, used for sterilizing shackling equipment, and elevated work platforms must have guardrails. Railings should also be checked to see that they are securely attached to walls.

51.6.10 Floors

Employers should install a nonskid flooring material or rubberized cushioned floor mats at all work stations for workers to stand on, especially in areas where hand knives and power tools are used.

51.6.11 Wiring

All electrical wiring should be checked periodically for cracking, fraying, or other defects, and all electrical equipment should be grounded.

51.6.12 Equipment and Machine Guarding

Equipment used to hold and move meat and items such as shackles, conveyors, and hooks should be checked frequently and repaired. Equipment that poses a hazardous energy source should, when not in use, be subject to lock-out and tag-out procedures. This assures that workers inspecting or maintaining equipment are not injured by start-up of the equipment. All equipment that poses a hazard should be guarded.

51.6.13 Local Exhaust Ventilation

A preferred control method for removing air contaminants from the workplace is local exhaust ventilation. This control is located at the source of the generation of contaminants, and captures, rather than dilutes, the hazardous substances before they escape into the workplace environment.

51.6.14 General Ventilation

General, or dilution, ventilation systems are also recommended because they add or remove air from the workplace to keep the concentration of air contaminants below hazardous levels. General ventilation consists of air flow through open windows or doors, fans, and roof ventilators. General ventilation control only dilutes air contaminants, unlike local exhaust ventilation which removes air contaminants. When using general ventilation systems, care should be taken not to recirculate the air contaminants throughout the workplace.

51.6.15 Administrative Controls

An employer also might decide to use administrative controls to minimize the risk of carpal tunnel syndrome, back and shoulder injuries, and exposure to toxic substances. One type of administrative control would be to reduce employee work periods in which excessive repetitive wrist bending is necessary or when the worker is exposed to hazardous substances.

51.6.16 Work Practices

Safe work practices are essential in helping to maintain a safe and healthful work environment. Workers must therefore be encouraged and be given sufficient time and equipment to keep surface clean and orderly.

To do this, spills must be cleaned up immediately. Water, blood, or grease on floors will cause falls. Also, wet-working conditions pose a serious threat of electrocution. Periods during the day should also be set aside for general housekeeping, and constant surveillance should be kept to spot slippery areas. Nonskid floor mats can also be used successfully in potentially dangerous areas. Knives left carelessly in sinks or on counters can cause serious accidents. Knives should be kept sharpened at all times. Dull knives can cause serious safety hazards and worker fatigue. Equipment such as the band saw and the bacon press must be cleaned with the power off and locked out, and tagged out. Workers should use only tools and equipment with which they are familiar. Moreover, employers should check refrigeration systems regularly for leaks and should make sure that hazardous substances, such as ammonia, are identified by appropriate hazard warnings (labels, signs, etc.).

Employers should make handwashing facilities readily available to employees working with or near toxic substances. It is equally important that handwashing facilities be made available for workers who handle meat without the use of protective gloves. Prompt handwashing and the use of disposable hand towels will help in preventing the spread of infectious diseases.

51.6.17 Protective Clothing and Equipment

Since slippery floors are a major cause of falls, protective clothing such as safety shoes or boots with toe guards and slip-resistant soles must be worn by workers. To help reduce the spread of infectious diseases, protective gloves should be worn when workers handle meat. Workers who use cleaning compounds must also wear protective gloves to prevent chemical burns. In addition, workers who use knives must be provided with metal mesh gloves and aprons, and wrist and forearm guards to protect them from knife cuts.

Workers performing hoisting and shackling operations should be protected with safety helmets that meet the specifications of American National Standard Requirements for Protective Head Wear for Industrial Workers as well as a barricade or shield assembly. These safeguards can prevent injury from

falling or moving animals and/or materials. In addition, removing the worker from the immediate area during hoisting operations is recommended.

The employer must furnish employees with proper personal protective equipment required for his or her specific work operation and exposure. For example, in the event of exposure to toxic chemicals, a worker must be provided with a suitable respirator to prevent inhalation of harmful substances.

In addition, adjustable work stands should be made available to accommodate for worker height to minimize the possibility of back strain.

Machines and equipment found in meatpacking plants produce a high level of noise; in such circumstances, workers must be provided with ear plugs. The employer may be required to provide workers with face shields or goggles when workers mix or handle cleaners. The use of this equipment will prevent chemical burns to the face and eyes. Goggles may also be required during the boning, trimming, and cutting operations to prevent foreign objects from entering the workers' eyes.

51.6.18 Related Issues

51.6.18.1 Training and Education

BLS studies show that many workers are injured because they often do not receive the safety training they need, even on jobs involving dangerous equipment where training is clearly essential. These studies also show that younger, and especially new, employees are most at risk because they are not taught the necessary safety measures before they begin work. More experienced workers, on the other hand, are injured because the task becomes routine, and they are not as cautious as they might be otherwise.

It is essential, therefore, that employers develop, implement, and maintain at the workplace a written comprehensive training program for all employees. A comprehensive, well-organized training program helps the employer to educate workers in safe work practices and techniques, and helps demonstrate the employer's concern for, and commitment to, safe work practices.

The training program should inform workers about safety and health hazards and their prevention, the proper use and maintenance of equipment, any appropriate work practices, a medical surveillance program, and emergency situations.

Once the employer has developed and implemented the safety and health program, he should choose a person who is committed to workplace safety and health to manage the program. This individual should have time to devote to developing and managing the program and must be willing to take on the responsibility and the accountability that goes with operating an effective program.

The employer should also make all employees familiar with their surroundings and work environment. Furthermore, employers should train workers annually in their work tasks or in new job assignments that expose them to new hazards. New employees should be trained at the time of initial assignment, and annually thereafter.

More training is needed when new equipment, materials, or processes are introduced, when procedures are updated or revised, or when employee performance is inadequate.

51.6.18.2 First-Aid Training

At least one person on each shift should be trained and certified in first aid. First-aid training should include, as a minimum, completion of an approved first-aid training course. Moreover, proper first-aid supplies must be readily available for emergency use. Prearranged ambulance services should also be available for any emergency.

51.6.19 Emergency Response

Proper planning for emergencies is necessary to minimize the employee injury. It is important, therefore, that employers in the meat industry develop and implement a written emergency action plan. The plan should include elements such as (1) emergency escape procedures and emergency escape route assignments, (2) procedures for employees to follow who remain to perform (or shut down) critical plant

operations prior to evacuation, (3) procedures to account for all employees after emergency evacuation has been completed, (4) assignment of rescue and medical duties to those employees who are to perform them and the preferred means for reporting emergencies, and (5) names and regular job titles of persons or departments to be contacted for further information or explanation of duties under the plan. Emergency phone numbers should also be posted in a conspicuous place near or on telephones.

51.6.20 Hazard Communication

OSHA's Hazard Communication Standard requires the employer to establish a written hazard communication program to transmit information on the chemical hazards to which employees are exposed. The program must include container labeling and other forms of warning and must provide exposed workers with material safety data sheets (MSDS's). The MSDS details the substance's properties and the nature of the hazard. The standard further requires chemical hazard training for exposed workers.

In addition, workers who handle only sealed containers of chemicals are required to keep labels affixed to incoming containers, are to be provided access to MSDS's while the material is in the workplace, and are to be trained on how to protect themselves against hazards.

51.6.21 Access to Exposure and Medical Records

In 1988, OSHA issued a standard that requires employers to provide employees with information to assist in the management of their own safety and health. The standard, "Access to Employee Exposure and Medical Records," permits direct access by employees or their designated representatives and by OSHA to employer-maintained exposure and medical records. This access is designed to yield both direct and indirect improvements in the detection, treatment, and prevention of occupational disease. Access to these records should result in a decreased incidence of occupational exposure and should aid in designing and implementing new control measures.

51.6.22 Record Keeping

OSHA requires employers with 11 or more employees to prepare and maintain pertinent injury and illness records of accidents affecting their employees. Moreover, all employers are required to report to the nearest OSHA office, within 48 h, all accidents resulting in a work-related death or in five or more hospitalizations. The report may be either oral or written.

The employer is also required to maintain occupational injury and illness records at each workplace. Records must be retained for five calendar years following the end of the year to which they relate and may be inspected and copied at any reasonable time by authorized Federal or State government representatives.

These records are important to the employer in analyzing the effectiveness of safety and health programs. They are also important to OSHA inspectors in deciding whether to conduct a complete workplace inspection and, if so, where to concentrate their attention.

51.6.23 Employer and Employee Responsibilities

An employer's commitment to a safe and healthful environment is essential in the reduction of workplace injury and illness. This commitment can be demonstrated through personal concern for employee safety and health, by the priority placed on safety and health issues, and by setting good examples for workplace safety and health. Employers should also take any necessary corrective action after an inspection or accident. They should assure that appropriate channels of communication exist between to allow information and feedback on safety and health concerns and performance. In addition, regular self-inspections of the workplace will further help prevent hazards by assuring that established safe work practices are being followed and that unsafe conditions or procedures are identified and corrected properly. These inspections are in addition to the everyday safety and health checks that are part of the routine duties of supervisors.

Since workers are also accountable for safety and health, it is extremely important that they too have a strong commitment to workplace safety and health. Workers should immediately inform their supervisor

or their employer of any hazards that exist in the workplace and of the conditions, equipment, and procedures that would be potentially hazardous. Workers should also understand what the safety and health program is all about, why it is important to them, and how it affects their work.

Finally, employers who want help in recognizing and correcting safety and health hazards and in improving their safety and health programs can receive assistance from a free consultation service largely funded by the Occupational Safety and Health administration. The service is delivered by State governments using well-trained professional staff. The service offers advice and help in correcting problems and in maintaining continued effective protection. In addition to employers identify and correct specific hazards, consultants provide guidance in establishing or improving an effective safety and health program and offer training and education for the company, the supervisors, and the employees. Such consultation is a cooperative approach to solving safety and health problems in the workplace. As a voluntary activity, it is neither automatic nor expected, it must be requested.

Appendix I: A Discussion of Stunned and Nonstunned Slaughter[*]

Joe M. Regenstein

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Purpose: This document critically evaluates what we know and what we do not know when comparing diverse religious and secular slaughter practices. We provide a short, consumer-friendly discussion without references of some of the issues that arise with respect to evaluating different methods of slaughter of mammals, specifically kosher, halal, and secular approaches. We suggest where future efforts ought to be focused, both from a practical public policy concern and from a scientific research point of view. The purpose is to recognize that many methods of slaughter are justified on cultural, secular, and religious grounds, but that all methods should be evaluated by rigorous, comparable scientific criteria. Under the broad umbrellas of religious and secular methods, there are many practices. Only the best practices of any method, understood as the most humane both to animals and to workers, should be encouraged.

Authors' Backgrounds: the authors are all concerned with improving animal welfare and slaughter industry practices, with respect to both meat animals and workers. They have been active in that area for many years. As a group, the authors are trained both scientifically and religiously in matters related to animal slaughter.

Prepared by an International Group of Scientists and Religious Leaders.[†]

* An earlier copy of this appendix appears on the American Meat Institute's web site (http://www.meatami.com) as part of the 2010 Animal Welfare Conference held in Kansas City, MO, March 26, 2010.

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I.1 Introduction

Food is an increasingly contested public issue. Environmental, economic, and social impacts force us to question how best to produce, distribute, and consume food. Our choices in developed countries are shaped only loosely by biological necessity, but rather by cultural histories, public policies, religious law, and a seemingly endless array of personal preferences. That we may all eat well and thrive is a goal of any universal ethical food system, religious or secular.

One of the most contested issues is how animals are slaughtered for food. The issue is not always based on rational ideas, nor must it be. We need not agree about how we justify different forms of religious or secular slaughter, but we should be able to agree on how we study, assess, and compare those forms. The different ways in which we take the life of animals embodies the ways in which we respect life. Whether we are vegetarians or meat eaters, we eat death; we eat that which has lived and died by our hand or failed to grow anew, again, by our hand. With the slaughter of animals, we actively take the life of a living animal and use its flesh to meet our needs for nourishment.

Our purpose in this discussion is to promote more humane slaughter, a slaughter that results in better treatment of both animals and the human workers involved in the process, as evidenced by a reduction in pain, suffering, and the frequency of mistakes. We accept the idea that many people will continue to include animals as part of what they "need" for a diet of "good food." To achieve good food for all, both the animals and workers deserve to benefit from high standards, as they provide this good food for consumers.

I.2 Background: Discomfort Shapes Our Thinking about Animal Slaughter

Many folks really do not want to think about the topic of the slaughter of animals. Yet, we are not concerned about the death of plants or the insects and mammals that die in their harvest. This is partially due to the known sentience of food animals, and our distance from these other deaths. We are often good at segmenting our lives and roles so that we do not have to consider this issue, especially as we eat.

In modern societies, segmentation is so thorough that the reality of slaughter is largely invisible to the average consumer. Where ignorance prevails and there is limited outside concern and oversight, standards for humane slaughter are likely to be lax and poorly enforced. In response to bad practices, righteous reactions to brutality are more likely to fuel attacks against animal slaughter rather than focusing on the development of more humane practices, precisely because we may not have a good idea of what is possible or desirable; thus, wasting an opportunity to promote positive change.

The issue of slaughter evokes our personal reactions about killing. At our emotional core, the practices of killing animals for food produces a primal response that is different only in degree to our reactions to the killing of human beings. All of these animal slaughter methods—stunning or killing with a bolt, clubbing or electrical jolt, gassing to unconsciousness or death by severing the arteries and veins in the neck to bleed to death—produce a visceral response in many of us precisely because they evoke a horror that touches us. These various methods have at one time or another been used as a cruel punishment for humans, in legal executions and for the mass murder of humans. Each method evokes feelings of brutality, barbarism, or genocide to someone or some group. Yet each method also evokes for different groups feelings of compassion and respect for the efforts they represent to minimize the inevitable pain of death.

To address animal slaughter, we must also address the emotions it provokes in human beings as well as understanding the techniques and science by which each method accomplishes its goal and how each method affects the reactions of different animals under different circumstances. Further, as human beings, what we need to sort through is the degree to which there should be a leveling reaction of our

human emotions, that is, to establish clear justification for what we can and will do to animals raised for food that we should not do, and will not be permitted to be done, to human beings. We find these justifications in the teachings of the world's historic religions and in secular public policies. We find science useful in assessing and comparing these diverse justifications to the extent that the issues underlying the teachings are scientific and technical, and evaluated in a holistic manner.

There is no consensus, nor is there ever likely to be one, on what is the best method for treating animals well under varying circumstances. We need to understand and value a range of different approaches to slaughter, including religious ones and secular ones. The diversity within both the secular and religious contexts makes writing this document tricky. Standards for evidence and argument vary depending upon the degree of religious observance and the specific religious body within Judaism, Islam, and Christianity. Atheists and agnostics may not find theistic argumentation justifiable, but they also have a wide array of standards for evidence in those traditions.

In general, Christians do not place much emphasis on food laws, but there are notable exceptions such as where vegetarianism holds, where the Jewish law is loosely observed, and even one Pauline scriptural endorsement of kosher meat over other forms of meat. And there are self-identified Jews who do not keep kosher and Muslims who do not observe halal, but the purpose of this document is to address the larger groups who may not have any religious food laws, or who may be observant to varying degrees.

We do not anticipate a least common denominator basis for offering evidence or argumentation. It is our failure as a community to find such a consensus that occasions this work. Our practices and public policies need to recognize that there are many ways to do what is moral and humane. We also need to recognize that there is a great deal of unjustifiable, inconsistent and even poor practices within well established religious or secular approaches to slaughter, and even within specific slaughter facilities. The purpose of this appendix is to recognize the need for principled differences regarding the justification of diverse forms of slaughter practices, while raising the bar for more humane treatment for all slaughter practices.

I.3 The Relationship of Stunning to Death

Stunning is the act of rendering an animal unconscious, or insensible. The latter term, although generally synonymous with unconscious, puts the focus on ways to measure the state of the animal at the time. Historically, stunning has been adopted by Western nations because of a widely held assumption that stunning makes for a more humane death. It is believed that rendering an animal unconscious or insensible prior to death makes the experience of death less stressful and painful to the animal. There is, however, no peer reviewed and repeated research to support this claim or to support the counterclaim that rapid exsanguination without stunning produces the same effect as an intentional stun. Stunning has been done prior to exsanguination with death coming from cutting the major blood vessels. However, from an animal welfare point of view, insensibility is the critical factor.

Stunning methods may also produce death on their own, depending upon the specific force, duration, or intensity of the treatment. Some advocates in Western nations, without sound research regarding the actual impact on the animal, have promoted using stunning techniques to cause death. As a consequence, some believe that with the heart not beating the process of exsanguination is neither as quick nor as thorough. Again, this has not been properly tested. But, from a religious point of view, the practice of stunning is not accepted by devout Muslims and Jews. Although some Muslims and Jews will say that their method leads to more blood being removed, that is actually not part of either religious requirement.

There are four major formats that are used in Western countries for stunning and/or death.

- The *first* method is a blow to the head. This is normally done in modern abattoirs (slaughterhouses) using a captive bolt gun that may either penetrate the skull or not. In both cases, the skull is fractured. Thus, the animal may be unable to recover from this procedure even if it is done poorly and does not lead to instant insensibility, and can lead to up to 2.5 minutes of massive brain activity. (Is this perhaps an indication of a potential aversiveness of this method?)

- The *second* form of stunning in common use is electrical stunning—this also potentially painful method can range from a very mild stun that is sufficient to cause insensibility (sometimes used for Muslim halal slaughter for those Muslim communities where stunning is accepted) to the electrical stunning normally used in North America to the European stunning, with higher voltages and amperages, that may kill as many as 30% of the animals according to Dr. Grandin (T. Grandin, pers comm.) and may be aversive for a short time period during the procedure. In fact, some European animal welfare organizations specifically advocate a strong enough stun to cause death.
- The *third* method is the use of various gas mixtures, usually incorporating carbon dioxide and/or one or more of the inert gases such as nitrogen or argon. The idea is to deplete the oxygen necessary to function. Animals are dealt with in batches, and because different animals within a single batch of animals may react differently, it is not clear how aversive any one mixture is to each specific animal. However, the UN's international animal health organization (OIE) indicates in their Guidelines that CO_2 is aversive and does not lead to instant insensibility.
- The *fourth* method, which has become synonymous with the religious slaughter endorsed by traditional Muslim and Jewish practices, requires a quick and deep horizontal cut across the neck of an animal that has not been otherwise stunned, cutting the arteries and veins, as well as the trachea and esophagus, but not severing the spine. The continued functioning of the nervous system permits the animal's heart to beat and to pump blood out. If the cut is done well and aggressively with time provided for the initial blood loss, then the rapid loss of blood leads to rapid unconsciousness and subsequent rapid loss of sensibility. This *per se* can constitute a stun (unconsciousness) followed by death (Rosen 2004).

To stun and kill any animal well, using any of the four methods described above, requires attention to four prerequisite conditions: (1) having a relatively calm animal, (2) having a skillful and focused slaughterer, (3) having a physical facility that is designed and maintained to reduce agitation in both the animals and the staff, and (4) having a commitment by management to the continuous application of these standards for animal handling and for labor practices that leads to immediate identification and correction of problems before they create an inhumane environment.

Historically, these conditions may have been met by those who raised and slaughtered their own livestock, whose compassion for the animal and themselves may have made them gentle, quick, and willing to step back when the animal, the setting, or the slaughterer was not ready. More often than not, slaughter would be with a knife to the neck, often of an unstunned animal. In a similar fashion, a local slaughterer would know the animal indirectly by virtue of the care received from the one who raised it, who was often present and would know how to treat the animal well.

But there is no room for nostalgia or a saccharin view of the past. It is just as easy, for those of us who have witnessed slaughter, to remember those who were made uncomfortable and unable to kill what they had raised, so much so that they botched the act, causing pain and suffering to the animal, themselves and to everyone involved. Or in their lack of skill, or proper equipment or ambivalence failed to have the necessary sharp knife or confined space to restrain the animal. Or who worked with too much haste, not really paying attention. We can also remember those who beat, or relentlessly stunned, their livestock, and who may then wonder why their animals were not as good meat producers or compliant as other people's livestock. And we can remember slaughterers whose brutality was only held in control by their need for a paycheck. Still, not everything depends upon human agency. The temperament of an animal varies every bit as much as a person or a physical setting, making each slaughter unique.

In more modern times, and even earlier with the advent of large cities, the restraining judgment of family and neighbors looking on during slaughter has given way to slaughterhouses that are intentionally located largely outside of the public eye (except for any limited government or religious oversight), that employ persons who will not see the animal until they slaughter it and who will not know its origins. Those who do the slaughter may not share any attributes of culture, religion, economic class, or status with those who produced or consume these animal products. All of these unknowns have made it more difficult, but not impossible, to handle well the stunning and killing of an animal.

Fortunately, there is growing interest among religious bodies, consumer groups, and large corporate purchasers to establish more humane slaughterhouse practices, enforceable public policies, and effective monitoring strategies. Yet what makes the slaughter process particularly difficult to assess is the relative absence of high-quality scientific research into each of the component processes associated with stunning and death. We need such research to evaluate stunning and its impact on unconsciousness, insensibility, and death for all four types of slaughter and to identify superior practices within each type of slaughter format.

The question is also more than a matter of scientific evidence. Diverse religious and secular, consumer, and business groups have different and often competing interpretations of what constitutes a good, humane slaughter practice. Further, the force of public policy when setting slaughter standards has often been wielded as a tool of coercion by secular or religious majorities to the exclusion of the rights of minority populations. What we have here are competing governance interests that function simultaneously, none of which will disappear or be thoroughly intimidated by the other. The larger question is whether or not there are times when some secular approaches ought to trump some religious approaches, or when some religious approaches ought to trump some secular or other religious approaches. These are only serious questions in settings where there is sufficient political acceptance of diversity to encourage groups to pursue and justify different sorts of lives. For the purpose of this appendix, we are limiting ourselves to the tensions between secular slaughter and religious slaughter (noting that there are varied kosher and halal practices). How can the separation of church and state operate when both have profound and different interests in the slaughter of mammals? We live in a world where multiple standards of accountability and justification of practice are at play.

We begin with a description of these interests, and note the inevitable need for multiple standards. We then turn to address the specific scientific questions, both across and within standard formats that will require further research.

I.4 Identify Requirements for Diverse Standards for Slaughter

What if meat industry standards were set by true consumer demand (i.e., the price consumers are willing to pay for a product produced and processed in a specific manner)? This would have several negative consequences. The absence of both secular and religious interests in a safe, high-quality meat supply could lead to more food and health crises. It would become more difficult to identify or promote more humane slaughter standards, either secular or religious, when final sale price alone might be the major determining factor for a large segment of the market.

Finally, markets in which all meat was identified with symbols representing the type of slaughter and/or religious endorsements might create an easy target for a prejudicial backlash against minority slaughter methods and the practices of religious groups. This is especially a concern for consumers of religiously slaughtered meats where, for example, European animal rights activists have been more successful in deriding religious slaughter with an unsubstantiated claim that it is inherently more inhumane than stunned slaughter. As a consequence, religious slaughter has been used as a wedge issue in a larger effort to altogether eliminate animal slaughter for food.

We must identify and clarify the diverse standards for slaughter if we are to successfully address the health and safety of the meat food system, the promotion of more rigorous standards of animal welfare and worker welfare and the accommodation of differences in moral and religious judgment.

I.4.1 The Concerns of Jewish and Muslim Consumers for Religious Slaughter

For people of the Jewish and Muslim faiths who wish to eat meat, the method of slaughtering animals is an important component of their religious belief systems—how the slaughter is done determines whether they are allowed to consume the resulting meat or not. This relationship between slaughter and the ability to eat meat is absolute, regardless of the secular laws or policies of the nation where they live, or the common practices of nearby peoples of other religious or cultural traditions.

Kosher and halal food practices form the basis for much of the religious regulation applied to foods in the United States, the Middle East, Africa, and Asia and increasingly, in the rest of the world. Auditing

and certifying that food production has followed these religious laws is highly developed within Judaism and increasingly so within Islam. While Jews are only 0.2% of the world's population and Muslims 19.6%, their food practices affect food products and food labels around the globe, setting a standard for food product certification processes that may be emulated by other religious and secular groups.

Religious standards are simply a fact of life in the worldwide food industry. When one adds the numbers of adherents of religions that esteem vegetarian or vegan practices, between a third and a half of the world's population honor, at least in part, religious governance practices with respect to food. Many religious laws were actually easier to carry out or were trivial when Jewish or Muslim communities lived in isolation from others. Nowadays, the food industry, in trying to serve these communities' needs, must deal with issues of operating in ways that are acceptable to multiple religious groups, which often present some interesting practical challenges.

The kosher and halal laws have a substantial overlap concerning the details of the actual act of slaughter, including the method of slaughter by severing of the neck except for the spinal cord, the establishment of permitted animals, and the prohibition of using blood as food or a food ingredient.

Serious adherents of both religions believe that an animal may not be otherwise stunned prior to slaughter. In the Jewish community, all forms of stunning have been disqualified by the recognized authorities in Jewish religious law. Although in the past a few rabbis attempted to promote other views, these views have been rejected by those whose rulings are taken as authoritative by the majority of Orthodox Jews.

Within the Muslim community, some sectors of this 1.5 billion member group will accept stunning (sometimes based on both scientific and religious misinformation) and a few within the community even advocate stunning, although the need to establish that the animal is alive at the actual time of slaughter complicates the process. However, it is important as we open this discussion to recognize that there is significant disagreement on this issue and we hope to respectfully discuss key issues reflective of both stunned and nonstunned slaughter.

Unfortunately, some governments and other groups have jumped to the conclusion that without stunning, animals suffer more than with stunning. This conclusion is not supported by well done scientific research. Because good animal welfare is a fundamental requirement of both of these faiths, people from both of these communities find a requirement for stunning difficult to accept, especially when properly done scientific evidence does not support such an interpretation. A key failure of the scientific literature has been to fail to separate the factors related to the preparation of the animal for slaughter from the actual impact of the slaughter itself when done properly, that is, to assure that good equipment, good animal handling, and properly trained personnel are used before addressing the impact of the slaughter itself. In many cases, the basic scientific necessity of having a sufficiently detailed description of the process being studied in a particular scientific report has not been met, that is, it is impossible to even begin the process of duplicating the results. This unfortunately seems to reflect a bias on the part of the scientific journals to accept articles that follow their preconceived assumptions and to not challenge the work sufficiently to insure proper peer review.

With respect to kosher mammals, only animals with a full split hoof and which also chew their cud are permitted, that is, all of the allowed mammals are ruminants. Thus, the focus is on the major red meat animals, for example, cattle, sheep, goats, buffalo/bison, and deer. This is a fairly small category of animals, making the choice of meats somewhat restrictive. Muslims have a wider range of acceptable animals, but eliminate all of the carnivorous animals and specifically ban the pig. Practically, the most important ban because of its importance in commercial trade is that for the pig—for Jews because it is not a ruminant and for Muslims because it is Quoranically defined as unclean (Najis).

Both religions view blood as the "life" fluid; it is not to be consumed. Slaughter must be done in such a way that the animal bleeds out and dies in a humane manner. Taking the life of an animal is a privilege that G-d has given to humans with some restrictions (e.g., "Thou may take of thy herd and they flock as I have commanded thee," *Deuteronomy* 12:21). Slaughter needs to be done with respect for the animal and with technical proficiency. Jews assign this important task to specially trained religious male slaughter men. Muslims permit halal slaughter by a knowledgeable and practicing Muslim of sound mind who is aware of the responsibility associated with slaughter. Common Muslim traditions expect every qualified adult to slaughter at least one animal a year.

Both require a prayer before slaughter, that is, the Jewish slaughter man says the prayer following a format that is used for all blessings in Judaism just as he begins the slaughter. The Muslim says a prayer, referring to the greatness of G-d over each animal at the actual time of slaughter.

For Jews, the rules regarding permitted animals are mainly an issue of their food use. The use of slaughtered animal products for purposes other than food is not an issue. So a leather belt, even from either a nonkosher cow or even from a pig presents no problems. On the other hand, for many Muslims, the strong prohibitions against the pig in the Quoran have led Muslims to prefer an almost total avoidance of the pig products in all shapes and forms. This can be a challenge for a multicultural society, where some parts of society, most notably Christians and secularists, have come to use the pig as an important part of their food culture. (The use of leather products and goods, such as shoes, belts, seats, hand bags and shoes, even from potentially "halal" animals has become a topic of question as to whether they were sources from animals that had been slaughtered halal.)

I.4.2 Christian Food Traditions Are at Odds with Islam and Judaism

In general, Christianity has very little formal concern for religious food laws. While maintaining a core of teachings promoting hospitality around food, feeding the poor, and condemning gluttony, much of the rhetoric of Christianity proclaims freedom from any religious law such as the food laws as a spiritual and religious ideal. Obedience to Jesus Christ makes a Christian's belief in God a matter of Christianity. The early church community replaced the law with obedience to God through Jesus Christ. The assumption was that obedience to the law was a matter of legalistic personal vanity, rather than personal discipline that reflects a mature respect for the Supreme Being. Many Christians clearly do not understand the role of religious law in Judaism or Islam, and neither is the Christian spiritual discipline well understood by most Jews or Muslims.

In the case of both Judaism and Islam, constant attention to what enters the mouth throughout the day is an on-going part of life and serves to constantly remind adherents of their relationship to G-d. Participants need to constantly strive to meet the religious strictures that are subject to divine evaluation on the Day of Judgment. By way of contrast, because of the early Christian conflicts over authority with Judaism, the moral status of a Christian with respect to the Supreme Being is more dependent upon what emanates from G-d's grace, and from an individual's actions or an absence of actions within one's community, and that community's response, than what enters an individual's body.

Many scholars believe that one of the ways that Christians differentiated themselves from Jews and Muslims from the Middle Ages forward was by making central to the Christian diets foods that Jews and Muslims would have found abhorrent. The eating of pork and blood sausage are two prime examples. On the other hand, Christians and others benefited from the sale of animal products that initially were processed as kosher but were subsequently identified as not acceptable with respect to the postslaughter religious inspection.

Within Christianity, veneration of blood, as in the blood of Christ, which is remembered symbolically in the Communion service (The blood of Christ shed for you; The body of Christ broken for you) and literally in the Eucharist is an act that is experienced as extremely odd and disturbing to Muslims and Jews (as well as to many secularist and less devout Christians). A more neutral, and dare say, "sanguine" approach to these differences may be the following: to hold something as sacred produces a strong reaction. In Judaism and Islam, the sacredness of blood results in it being kept away from and outside everyday human life. In Christianity, there is an equally strong reaction that runs in the exact opposite direction. Blood, symbolized in either the Eucharist or Communion, becomes central to daily human life and the concept of being in a community with one another. The central issue for Christians is not that food products should be made from blood, such as blood sausage, but rather that the notion of wholeness and community is sanctified by blood. No manner of scientific or empirical research can shake these determinations, because they are rooted in strong and well-defined meanings that are different in the different groups.

While profound prejudice runs in both directions, Christian majorities and monarchs, and their governments up through the twentieth century have instituted laws to limit Jewish and Muslim practices. Violence toward both Muslims and Jews persists to this day among some fringes of Christianity. But the most virulent anti-Semitism and Islamophobia may be perpetuated by agnostics and atheists of countries

that formerly had a Christian majority. But in a more pervasive and perhaps benign way, many Christians simply do not understand the spiritual devotion of Jews and Muslims that produces the revulsion for the idea of eating pork or handling blood.

When slaughter was done locally, in many cases the Christian attitudes toward slaughter were historically closer to Jewish and Muslim practice, that is, stunning was not the normal practice. Anecdotally, Jewish and Muslim slaughter practices on farms used many of the same basic techniques as Christians until the late-nineteenth or early twentieth centuries. What has happened in Christian majority nations, such as the United States, and formerly Christian majority nations such as many in Western Europe, is that there has been an effort to make slaughter less intimate and more a matter of establishing a presumed superior, more humane technology than occurs by slaughter with a knife. This process of slaughter becoming less and less visible coincided with urbanization and the removal of most citizens from closeness to the growing and slaughtering of animals, increasing affluence and a tendency to approach death, in general, in a technocratic way.

I.4.3 The Challenge of Slaughter Practices for Public Policy in a Secular Government

Religious slaughter has been an issue of contention in Western Nations for over a century. Most Western nations explicitly endorse the practice of first stunning the animal prior to slaughter, and have often entertained bans on religious slaughter. The policies are based on an assumption that stunning is more humane than a religious slaughter. The relevant scientific research remains to be done; the policy is not supported by sound evidence as discussed earlier.

Because slaughter is largely a nonissue for Christians and to the best of our knowledge, there are no Christian movements that have ever condemned religious slaughter on religious grounds, that is, based on theology, it is not clear whether the bans initiated by heavily secular governments reflect actions that are primarily antireligious in nature or are more narrowly anti-Semitic and Islamophobic. The most virulent anti-Semitic and Islamophobic actions, both anti-Jewish and anti-Muslim policies, have been unleashed by avowedly secular governments in nations with majority secular populations, from the early mid-twentieth century to the present.

Clearly, since the relevant research has not been done, favoring one method over another would seem to violate the notions surrounding sound policy-making in a democracy. And even if there are clearly better methods than others, within a democracy it is quite imaginable that different standards might obtain to meet the fundamentally different needs of different communities as long as each worked to optimize the animal welfare aspects of their requirements.

Yet, a third concern exists which promises to reconcile the prior two. The formal governmental interest is in setting universal, national standards. Yet there will always be a governance role for religious or cultural communities or groups of consumers that band together to meet their own needs. The government has a serious interest in the quality of the meat supply and in promoting the humane treatment of animals, as well as of slaughterhouse workers. If a roughly equal level of humane treatment of animals and workers can be obtained by different methods, then the government ought to be indifferent to which method is used, as long as the standards are maintained and each standard for each method is clearly researched and documented to show that animal welfare has been optimized.

I.5 Focus on Research-Based Standards, Regardless of Slaughter Method

A key component of good animal welfare for humane slaughter is to present an animal that is not stressed at the time of slaughter. This takes a lot of effort, starting with the animal husbandry of the animal from birth through to the time it is ready to be taken to slaughter, the transport, holding and moving of the animal to slaughter, and then the actual set up for moving and handling animals in the slaughterhouse. To do this well requires an on-going commitment of management, continual training and monitoring of workers, and the proper selection and maintenance of equipment and facilities.

I.5.1 Compassion, Stress-Reduction and Animal Welfare Prior to Slaughter

When an animal is not comfortable, it will respond both behaviorally and with changes in its biochemistry. These can be measured, but the interpretations of these measurements are not always clear. Obviously, stress is a part of life for all humans and animals, and the question of what constitutes unacceptable stress remains to be more carefully defined. Although this is only part of the answer, it is important to note that in most cases, production decreases with obvious stressors, for example, too cold, too hot, bad air quality, and so on, so that in many cases animals can indicate their stress by decreases in production traits, which from a farmer's point of view means that they usually do not want to see such stresses. In some cases, however, economic benefits may outweigh the stress-induced production losses for the individual animals. For these situations, it is necessary that everyone involved works together to eliminate these situations by creating appropriate industry standards that require all producers to avoid such situations. In most cases, thankfully, good animal welfare is actually more productive.

Preslaughter handling of animals is common to *all forms of slaughter*, so that deficiencies in preslaughter animal welfare are common to *all forms of slaughter*, both stunned and nonstunned. The British Farm Animal Welfare Council has stated the principle that the greater the number of animals affected, the greater the extent to which animal welfare is compromised—that is, each offense is treated by them as essentially additive and a separate "charge" is leveled against the perpetrators for each infraction. Because preslaughter handling affects almost all food production animals, it is all the more important that animal welfare during preslaughter handling be optimized for all forms of slaughter.

Dealing with all of these issues are major topics in their own right, but will not be discussed here, although references for additional reading/listening will be provided. A great deal of the current governmental and nongovernmental organizational efforts in the area of farm animal welfare are focused on improving how these details are dealt with across the board, from farm to slaughterhouse. The quality of preslaughter handling, in turn, will affect the outcome of the slaughter.

In this area, there is nothing different about preparing for religious or nonreligious slaughter and excellence in preslaughter handling is strongly supported by all religious communities. (Jewish, Muslim, and Christian scriptures, for example, all have very strong animal welfare statements.) Yet the current literature on different methods of slaughter, especially religious slaughter, does not assess clearly the animal welfare of the animals and care taking with them leading up to the time of the slaughter in different facilities. Animals destined for different slaughter methods may need to be handled in different ways. This issue needs to be researched, evaluated, and accounted for, that is, how were all of the other steps leading up to slaughter or stunning carried out and, therefore, what is the emotional and physical state of the animal at the time it is presented for slaughter or stunning. Do variations in preparation for slaughter lead to differences in outcomes? How are these objectively evaluated and quantified? Everyone involved with animal agriculture should support such research properly done in accordance with scientific standards for detailed descriptions of the systems used.

The variables associated with mal-stress need to be isolated and studied before the effect of a method of slaughter is studied. It is also important that the various plants and the various methods that are actually used for religious slaughter be carefully identified and evaluated separately and against each other. Without that information, we cannot determine which methods are preferable or optional and what specific problems may need to be addressed with each different method.

The people who work with animals are also part of the animal environment and the workers experiences of stress may directly result in increased prodding and agitated behavior toward the animals, increasing animal stress. While there may well be generally optimal environmental conditions for workers when handling animals, there may be a need to recognize the specific limits of individual workers whose reactions become compromised either with extended time or at different points in the process, and in different ways in different types of physical facilities or with different animals. Worker orientation, training, evaluation, retraining, compensation, and bonuses need to be evaluated relative to their impact on animal welfare at the processing plant and during transportation in addition to the farm level, but without government interference.

I.5.2 Key Issues to Assess When Evaluating Methods for Stunning and Slaughter

Evaluating a process that is rapid and, in the end, irreversible, is difficult but not impossible. The challenge with respect to assessing *stress* is that it requires further research, as measuring stress at the actual time of stunning and/or slaughter becomes more complicated to measure accurately at that time because the blood volume is changing dramatically and nervous function is rapidly changing.

To assess whether a slaughter experience was humane, we must address and define several concepts: *conscious* (and the opposite, unconscious), *sensible* (and the opposite, insensible), and *living* (and the opposite, dead). The last of these pairs is often the hardest to deal with and in fact, it is often controversial in modern times with respect to brainstem versus heart versus sensible death. Our capacity to measure death is more precise than our ability to simply recognize it. The recognition of death will be dealt with later, in the section on cultural and religious meaning.

Conscious is a state where the brain is aware of what is happening and can react. An unconscious animal is not aware of what is happening. This means that although the pain receptors may be activated, that is, the initial biological sensing of something that is unpleasant, the signal from the site where the pain is occurring is not processed at the highest levels of the brain, where "consciousness" resides. From a scientific point of view, an unconscious animal is not dead, and is still giving off some signals as measured by various brain wave techniques, but has lost the ability to perceive these signals.

Insensible, which is a deeper stage of unconsciousness, although similar in meaning to unconscious, operationally means that the animal is not able to respond and it is assumed that the pain is not processed at all through the upper brain to an external signal. It is the term used to reflect a testable state. For maximum insensibility in animals, which should focus on the head, the lack of an eye blinking response following a "tap" near the eyes is often taken as the point of full insensibility. However, the response to an external poke on the other parts of the body is believed to be coming from a lower level of the brain, so that it remains measurable long after upper brain function where consciousness resides may have ceased to function. Therefore, the only responses that are relevant are those applied to and responded by the nose or eyes.

What this means is that an animal may become unconscious relatively rapidly and yet remain "sensible" for longer. To the best of our knowledge, the perception of pain is tied more closely to consciousness than it is to insensibility, but more research work is needed in this area. Operationally (i.e., practically in a slaughterhouse), insensibility is often used as the definition of when further processing of an animal can take place.

Pain is another concept that needs to be considered. Pain is probably best defined as a negative physiological response to an outside signal; that is, to a noxious stimulus. Pain is the stimulation of the "pain receptors." However, there is evidence that with a very sharp knife, the cut itself does not trigger these receptors, or, at least you will trigger a minimal number of pain receptors and it is possible that endorphins, an opiate associated with runner's high, may be released and overwhelm the limited pain receptors activated. Again, more research work is needed in this area.

I.5.3 Addressing Pain, Consciousness, and Insensibility When Using Different Slaughter Methods

Efforts are already under way to render diverse slaughter practices more humane, even if the confirming research has yet to be done. Many of these efforts have focused on the obvious needs for improving the technology of the slaughter stunning or killing equipment.

With captive bolt types of equipment (those that either fracture or penetrate the skull), the more stringent animal welfare standards being proposed in the United States at this time would permit a certain percentage (around 5%) to be missed on the first try. This can be very stressful for an animal that was missed and it is not clear at this time what level of failure leads to what degree of pain and it is not clear if there is any short-term pain in the realm of less than a second associated with the successful procedure, which leads to immediate insensibility. This needs to be studied further.

While fracturing or crushing the skull is presumably painful, it is not known how the pain of this method compares with others. It is known that one form of penetrating captive bolt has been banned

because it led to brain tissue entering the blood stream, which might then transmit BSE (bovine spongiform encephalopathy; i.e., what is commonly referred to as "mad cow disease"). So the evaluation of the stun/kill method may need to include other concerns, as well.

Temple Grandin has also noted anecdotally that the person operating the captive bolt equipment can work at a high level of accuracy for possibly up to 5, maybe 6 h, but after that point, in almost all cases fatigue sets in, accuracy diminishes and the person's attitude becomes one of just pushing himself to get through to the end of the shift. Stress on the worker likely leads to additional stress on the animal and a higher failure rate.

Electrical shock stunning, which in its mild form is approved by some Muslim authorities, but even in the less mild forms, it may kill up to 30% of the animals, which is not acceptable to Muslims. Questions that can be asked include: Is preparation for electrical stunning more stressful? What is the rate of ineffective stuns? What are the individual animal's differences in their response? What are the consequences of re-stunning? What impact might improvements in equipment make? What are the effects of this process on workers? (Obviously, some of these questions also need to be asked in great detail for the other methods being discussed.)

Use of CO_2 gas would presumably lead not only to stunning but also to asphyxiation. How painful is asphyxiation? What is the temporal sequence of these events and how does that impact the evaluation? What would the addition of other gases do to the process and to the meat from the slaughter? Again the same questions asked for electrical stunning can be asked here. It is known that the use of gas for some breeds of pigs is much less successful than for other breeds.

Historically, both Judaism and Islam provided far more humane guidelines for conducting slaughter than existed for the other techniques being used when the rules were promulgated hundreds and thousands of years ago. The other slaughter techniques of today may have become more comparable to those of religious slaughter over the centuries. Rapid exsanguination may induce a rapid loss of consciousness similar to stunning and be followed by death. This claim needs to be studied to ascertain the time it takes to reach both unconsciousness and then insensibility and to determine how these times can best be realistically minimized.

In reality, today we must recognize that the actual practices of religious slaughter are extremely varied among religious groups and from facility to facility. This requires that each facility and each technique be independently evaluated against a standard that respects the religion and is deemed by the proper experts to be "best practices." The American Meat Institute's standard for slaughter, which includes religious slaughter, is a widely accepted standard that is accepted by almost all of the animal activist groups. To meet religious slaughter standards, the animal is directly cut at the neck and allowed to bleed until it becomes insensible. In most cases it is preferable from an animal welfare point of view that the religious slaughter be done in the upright position, which is the preference of the American Meat Institute slaughter recommendations although upside down is recognized while shackling and hoisting is not.

The turning of the animal upside down for some religious slaughter that is currently being done will have to be researched and may have to be phased out over time or at least brought up to the highest animal welfare standards consistent with the method and might only be permitted where it is a religious requirement of those for whom the meat is intended. Some methods, such as shackling and hoisting and its variants, do not reach a reasonable level of animal welfare and will have to be phased out. A well-trained religious slaughter-person can do the slaughter properly, quickly, and repeatedly to minimize the time to both unconsciousness and insensibility. Some slaughter-persons may not be able to meet this requirement and should probably be assigned to other activities.

A key to both good shechita (Jewish) and good halal (Muslim) slaughter being painless seems to be that the knife used to make the cut be VERY sharp and that rapid blood loss occurs along with assuring the absence of any "ballooning" of the severed ends of the neck arteries. Such "aneurysms" might increase the time to unconsciousness and insensibility by slowing blood loss. While the idea of a painless cut may seem odd, most of us need only think about the discovery of blood, even copious blood, from a previously unnoticed cut.

Each of the methods for slaughter requires well-trained slaughterers. The quality of a religious slaughter may be more highly dependent upon people skills than other methods. With poorly trained slaughter men, no slaughter may be acceptable. But with well-trained slaughter men using a very sharp knife with the proper design of the knife and the equipment for holding a calm animal, religious slaughter may in fact be

superior to the other methods. This needs to be more fully and objectively researched. The key point is that when evaluating slaughter systems, bad systems (incorporating all of the above types of issues) need to be improved. What is inherent to each type of slaughter can only be evaluated on the best systems.

By way of illustration, the following is a statement by Dr. Grandin about a kosher slaughter plant that is doing things right, partly thanks to her practical work with the plant:

> Recently, I participated in a ritual kosher slaughter—in this ritual, the way it was meant to be done, I must say. This was at a plant where the management really understood the importance and significance of what they were doing, and communicated this to their employees—and to the animals as well, I believe. As each steer entered the kosher restraining box, I manipulated the controls to gently position the animal.
>
> After some practice, I learned that the animals would stand quietly and not resist being restrained if I eased the chin-lift up under the animal's chin. Jerking the controls or causing the apparatus to make sudden movements made the cattle jump … Some cattle were held so loosely by the head-holder and the rear pusher gate that they could easily have pulled away from the rabbi's knife. I was relieved and surprised to discover that the animals don't even feel the super-sharp blade as it touches their skin. They made no attempt to pull away. I felt peaceful and calm. (Regenstein and Grandin 1992, pp. 156–157)

I.6 Work for Precise, Humane Slaughter Standards across and within Respective Communities

Death is the minimal acceptable outcome of a successful slaughter process. Death with as little pain and suffering as possible, for the animal and all the people involved in watching/participating in the process, is most desired. While we may disagree about the best methods to achieve the result, humane professionals will agree with the desired objective and promote all procedures that achieve that outcome.

I.6.1 Minimizing Pain and Suffering

With this goal, in this context, it is understandable that some groups, who at this point are from the most affluent societies of Northern Europe, are promoting the anesthetization of animals prior to slaughter. Whether this is primarily to relieve the pain of the animal or to relieve the guilt and sympathetic suffering of meat eaters and workers is an open question. This is particularly true if these concerns are expressed in secular overtones (e.g., "an animal has a right …"). (Without specific and detailed knowledge of the impact of such interventions on the animal and the meat, it cannot be said whether it is a fool's errand or an expensive, but perhaps legitimate option.) Both human and animal concerns are important. Yet the idea of gassing or anesthetizing groups of animals provokes in other people the memories of horror from the Holocaust and the use of poisonous gas in warfare. This in addition to the fact the process may carry some degree of danger to humans, pollute the environment, and, depending upon the gas, may be aversive and traumatize the animal.

With 10 billion animals slaughtered every year in the United States and Europe (8–9 billion of them poultry), there is a huge industry in animal death. It is hard to conceive of death on such a scale. What we are trying to show is that there may be as many ways to be careful in death as there are ways to be careless in death. Christians, secularists, Jews and Muslims and others may all end up making very different judgments regarding humane slaughter, whether it is possible or how it is possible, as we do with the respect for and the use of blood. And we may substantially agree as to what denotes more, as opposed to less, humane practices, in the same way that we may agree about the scientific requirements for the biologically safe handling of blood and blood products.

I.6.2 Suffering

On the issue of suffering we can do much more research, but the framing of the issue is largely one of giving it meaning in the context of moral values, religious law and faith. Suffering is the experience of

loss. It is a complex response that assumes expectations or hopes that are not met and will not be met in the near future if ever; it is an affective response and, as such, it requires a degree of brain function that requires integrating knowledge and looking into the future and being able to recognize that there are longer term consequences. We know very little about the affective life of animals, especially in terms of the higher order emotions. However, it is not mere anthropomorphism that allows empathy for an animal in obvious pain or otherwise suffering.

Clear proof that any of the animals subject to commercial slaughter have this level of comprehension with respect to the slaughter process has not been shown to date, so for now it appears that they do not suffer, specifically with respect to slaughter, including with regard to their own deaths. We do know that animals that have lived lifetimes or years together react to the sudden absence or death of another in a way that implies suffering or grief. Except for the after-birth, most livestock are not routinely in contact with blood, neither would they associate it with death. Stresses can be perceived by animals, so careful handling will contribute to their well-being during the preslaughter and slaughter processes. It is known that cattle will lick the blood of a recently slaughtered animal. It is also known that sheep will jump to "get ahead" of the animal in front of them at the slaughter point! Unless the animal had witnessed death before, we would not anticipate that an animal would have any association with the surrounding chaos, except that flight may be a response to anxiety, when going forward is the only available option.

I.6.3 The Complexity of Death

The whole issue of when death occurs may be definable with a scientific precision that is more exact than what the experience and practical aspects of slaughter may allow. The issue of postslaughter activity must be considered. Some authorities, in both the Jewish and Muslim religious communities permit postslaughter stunning of animals. While this action may seem odd or unnecessary to those for whom slaughter simply means death, it is an important concern to those who recognize death as a process. Stunning after religious slaughter may lead to more rapid insensibility, although rapid bleeding if the cut is done properly will lead to rapid unconsciousness. Beyond this, the Muslim community does not permit anything further to be done to the animal until it has fully expired, until it is dead, defined by the cessation of any heartbeat. However, there is also a category in Jewish law of "religious death," that is, once the animal's throat has been slit, the animal is "dead" and one may do anything to the animal—except for eating it. This is an area where the rabbis will need to work with the animal welfare community to define what is and is not acceptable during this interim period. For example, when is the eye reflex and other meaningful reflexes gone so that further processing can proceed?

I.6.4 Putting Death in Perspective

Participation in the larger commercial marketplace, which encompasses many different slaughter methods will require both the religious community and secular communities to work together to assure that all of their different practices take advantage of the best available methodology and equipment while still remaining faithful to their unique religious requirements or secular sensibilities.

We mentioned earlier the history of anti-Semitism and Islamophobia that led to efforts to ban religious slaughter in Europe. But some methods of permitting religious slaughter have been used toward anti-Semitic ends, such as when Jews were not permitted sell animal products that failed some aspect of kosher inspection as nonkosher. By singling them out for special labeling, the aim is to put market pressure to inhibit the purchase of these meats. This action took away an essential market for nonkosher meats and undermined market integration across religious differences while producing great waste and increased food costs for Jews. While different communities are going to value different methods of slaughter, any system of labels that might simply indicate the method by which any animal was slaughtered may be used as the basis for prejudice against the community that favors that type of slaughter, specifically Jews or Muslims. While labeling has proven useful in educating consumers about some aspects of their food supply, in the case of slaughter labeling, it might not just be the method that is being evaluated. The point is to recognize that addressing slaughter techniques using labeling with an eye to educating consumers and the public raises challenges of historic prejudice that are extremely complex to address. Other

mechanisms such as this educational piece might be more appropriate. (The reality is that people need to be reeducated as to the realities as well as industry practices, as they/we have become desensitized over the years. The ability to have correct information and to make proper decisions must be restored.)

Because so much of our food industry is a death industry (technically, all are thus except animal products such as milk, eggs, honey, although the producing animal eventually becomes part of the "death industry"), we need to recognize how the technology for animal slaughter may or may not relate symbolically to or be used in times of war and unrest to carry out human slaughter. This may seem trivial, but much of what makes the topic of animal slaughter so horrid, beyond its own violence and messiness, is the implicit threat that we humans, too, could be slaughtered.

Yet more importantly than these associations, we need to reconsider the role of meat in our diets. Not that this discussion is meant to suggest that we need to abandon meat or animal products, but rather that we may need to more fully respect the life of animals that we encounter in death, and whose death brings renewed life to our bodies.

I.7 Conclusions

First, while there are many religious, secular, and cultural approaches to slaughter, we have very little evidence based on rigorous, comparable evaluations of general methods and specific practices evaluating each approach. In a world of religious, secular, and cultural diversity, we should expect that many methods of slaughter will be justified. Both stunned and nonstunned slaughter is justified to significant, and usually different, groups, for different types of reasons. The goal that we hope would be accepted at this time is to try to work together to improve slaughter in all of its aspects, both with stunned and nonstunned slaughter.

Second, regardless of our preferred methods, we all need to learn to accept the documentation of bad outcomes based on agreed upon scientific measurements that show that an entire integrated system may not be working properly. There are many slaughter systems, both religious and nonreligious that are currently being evaluated that are not animal welfare friendly. There is work to be done to improve all of these systems. It is vital to stress that the humane treatment of animals from start to finish in the meat industry does not depend upon getting the rabbis or Muslim clerics to endorse stunning.

Third, much of the work for humane slaughter may well need to focus on all the other things that need to be improved prior to the actual aspects at the moment of slaughter. At this point in time, there remains a lot of work to be done to improve animal welfare, but those involved in this appendix believe strongly that the evidence strongly favoring any one system or approach does not exist. Each system should be optimized. Fourth, much greater attention needs to be paid to the details at the point of stunning, where this procedure is done, or the actual unstunned slaughter, where this procedure is done. In fact, there may be factors related to religious slaughter that would make it favorable, at least in some settings, when compared to slaughter with stunning. Researchers and policy makers should remain open minded to the fact that when done right, religious slaughter may actually be better in terms of animal welfare than slaughter with stunning. Yet stunning, as a method, may be justified as well, on other grounds, for cultural or secular reasons. Fifth, with respect to nonstunned slaughter, important segments of both the Jewish and Muslim communities are committed to working with the scientific animal welfare community to optimize the process and to replace some of the methods currently used to restrain animals that are totally unacceptable from an animal welfare point of view. New equipment, better knives, and better training are all aspects that are being explored and seriously worked on by the Jewish and Muslim community in conjunction with the scientific community.

Finally, any effort to engage the religious communities on this issue will require fluency in the specific governance practices of each group to develop the proper procedures for processes prior to and after the actual slaughter. There must be clear guidelines that are accepted within the Orthodox Jewish and Muslim communities about how to harmonize humane slaughter with the ritual requirements of each religious community. Many of the people involved in this appendix are working on these efforts.

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