NITRITE CURING OF MEAT

The *N*-Nitrosamine Problem and Nitrite Alternatives

by

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PREFACE

Meat has been treated for centuries with rock salt as a means of preservation. However, only one century has passed since the German researchers, Polenske in 1891, Kisskalt in 1899 and Lehmann in 1899 discovered that the active component in the curing process was nitrite. It is interesting to look back over this century to see what actually transpired in a hundred years. How much more do we really know about nitrite, its chemistry and its preservative effect on meat than we did a century ago? What were the milestones achieved, if any? Have we learned things about nitrite that perhaps we didn't want to know, and has technical advancement in terms of processing by meat packers resulted in the loss of certain traditional old-world meat products?

In the beginning, the role of nitrite as a meat curing agent was revealed and shortly thereafter, government regulators placed guidelines on the level of nitrite and nitrate permitted for use in cured meat formulations. The importance of salt in combination with nitrite as an antibotulinal agent and the limited understanding of its mode of action was a key event during this century. In the late 1960s and early 1970s, the development of the so-called "nitrite problem" surfaced on account of the detection of *N*-nitrosamines in processed meats. The industry was in an uproar, and the issue rose to the very pinnacle of interest, for both the public and research scientist. A major technical advance in the analytical technique for *N*-nitrosamine detection was achieved when Thermo Electron of Waltham, Massachusetts introduced the thermal energy analyzer (TEA). This unit was a watershed in *N*-nitrosamine research because it became possible to screen a large number of samples with only a minimum preparation. As an analytical tool, the selectivity of the instrument for detecting sub μg/kg quantities of *N*-nitroso compounds in complex biological materials and foodstuffs without the elaborate clean-up procedures was far beyond anything else available. Today the TEA is recognized as a cornerstone for analytical *N*-nitrosamine detection. In terms of nitrite chemistry, the controversy over the identity of the pigment of thermally processed nitrite-cured meat raged on for decades. Early studies suggested that the pigment of cooked cured-meat was a dinitrosyl protoheme complex. Yet, a number of scientists were able to unravel some of the earlier conclusions presented in the scientific literature and provide compelling evidence to support their hypothesis that the pigment of nitrite-cured meat was indeed a mononitrosyl ferrohemochrome. The role of nitrite in revealing the desired and unique flavor of cured products, perhaps by suppressing the formation of lipid oxidation products, was another development in revealing other properties of nitrite. Above all, the antimicrobial role of nitrite, together with salt, had a major influence on the popularity of nitrite/nitrate in food preservation.

This book presents a review of the desirable attributes that sodium nitrite confers to meat during processing, as well as drawbacks of nitrite usage, *i.e.*, the presence of *N*-nitroso compounds, particularly *N*-nitrosamines. Furthermore, the book provides solutions with regard to curing of meat without the use of nitrite. An examination of a multicomponent nitrite-free curing system entailing the color, flavor, and microbial protection of such a system is presented in Chapter 9. The book has been divided into the following chapters.

Chapter 1 begins with a general introduction of what cured meats are, followed by an overview of the main benefits and drawbacks nitrite affords meat and meat products.

Chapter 2 contains a review of the history of the curing process. Issues on how curing began, techniques used then and today, and the discovery of nitrite as the active agent are addressed. This chapter will also introduce the characteristic attributes which nitrite affords meat and will lay the foundation for the remainder of the book.

Chapter 3 deals with the color characteristics of meat and meat products with particular emphasis placed on the chemistry of muscle pigments. The color of fresh meats, how nitrite modifies it and the color of the product after thermal processing will be described. A review of the controversy surrounding the exact chemical structure of the cooked cured-meat pigment is reported, as well as evidence which supports the view that the cooked cured-meat pigment is a mononitrosylheme complex.

Chapter 4 begins with a brief introduction on what is meant by the oxidative stability of meat lipids and further details how lipid oxidation in uncured frozen and cooked meat proceeds. Nitrite's role in curbing meat flavor deterioration (MFD), previously referred to as warmed-over flavor (WOF), and the proposed mechanism(s) of nitrite's antioxidative efficacy are addressed. A review of the classical 2-thiobarbituric acid (TBA) test, used for assessing the extent of lipid oxidation in meats, follows. The difficulties raised by nitrite in the TBA test is accounted, and an alternative approach for assessing the oxidative status of cured meat systems is furnished.

Chapter 5 deals with the flavor of uncured cooked meat and places particular emphasis on the volatile flavor compounds mainly responsible. The relationship between nitrite and cured-meat flavor is explored and the chapter concludes with a simplistic view on how the basic flavor of cooked meat, species differentiation and MFD may be interrelated.

Chapter 6 discusses the microbial status of cooked meat with concerns over possible contamination by *Clostridium botulinum* spores. A general review of the microbiology of *Clostridium botulinum*, with regard to how it proliferates and how nitrite exerts a concentration-dependent antimicrobial action in combination with added sodium chloride and adjuncts is recounted. A more detailed review of the bacteriostatic properties of nitrite follows.

Chapter 7 explores the fate of nitrite in the meat matrix. The reactive nitrite anion exists in various forms in meat; it may be converted to nitrogen gas, nitrate, nitrous acid, its anhydride dinitrogen trioxide, or it may react with heme-based muscle pigments, protein, lipid and sulfhydryl-containing compounds in the meat matrix.

Chapter 8 deals with the potential hazards of nitrite usage in meat. A review of the *N*-nitrosamine story is reported and covers how *N*-nitrosamines are formed, and their prevalence in various nitrite-cured meat and meat products. A discussion on current meat industry regulations for nitrite usage, which includes why nitrite usage in fish is banned, as well as various means to prevent and retard *N*-nitrosamine formation, follows. This chapter also includes *N*-nitrosamine inhibitors which are available to the meat processor, and the impact of a nitrite-ban to the industry.

Finally, Chapter 9 considers possible substitutes for nitrite with regard to the use of a multicomponent nitrite-free curing system. This chapter considers color characteristics, antioxidant properties, flavor characteristics and antimicrobial choices for nitrite alternatives. The section on color attributes of nitrite-free meats deals with various food-grade dyes and pigments available for addition to meat with a review of their advantages and limitations. A discussion of why the cooked cured-meat pigment itself, performed outside of the meat matrix, is the only appropriate alternative for nitrite-free curing of meat is described in detail. The section on antioxidant properties deals with synthetic and natural antioxidants available for use in meat to mimic the effect of nitrite. A discussion on these antioxidants, as well as chelators and synergism noted between combinations is reported. The section on flavor characteristics of nitrite-free meat considers sensory studies already performed on such systems, as well as the role of salt and smoking to the flavor. The last section in this chapter reviews the various food-grade antimicrobial agents available for use in nitrite-free curing of meat and evaluates those which have been tested, those which are promising, and those which are not. A concluding section on the multicomponent nitrite-free curing package and its usefulness to the industry is provided.

The book thus presents a state-of-the-art account of nitrite, the *N*-nitrosamine problem and nitrite-free meat curing alternatives which would be of interest to meat scientists, government regulators and the industry. Food scientists, nutritionists and biochemists would also find this book informative and useful for inclusion as materials to be covered in a graduate meat science or food chemistry course.

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CONTENTS

CHAPTER 1

INTRODUCTION

Prior to the availability of refrigeration, foods, particularly fish and meat, were preserved by salting, marinating or pickling. Through a decrease in water activity, meat and fish were protected against microbial spoilage and other deteriorative processes. It was the process of treating meat with rock salt that led to modern curing practices (Cassens 1990). Thus, meat curing, historically defined as the addition of salt (sodium chloride) to meat, is now referred to as the intentional addition of nitrite and salt to meat. Although meat constitutes a major ingredient in such products, color stabilizers, sweetening agents, non-meat extenders, seasonings, acidulants, smoke and other adjuncts might be added to enhance the quality of products or to reduce cost.

Cured meats represent a large portion of the processed meat products consumed in North America. These processed meats are attractive in their color, flavor, texture and are popular because they combine variety with convenience of relatively long shelf-life and storage stability. Nitrite might also have an influence on the texture of finished meat products by cross-linking of meat proteins. Most importantly, nitrite, together with sodium chloride, inhibits the production of the neurotoxin by *Clostridium botulinum*, thus preventing food poisoning and botulism.

Despite its numerous benefits and multifunctional properties in processed meat products, nitrite has often been a source of concern due to its role in the formation of *N*-nitrosamines which are known carcinogens in a variety of animal species. These compounds are formed from the reaction of nitrite with free amino acids and amines in meat products under high temperatures experienced during frying in certain cured meats. Since it is difficult to control the level of endogenous factors, such as amino acids and amines, a reduction in the level of nitrite added to products or specifics of reaction and process conditions might be necessary. Thus, the allowable level of nitrite addition in cured meats has been reduced to a maximum of 150 to 200 mg/kg in different products; processors have voluntarily reduced these levels even further. In addition, meat curing adjuncts are kept separately prior to their addition to meat so that there is no reaction between nitrite and spices and other ingredients in order to avoid accidental formation of *N*-nitrosamines in products. Thus, the meat industry has responded adequately and responsibly to concerns expressed about nitrite and control of *N*-nitrosamine formation in processed meats. Nonetheless, a recent study has recommended that excessive consumption of hot dogs and cured

products be avoided in order to prevent occurrence of leukemia in children, among others (Peters *et al.* 1994; Blot *et al.* 1999). However, it should also be noted that humans excrete non-carcinogenic *N*-nitrosoproline in their urine, thus demonstrating that such compounds are also formed within the body (Loeppky 1994).

Cured Meat Products and Residual Nitrite

Among cured meats that are available in the market, hot dogs, other sausages and frankfurters, salami, bologna, pepperoni, as well as ham, bacon and corned beef are considered as major products. These products could be prepared either by direct addition of nitrite and other ingredients to the systems, such as those in emulsified-type products, or by pickling in a cure solution. In addition, injection of pickle solution into solid cuts of meat, as well as dry curing in which products are rubbed or packed in dry ingredients are common; fermented sausages may also be produced.

Depending on the type of product and production procedures employed, the amount of residual nitrite that is present in products may vary. Since nitrite is extremely toxic to man, causing methemoglobinemia and even death at relatively high doses, as well as being a precursor of carcinogenic *N*-nitrosamines, its usage for processing of cured meat is strictly controlled by government regulations and monitored by both government and industry. In North America, the permitted level of nitrite addition to products varies between 150 and 200 mg/kg, but imported products sometimes contain high residual nitrite which indicates that more than 200 mg/kg nitrite might have been added to meats during curing. Sen and Baddoo (1997) published a recent paper which summarized the residual level of nitrite in products available in the marketplace in Canada. Of the 197 samples surveyed in 1972, the average residual nitrite varied between zero and 252 mg/kg, but in 1983–1985 of 659 samples examined, values were in the range of 0–275 mg/kg. In 1993–1995, the value was 1–145 mg/kg in 76 samples and in 1996 it was 4–68 in 35 samples tested. Therefore, it appears that the residual nitrite levels in the Canadian cured products have decreased over the past 20–25 years, albeit slightly. However, the incidences of nitrite residues greater than 100 mg/kg have sharply decreased. Similar trends in residual nitrite might be evident for supply of cured meats in the USA and elsewhere in North America. The amounts of residual nitrite present in cured meats in Canada was found to depend on the type of product examined (Table 1.1). It should be noted that the residual amount of nitrite and subsequent production of *N*-nitrosamines in products are affected primarily by the preparation method employed. Thus, frying, broiling, boiling, etc., may exert different effects on the rate of production and level of these carcinogens in products. The interest in studying the formation and occurrence of *N*-nitrosa-

mines in cured meats and other foods stems from the absolute nature of the U.S. Food and Drug Regulations and other regulatory agencies in Canada and Europe which prohibit the use of any food additive, that is either in itself carcinogenic or produces carcinogens. Therefore, it is only reasonable that the usage of nitrite in cured meats be reduced, or even phased out if effective and safe substitutes are found. Alternatively, nitrite-free curing of meat may represent a niche market for consumers who do wish to have these products available to them.

TABLE 1.1
RESIDUAL NITRITE (mg/kg) IN SELECTED CURED MEAT PRODUCTS IN CANADA[1]

Product	1983-1985	1993-1995
Bacon	0-178 (33.7)	7-81 (33.8)
Hot dogs	1-178 (60.9)	23.112 (65.5)
Sausages	1-132 (33.8)	4-145 (30.2)
Hams	4-146 (48.9)	—
Bologna	0-137 (65.5)	—
Pepperoni	10-206 (62.5)	10-58 (35)
Overall	0-275 (43.6)	1-145 (30.8)

[1] Adapted from Sen and Baddoo (1997). Values in parentheses denote mean values based on total number of samples analyzed.

Cured Meats and *N*-nitrosamines

The key examples of *N*-nitrosamines that are found in some thermally cured products include *N*-nitrosodimethylamine (NDMA) and *N*-nitrosopyrrolidine (NPYR). These compounds are known to be carcinogenic, mutagenic and teratogenic in experimental animals. Of the more than 300 *N*-nitroso compounds that have been tested in different animals, greater than 90% of them have been shown to cause cancer (Preussmann and Stewart 1984; Tricker and Preussmann 1991). Although the carcinogenicity of *N*-nitrosamines in humans cannot be tested, epidemiological studies have suggested a possible link to the incidence of various cancers in humans.

The levels of *N*-nitrosamines in cured meats are in the parts per billion (μg/kg) range, if present (Walker 1990). Among cured products, fried bacon has consistently shown the presence of NDMA and NPYR at mean levels of up to 3 and 25 μg/kg, respectively (Glória *et al.* 1997). Use of elastic rubber netting in packaged meats must also be avoided as nitrite might react with amine additives used in them as vulcanization accelerators (Sen *et al.* 1987, 1988). Thus, *N*-nitrosodiisobutylamine (NDiBA) and *N*-nitrosodibenzylamine (NDBzA) might be present at levels of 4.6–33.5 and 52.3–739.9 μg/kg, respectively, on

the outer surface of the netted hams (Fiddler *et al.* 1997). Therefore, caution must be exercised to avoid accidental formation of *N*-nitrosamines in products.

Benefits and Drawbacks of Nitrite: New Trends and Prospects

In response to the nitrite-nitrosamine problem, the industry reduced the level of nitrite used in preparation of a variety of products. The current trends, however, have concentrated in production of low-nitrite, low-fat, low- salt, and all-meat products. In addition, all-meat emulsion-type products are being introduced into the market. Although the *N*-nitrosamine scare has died down, nonetheless, nitrite-free curing of meat might still be attractive in view of the fact that many of the effects of nitrite can be easily duplicated by the presence of adjuncts, together with refrigeration. The typical color of the products might also be reproduced by the addition of the preformed pigment present in cured meats. However, offering of these novel curing techniques awaits regulatory approval and might first be initiated in niche markets and in connection with functional foods and all natural products, before they could be used industrially.

REFERENCES

Blot, W.J., Henderson, B.E. and Boice, Jr., J.D. 1999. Childhood cancer in relation to cured meat intake: review of the epidemiological evidence. Nutr. Cancer **34**, 111–118.

Cassens, R.G. 1990. Nitrite-Cured Meat: A Food Safety Issue in Perspective. Food & Nutrition Press, Trumbull, CT.

Fiddler, W., Pensabene, J.W., Gates, R.A., Custer, C., Yoffe, A. and Phillipo, T. 1997. *N*-nitrosodibenzylamine in boneless hams processed in elastic rubber nettings. *JAOAC Int.* **80**, 353–358.

Glória, M.B.A., Barbour, J.F. and Scanlan, R.A. 1997. Volatile nitrosamines in fried bacon. *J. Agric. Food Chem.* **45**, 1816–1818.

Loeppky, R.N. 1994. Nitrosamine and *N*-nitroso compound chemistry and biochemistry. In *Nitrosamines and Related N-Nitroso Compounds: Chemistry and Biochemistry*, ed. R.N. Loeppky and C.J. Michejda. ACS Symposium Series 553. American Chemical Society, Washington, DC, pp. 1–18.

Peters, J.M., Preston-Martin, S., London, S.J., Bowman, J.D., Buckley, J.D. and Thomas, D.C. 1994. Processed meats and risk of childhood leukemia (California, USA). *Can. Causes Contr.* **5**, 195–202.

Preussmann, R. and Stewart, B.W. 1984. *N*-nitroso carcinogens. In *Chemical Carcinogens,* Second Edition, Volume 2, ed. C.E. Searle. ACS Monograph 182. American Chemical Society, Washington, DC, pp. 643–828.

Sen, N.P. and Baddoo, P.A. 1997. Trends in the levels of residual nitrite in Canadian cured meat products over the past 25 years. *J. Agric. Food Chem.* **45**, 4714–4718.

Sen, N.P., Baddoo, P.A. and Seaman, S.W. 1987. Volatile nitrosamines in cured meats packaged in elastic rubber nettings. *J. Agric. Food Chem.* **35**, 346–350.

Sen, N.P., Seaman, S.W., Baddoo, P.A. and Weber, D. 1988. Further studies on the formation of nitrosamines in cured pork products packaged in elastic rubber nettings. *J. Food Sci.* **53**, 731–734, 738.

Tricker, A.R. and Preussmann, R. 1991. Carcinogenic *N*-nitrosamines in the diet: occurrence, formation, mechanism and carcinogenic potential. *Mutat. Res.* **259**, 277–289.

Walker, R. 1990. Nitrates, nitrites and *N*-nitroso compounds: a review of the occurrence in food and diet and the toxicological implications. *Food Addit. Contam.* **7**, 717–768.

CHAPTER 2

HISTORY OF THE CURING PROCESS

The preservation of meat by way of curing is based in part upon the art as practiced through eons of time and perhaps to a far greater extent upon sound scientific principles developed since the turn of the century (Binkerd and Kolari 1975). The origin of nitrate usage, as saltpeter, in meat curing is lost in antiquity, but preservation of meat with salt preceded the intentional use of nitrate by many centuries. It was recognized that cuts of meat could be preserved by treating them with a salt solution or packing them in dry salt. These early processed meat products were prepared with one purpose in mind: their preservation for use in times of scarcity. Very early on, people learned that dried or heavily salted meat would not spoil as easily as its fresh counterpart (Hedrick *et al.* 1994). Salting prevented bacterial growth due to salt's direct inhibitory effect and because of the drying action it had on meat (*n.b.*, most bacteria require substantial amounts of moisture in order to survive and proliferate). Thus, rock salt was an important commodity long before the Christian era as it was routinely employed for muscle food preservation in ancient China, the Jewish Kingdom, Babylonia and Samaria (Jensen 1953).

In ancient Greece, salt obtained from "salt gardens" was used to preserve fish. The Romans learned the use of salt from the Greeks and continued this practice. Besides curing fish, the Romans preserved various types of meat, such as pork with pickles containing salt and other ingredients, thus, establishing a trade for these commodities in the Roman empire (Jensen 1954). In fact, a book dating from the reign of Augustus (63 B.C. - 14 A.D.) contains directions for the preservation of fresh meat with honey and cooked meat in a brine solution containing water, mustard, vinegar, rock salt and honey. Other recipes are listed for liver sausage, pork sausage and a round sausage. The latter product consisted of chopped pork, bacon, garlic, onions and pepper and was stuffed in a casing and smoked until the meat turned pink (Hedrick *et al.* 1994). As the use of salt as a meat preservative spread, it was found that high concentrations could promote the formation of an unattractive gray color within the lean muscle. Thus, a preference developed for "certain" salts that produced a pink color and special flavor in meat. It was nitrate impurities in the rock salt, which upon incorporation into the meat matrix and after reduction to nitrite by the postmortem reducing activity of the muscle tissue, that were truly responsible for the curing effect.

By medieval times, treating meat with salt, saltpeter and smoke was commonplace, and saltpeter's effect to "fix" the red color was well-recognized. Gradually, sweet pickle and sugar cures evolved as sucrose became available as a commodity of trade. Sugar added flavor to the meat and helped to counteract some of the harshness and hardening effects of salt. As the art progressed, the term "meat curing" eventually was understood as the addition of salt, sugar, spices, saltpeter (nitrate) or nitrite to meat for its preservation and flavor enhancement (Townsend and Olson 1987). Spices and other flavorings were added to achieve a distinctive brand flavor.

Toward the end of the nineteenth century, significant changes in meat curing had occurred. Various methods of curing, namely dry, wet or pickle cures and combinations of the two, were commonplace. Dry curing is the oldest technique and involves applying uniform and quantitated mixtures of salt, sugar, spices and sodium nitrate and/or nitrite to solid cuts of meat such as ham. The curing agents are rubbed in dry form over the surface of pieces of meat. The meat is then placed in a cooler at 2–4°C and permitted to cure. No water is added; hence, the curing agents are solubilized in the original moisture present in the muscle tissue. With time, slow penetration of the cure into the meat (*ca.* 2.5 cm/week) and micrococcal reduction of nitrate to nitrite affords the characteristic cured meat color and flavor of the product (Fox 1974). More than one application of the salt mixture is necessary to effect a cure, and the cuts must be "overhauled," turned over and restacked. This labor-intensive process requires a considerably longer period than that to cure comminuted meats. After curing is complete, the excess cure is washed off and the meat is placed under refrigeration (2–4°C) for 20–40 days to allow for salt equalization throughout. The pieces of meat are held in natural or air-conditioned drying chambers and ripened for a minimum of 6 months and often 12 months or more, depending on each country's tradition; details for the preparation of Spanish Serrano and Iberian hams are described below. The temperature is usually varied between 14 and 20°C at relative humidities of 70–90%. Complex biochemical reactions which are mainly proteolytic and lipolytic in nature occur and a characteristic flavor is developed (Flores and Toldrá 1993). Dry curing is used only for specialty items such as country-cured hams and bacon, as well as European-type dry cured hams such as Spanish Serrano and Iberian, Italian Parma and San Daniele prosciuttos or French Bayonne. These European hams are usually consumed raw unlike country-style hams in the U.S. and Westphalia hams in Germany which are smoked and then thermally processed before consumption (Toldrá and Flores 1998). Nevertheless, worldwide production of dry-cured products represents an important segment of the processed meat industry because these products possess unique flavor and texture attributes that apparently cannot be developed in any other way (Hedrick *et al.* 1994).

Pickle curing involves the immersion of whole cuts of meat into brine solutions that generally contain sodium nitrate or nitrite. Meats are then held in vats at 2–4°C for long periods of time to allow diffusion of the curing agents through the entire product. This process has severe limitations, especially for large pieces. Due to the high water activity, microbial growth and spoilage can arise from pickling even though the product is refrigerated and salt is present at an appreciable concentration. If sugar is included in the brine, it is referred to as a sweet pickle. Other adjuncts may be added to the pickle to enhance the flavor of the meat. At present, only specialty products such as neck bones, tails, pigs' feet and salt pork are cured in this manner (Hedrick *et al.* 1994).

The practice of pumping/injecting meat with a perforated needle originated in the late nineteenth century and greatly shortened the length of time required to cure meat. In ham, a pickle solution can be pumped directly into the vascular system; this technique, known as artery pumping, utilizes the arteries of the ham for distribution of the cure, but requires care so that blood vessels are not ruptured by excessive pumping pressures. There are problems, however, with this technique. Firstly, the arterial pathways in the muscle are not uniform and secondly after injection it is necessary to hold the ham under refrigerated conditions to permit not only equilibration of the cure, but also the fixation of the cured color; often a holding time of 5 to 7 days is required. Most important, the success of arterial injection is dependent on attentive work during slaughter and cutting, as well as subsequent handling procedures in order to guarantee that the arteries are left intact (Holland 1983).

Stitch pumping involves addition of pickle to the interior of meat by injection through a single orifice needle. By way of the many channels running throughout muscle tissues, the cure is rapidly distributed. Spray pumping and multiple injection are variations of the stitch method and use needles with many evenly spaced holes along their length to allow for more uniform distribution of the pickle. Injections are made at several sites as close to one another as possible. Afterward, tumbling and massaging subject the products to agitation and further accelerate the curing process by disrupting tissue membranes and hastening the distribution of cure ingredients.

Massaging and tumbling facilitate in the extraction of salt-soluble myofibrillar proteins, such as myosin, actin, and actomyosin, as well as water-soluble sarcoplasmic proteins through the rupture and loss in structural integrity of some muscle tissue. As a result of these processes, extracted myofibrillar proteins, fat and water from the brine form a creamy, gluey exudation (*i.e.*, an increase in viscosity of meat juices) which envelop pieces of meat and encourage their cohesion. During cooking, the protein matrix denatures and coagulates. It has been proposed that juiciness results from the coagulation of the salt-soluble proteins which in turn entrap moisture within particles of meat. Other researchers have suggested that the myofibrillar proteins immobilize the water

and reduce evaporation during thermal processing. In either case, improved yield and juiciness result (Holland 1983). Additional benefits from massaging and tumbling include uniformity of color development, as well as improved tenderness and palatability of meat products.

The process of multiple needle injection has become popular and such designed machines have ensured rapid, continuous processing of meat cuts. Unlike stitch pumping, multiple needle injection machines inject brine at hundreds of locations along the meat's surface resulting in a relatively rapid uniform distribution of the cure. Low pressure injection is favored over that of high pressure so as to reduce muscle tissue damage and loss of cure retention. To overcome the problems of recirculating the cure, injection needles have been designed to incorporate a valve type mechanism that opens only on the downward stroke and when in contact with meat. When there is no meat or when in contact with bone, the valve is closed. In the case of bone-in ham, injected meat pieces may need to be placed in vats and immersed in pickle for a period or tumbled to ensure a more even distribution of the cure throughout the ham. It is important to note, irrespective of the method employed, that the fundamental requirement is to distribute the cure throughout the entire piece of meat. Inadequate or uneven distribution can result in poor color development and a greater likelihood of spoilage. Bone sour in ham and gray areas in the interior of other meat products are examples of some problems that result from improper distribution of the curing mixture (Hedrick *et al.* 1994).

Dry-Cured Hams

In the Mediterranean area, dry-cured hams are very popular and are revered for their unique flavor as well as other characteristic sensory attributes. Two such products are the Spanish Serrano and Iberian hams, whose production in 1993 was *ca.* 181,500 tons (Toldrá *et al.* 1997*a*). During processing, there is a loss of water and diffusion of salt throughout the ham, leading to a gradual stabilization of the product due to the drop in water activity (Sabio *et al.* 1998). Simultaneously, there is a slow degradation of proteins and lipids that results in an accumulation of free amino acids and fatty acids, respectively. Details on the processing of Iberian and Serrano dry-cured hams are described in Table 2.1, but briefly consist of the following steps (Toldrá 1992; Flores and Toldrá 1993; Toldrá *et al.* 1997*b*):

(1) reception and classification of hams, and then presalting where a mixture of curing ingredients (*i.e.*, salt, nitrate and/or nitrite) and adjuncts (*i.e.*, ascorbic acid) are rubbed onto the lean muscle surface of the meat;
(2) salting, where hams are then placed fat side down, entirely surrounded by salt and arranged in single layers without touching one another. As there is

no water added, the curing agents slowly diffuse into the ham and are solubilized by the original moisture present in the muscle tissues. This period usually takes 8–10 days (*i.e.*, 1–1.5 days/kg weight) at temperatures between 2 and 4°C;

(3) during the post salting stage, a complete salt equalization within the hams takes place. The temperature is kept below 4°C for a period not less than 20 days, but not exceeding 2 months;

(4) the last and more complex stage is the ripening/drying stage. Hams are placed in natural or air-conditioned chambers and subjected to different time-temperature/relative humidity (RH) cycles. The temperature is usually maintained between 14 and 20°C with a RH decreasing from 90 to 70%. Aging of hams takes anywhere from 9 to 24 months. For example, the ripening period for Serrano hams is between 9 and 12 months and for Iberian hams it can be extended up to 18 or 24 months.

TABLE 2.1
SCHEME OF THE APPROXIMATE CONDITIONS FOR THE PROCESSING OF SERRANO AND IBERIAN DRY-CURED HAMS[1]

	Serrano Ham	Iberian Ham
Salting	T 0-4°C RH 75-95% t >0.65 and < 2d/kg	
Post Salting	T 0-6°C RH 70-95% t >40 and < 60d	
Dry-Curing		
first phase	T 6-16°C RH 70-95% t > 45d	T 6-16°C RH 60-80% t > 90d
second phase	T 16-24°C RH 70-95% t > 35d	T 16-26°C RH 55-85% t > 90d
third phase	T 24-34°C RH 70-95% t > 30d	T 12-22°C RH 60-90% t > 115d
fourth phase	T 12-20°C RH 70-95% t > 35d	
Total Time	t > 190d	t > 365d

[1] Abbreviations are: temperature, T; relative humidity, RH; and time in days, t. (Table from Toldrá *et al.* 1997*a*).

The quality of these two hams depends on the raw materials and the ripening conditions employed. Iberian dry-cured ham is produced from an autochthonous pig that is found in the southwestern region of Spain. These swine feed on pastures or stubble fields during their growing period (until 12–16 months, 55–75 kg) and their nutritional requirements are complemented with cereals, such as corn and barley. During the fattening period, three types of feeding regimes, known as montanera, recebo and cebo, are possible. For montanera, the basic food is the acorn (*Quercus ilex, Quercus rotundifolia* and *Quercus suber*) and the feeding period lasts from October to December or until a final weight of about 160 kg is achieved. For recebo, the acorn is complemented with cereals and mixed feeds. For cebo, only cereals and mixed feeds are used. Meat from acorn-fed pigs commands the highest price and the dry-cured hams so prepared offer a high degree of marbling (resulting from the finishing lipid-rich acorn diet), firm texture and exquisite characteristic flavor (Flores *et al.* 1988; López *et al.* 1992). The Serrano ham is produced from different crossbreedings of white pigs and has lower marbling, firm texture and a typical flavor. The intensity of the flavor can be controlled by the length of time the ham is allowed to ripen/dry. Complex biochemical reactions, mainly enzymatic, proteolytic and lipolytic in nature, occur during the dry curing process and contribute to the development of an adequate texture and characteristic flavor (Toldrá and Flores 1998).

Discovery of Nitrite as the True Meat Curing Ingredient

Today it is recognized that in order to cure meat, two ingredients must be used: salt and nitrite. Nitrite is the active agent in curing and all reactions taking place have some kind of relation with nitrite chemistry. However, for the production of dry-cured or fermented meat products, nitrate is required in this long ripening process for slow nitrite generation by bacterial reduction.

When nitrite *per se* was first used to cure meat is unknown, but classical studies in the latter half of the 19th century by Polenske (1891), Kisskalt (1899) and Lehmann (1899) demonstrated that nitrite, rather than nitrate, was the key ingredient in the curing process. Polenske (1891) provided the first technological advance in curing by concluding that the nitrite found in cured meats and curing pickle arose from bacterial reduction of nitrate. Shortly afterward, Kisskalt (1899) and Lehmann (1899) demonstrated that the typical color of cured meats was due to nitrite and not to nitrate. By 1901, Haldane had investigated the pigment responsible for the redness of cooked cured meats. He prepared nitrosylhemoglobin (NOHb) by adding nitrite to hemoglobin (Hb) and showed that its conversion to nitrosylhemochromogen upon thermal processing was the pigment responsible for the red color of cooked cured meat. Haldane (1901) further stated that the color change during cooking was a consequence of NOHb

decomposition into two constituents, namely hemin, the coloring group, and a denatured protein. Hoagland (1908) confirmed Haldane's findings and suggested that reduction of nitrate to nitrite, nitrous acid and nitric oxide by either bacterial or enzymatic action, or a combination of the two, was essential for NOHb formation.

This scientific knowledge led to the direct use of nitrite instead of nitrate, mostly because lower addition levels were needed to achieve the same degree of cure. By 1917, proprietary curing mixtures containing nitrite were marketed in Europe. At the same time, a U.S. patent was issued to Doran (1917) for nitrite usage in meat curing. Because data indicated that the nitrite content of meat cured by processes solely containing nitrate yielded extremely variable and, at times, high levels of nitrite in the product, the USDA permitted direct addition of nitrite to meat in early 1923. Studies by Kerr *et al.* (1926) revealed that the flavor and keeping quality of nitrite-cured meats were equal to those cured by traditional processes; judges were unable to distinguish meats cured by either method. A limit of a 200 ppm nitrite content in all finished meat products was established at this time. The products so cured included pork shoulders, loins, tongues, hams, and bacon, as well as corned and dried beef. On the basis of the results obtained in these experiments, the use of sodium nitrite to cure meats in federally inspected establishments was formally authorized by the USDA in 1925 (United States Department of Agriculture 1926).

During the 1930s, progress continued as meat processors adopted the use of nitrite to accelerate their cures. Surveys showed average nitrite levels of 100 ppm or less in finished products (Mighton 1936; Lewis 1937), but nitrate levels remained quite high. Stitch pumping was formally introduced in the 1930s (Fox 1974). This decade also saw the next technological advance, namely, the discovery that ascorbic acid would effectively reduce nitrite to nitric oxide (Karrer and Bendas 1934). It was not until the 1950s that ascorbic acid, ascorbate, or their isomers, erythorbic acid and erythorbate, were formally authorized for use in cures by the USDA (Hollenbeck 1956). These ingredients provide reducing conditions in meat and meat products which tend to speed up the chemical conversion of HNO_2 to NO, and nitric oxide's subsequent reaction with myoglobin. These adjuncts also serve as oxygen scavengers and help to prevent the fading of cured meat color in the presence of light and air.

The need to decrease curing time to meet increased demands for finished products led to the use of various acidulating agents during the 1960s (Karmas 1977). Glucono-δ-lactone (GDL), acid phosphates and citric acid were most common. Use of alkaline phosphates had the advantage of reducing excessive shrinkage or purge when the product was cooked; this was accomplished by increasing the water holding capacity of the meat protein. During this period, direct usage of nitric oxide gas for curing of meat was proposed (Shank 1965), but was found not to be commercially feasible. Emulsification and mixing under

vacuum of various comminuted meat formulations were also considered to speed up the process and to decrease the curing time.

Up to the early 1970s, the primary technological emphasis of nitrite usage had been to reduce the time required for curing as much as possible, in order to increase production capacity. Modern technology and scientific understanding had made it possible to utilize smaller quantities of nitrite while exercising vastly improved control over the curing of meat and meat products. Suddenly, the technological emphasis shifted to problem-solving with particular regard to *N*-nitrosamine production (Sebranek 1979).

Current Status of Processed Meats

Although meat curing processes, including smoking of meat, were designed for preservation without refrigeration, cured meats continue to have an important place in our diet. Current curing mixtures employed in North America contain salt, nitrite, nitrate (limited to certain types of products), reductants, phosphates, seasonings and other additives (*e.g.*, sweeteners, binders/fillers/extenders, gelling agents and smoke). Noteworthy is the fact that most curing ingredients these days are restricted in use by regulatory agencies. Nitrite addition levels are most stringently monitored in industrialized countries as a result of the *N*-nitrosamine scare of the 1970s. A general guide of Canadian practices to ingredients for sausage and processed meat manufacture has been compiled by Shand and Prefontaine (1995).

Salt is basic to all curing mixtures and remains the bulk ingredient even though the 1990s health-conscious consumer searches for low sodium-containing foodstuffs. In addition to its limited preservative action against microorganisms by lowering water, salt develops flavor and helps to solubilize proteins that are important for the emulsion stability of comminuted meat products. Sodium chloride levels vary from product to product. In typical formulations, the levels are 1.2–1.8% in bacon, 2–3% in hams, 1–2% in sausages and 2–4% in jerkies. Potassium chloride is used in the manufacture of sodium-reduced meat products and its concentration ranges from 0.4–0.7%; however, it can impart bitter and metallic flavors if used at greater than 0.75%. Although the salt content in meat products may be as high as 4%, this level is not sufficient to exert a complete bacteriostatic action. Consequently, other preservation techniques such as refrigeration, dehydration, acidification, thermal processing or smoking are needed.

Addition of alkaline phosphates such as sodium tripolyphosphate and sodium hexametaphosphate to ham, roast beef, sausage, solid meat cuts and poultry products aids in solubilizing proteins, particularly myofibrillar proteins, and therefore improves binding of comminuted and restructured-type meat products. Furthermore, phosphate usage in cures has been reported to increase pH, ionic

strength, moisture retention (*i.e.*, juiciness) and to assist in retarding the development of oxidative rancidity and improve the color and tenderness of finished products (Savich and Jansen 1957; Mahon *et al.* 1971; Smith *et al.* 1984). The maximum regulated level of phosphates in meat in Canada is 0.5% and typical usage for hams, bacon and cooked sausages is 0.3–0.5%, 0.2–0.25% and 0.3–0.4%, respectively. Metallic flavors in meat products have been noticed if phosphate levels are greater than 0.45%.

Sodium acid pyrophosphate is added to bacon and hams at a 0.05% level in some preparations to decrease pH and speed up cure development. Acid phosphates are typically not used in sausages as a rapid pH decline can cause emulsion breakdown. On the other hand, acidulants such as GDL, citric acid or its sodium salt are added to some sausage and fermented sausage formulations. Typical concentrations for GDL and citrate range from 0.1–0.75% and 0.1–0.5%, respectively. These acidifiers lower pH, contribute a desired tangy flavor and help color fixation in cured products. As a consequence of a pH decrease, sausage products containing GDL or citric acid should be stuffed and thermally processed in an expedient manner to prevent emulsion breakdown.

Sweeteners added to meat products include table sugar (*i.e.*, sucrose), brown sugar, dextrose, glucose solids, corn syrup solids and lactose. These carbohydrates add flavor, lower water activity, assist in browning reactions during thermal processing (*i.e.*, specifically dextrose), and help to moderate the astringency of salt in certain products. Lactose has little sweetening ability and may contribute bitterness in certain items, but is present in sausages only when nonfat dried milk is included in the formulation (Hedrick *et al.* 1994). Typical addition levels of lactose range from 0–3% in hams, 0.2–1% in bacon and up to 1% in sausages. Care in terminology should be noted. When professionals in the meat industry refer to glucose they usually mean glucose solids, a hydrolyzed starch product, and not dextrose itself.

Extenders, binders and fillers refer to nonmeat ingredients that are incorporated into sausage and loaf products. They can help improve meat batter stability, improve water binding capacity, enhance texture and flavor, reduce product shrinkage during thermal processing, improve slicing characteristics and reduce formulation costs (Hedrick *et al.* 1994). Extenders commonly used in sausage formulations are characterized by a high protein content and are either soybean or dried milk products. Both items impart a distinctive flavor to meat products and therefore this limits their usage. Yet, soy protein concentrate and isolated soy protein contain approximately 70 and 90% protein, respectively, and both possess a bland flavor. In Canada, soy protein flours/grits, concentrates and isolates are added to steakettes, burgers and cooked sausages at 0.5–5.0% levels for their water and fat binding properties, texture modification and to increase the overall protein content of the product.

Other extenders used in sausages and loaves include (1) cereal flours obtained from wheat, rye, barley, corn or rice; (2) starch extracted from these flours or from potatoes (either natural or modified); and (3) corn syrup or corn syrup solids. Flours from these cereals are high in starch and therefore can bind large amounts of water but are relatively low in protein. They are added to fresh sausages, steakettes, burgers and cooked sausages at concentrations ranging from 1-7% for water and fat binding, as well as purge control. Toasted wheat crumbs or cracker crumbs are added to fresh sausage, steakettes and burgers at levels from 1-6%. They minimize fry-away, absorb moisture and provide a source of nonmeat protein (*n.b.*, toasted wheat crumb contains 18% protein). Wheat flour and products thereof are much cheaper than soy counterparts, but unfortunately enzymatic activity of wheat flour deteriorates the color of fresh sausages and therefore wheat additives have to be used sparingly.

A texturized vegetable protein is generally produced through an extrusion/cooking process. These extruded products contain a mixture of soy flour with other additives, such as flavoring and coloring. Texturized vegetable proteins are used as extenders for ground beef and as binders and extenders in meat patties. Extended protein products reduce formulation costs, have a meat-like texture, hydrate rapidly and have an affinity for moisture retention. Besides burgers, they are useful in steakettes and pizza toppings.

Seasonings, including spices and their extracts, dried herbs, hydrolyzed plant and vegetable proteins and autolyzed yeast, do not enter in the curing reaction but do impart unique flavors and appearance to meat products. They are added to enhance or modify the existing flavor of a processed meat product and allow processors to create new products or to provide variety in existing ones. Salt and pepper form the basis for sausage seasoning formulations. Nevertheless, artistry is required for imaginative and successful employment of seasonings, and for this reason meat processors guard the secrecy of their seasoning formulations very closely. In addition to flavor improvement, seasonings can contribute to the preservation of meat. For example, certain spices and herbs possess antioxidant properties and thereby reduce the rate of oxidative rancidity development (Nakatani 1997). A list of some of the common spices used in meat products, their origin, and some typical examples of their use in processed meats are presented in Table 2.2 (Hedrick *et al.* 1994).

Of all the aforementioned additives to cured meats, nitrite is the most important one when used in sufficient quantities, but is harmful if used too freely. Nitrite is responsible for the typical color and flavor associated with cooked cured meat. It also acts as an antioxidant and retards the formation of *Clostridium botulinum* toxin. Addition of sodium nitrite to meat and meat products is highly regulated in the industrialized countries. In Canada for example, a maximum of 200 ppm (*i.e.*, 0.32 oz nitrite per 100 lb raw batch) is permitted in products such as cooked sausage, hams and corned beef. However,

TABLE 2.2
TYPES AND ORIGINS OF SPICES COMMONLY USED IN PROCESSED MEATS[1]

Spice	Part of Plant	Chief Regions Grown	Use
Allspice	Dried, nearly ripe fruit of *Pimenta officinalis*	Jamaica, Cuba, Haiti, Republic of Trinidad and Tobago	Bologna, pickled pigs' feet, head cheese
Anise (seed)	Dried ripe fruit of *Pimpinella anisum*	Russia, Germany, Scandinavia, Czech Republic, France, The Netherlands, Spain	Dry sausage, mortadella, pepperoni
Bay leaves (laurel leaves)	Dried leaf of *Laurus nobilis*	Mediterranean region, Greece, Italy, England	Pickle for pigs' feet, lamb, pork tongue
Cardamom	Dried ripe seeds of *Elettaria cardamomum*	Malabar coast of India, Sri Lanka, Guatemala	Frankfurters, liver sausage, head cheese
Cassia	Dried bark of *Cinnamomum cassia, C. loureirii, C. burmanni*	People's Republic of China, India, Indochina	Bologna, blood sausage
Celery seed	Dried ripe fruit of *Apium graveolens*	Southern Europe, India	Pork sausage
Cinnamon	Dried bark of *Cinnamomum zeylanicum* and *C. loureirii*	Sri Lanka, Sumatra, Java, Vietnam, Borneo, Malabar coast of India	Bologna, head sausage
Clove	Dried flower buds of *Eugenia caryophyllata*	Brazil, Sri Lanka, Tanzania, Malagasy Republic	Bologna, head cheese, liver sausage
Coriander	Dried ripe fruit of *Coriandrum sativum*	England, Germany, Czech Republic, Hungary, Russia, Morocco, Malta, India	Frankfurters, bologna, Polish sausage, luncheon specialities
Cumin	Dried ripe seeds of *Cuminum cyminum*	Southern Europe, India, Mediterranean areas of North Africa, Saudi Arabia, India, People's Republic of China	Curry powder
Garlic	Fresh bulb of *Allium sativum*	Sicily, Italy, Southern France, Mexico, South America, India, United States	Polish sausage, many types of smoked sausage
Ginger	Dried rhizome of *Zingiber officinale*	Jamaica, West Africa, West Indies	Pork sausage, frankfurters, corned beef
Mace	Dried waxy covering that partly encloses the seed of nutmeg, *Myristica fragrans*	Southern Asia, Indonesia and East Malaysia	Veal sausage, liver sausage, frankfurters, bologna

TABLE 2.2 Continued

Spice	Part of Plant	Chief Regions Grown	Use
Marjoram	Dried leaf of *Marjorana hortensis* or *Origanum vulgare*	Northern Africa, Greece, and other Mediterranean countries	Liver sausage, Polish sausage, head cheese
Mustard (black, white, yellow, brown, red)	Dried ripe seed of *Brassica nigra, B. juncea, B. alba*	China, Japan, India, Italy, Russia, The Netherlands, England, United States, Canada	Good in all sausage
Nutmeg	Dried and ground ripe seed of *Myristica fragrans*	Southern Asia, Indonesia and East Malaysia	Veal sausage, bologna, frankfurters, liver sausage, head cheese
Onion	Fresh bulb of *Allium cepa*	Worldwide	Liver sausage, head cheese, baked loaf
Paprika	Dried, unripe fruit of *Capsicum annuum*	Hungary, Spain, United States, Ethiopia	Frankfurters, Mexican sausage, dry sausage
Pepper (black)	Dried, unripe fruit of *Piper nigrum*	Singapore, Lampung, Sumatra, Penang, Sarawak, Thailand, India, Philippines, Indonesia, Tanzania	Bologna, Polish sausage, head cheese
Pepper (cayenne, red)	Dried ripe fruit of *Capsicum frutescens*	Tanzania, Japan, Mexico, United States	Frankfurters, bologna, veal sausage, smoked country sausage
Pepper (white)	Dried, ripe decorticated fruit of *Piper nigrum*	Singapore, Thailand	Good in all sausage
Pimento	Ripe, undried fruit of *Capsicum annuum*	Spain, United States	Loaves
Sage	Dried leaves of *Salvia officinalis*	Dalmatian coast, United States	Pork sausage, baked loaf
Thyme	Dried leaves and flowering tops of *Thymus vulgaris*	Mediterranean coast	Good in all sausage

[1]Table from Hedrick *et al.* 1994.

typical industrial levels in such products range from 150–180 ppm (*n.b.*, regulated levels are based on input to product formulation before any cooking, smoking or fermentation and are commonly added as a cure salt such as Prague powder). In the case of Canadian side bacon, the maximum regulated level is 120 ppm (*i.e.*, 0.19 oz nitrite per 100 lb). Nitrates are only permitted in specialty products that require a long cure such as dry or semidry sausages. In Canada, a maximum level of 200 ppm, in addition to the 200 ppm of nitrite, is allowed in such products. The characteristic attributes that nitrite imparts to meat, and ramifications and possible alternatives to its usage are discussed in the remaining chapters of this book.

REFERENCES

Binkerd, E.F. and Kolari, O.E. 1975. The history and use of nitrate and nitrite in the curing of meat. *Food Cosmet. Toxicol.* **13**, 655–661.

Doran, G.F. 1917. Art of curing meats. U.S. Patent 1,212,614.

Flores, J., Biron, C., Izquierdo, L. and Nieto, P. 1988. Characterization of green hams from Iberian pigs by fast analysis of subcutaneous fat. *Meat Sci.* **23**, 253–262.

Flores, M., Spanier, A.M. and Toldrá, F. 1998. Flavour analysis of dry-cured ham. In *Flavor of Meat, Meat Products and Seafoods, Second Edition*, ed. F. Shahidi. Blackie Academic and Professional, London, UK, pp. 320–341.

Flores, J. and Toldrá, F. 1993. Curing. In *Encyclopedia of Food Science, Food Technology and Nutrition*, ed. R. Macrae, R.K. Robinson and M.J. Sadlor. Academic Press, London, UK, pp. 1277–1282.

Fox, J.B. Jr. 1974. Role of cure accelerators. In *Proceedings of the Meat Industry Research Conference*. American Meat Institute Foundation, Arlington, VA, pp. 17–21.

Haldane, J. 1901. The red colour of salted meat. *J. Hygiene* **1**, 115–122.

Hedrick, H.B., Aberle, E.D., Forrest, J.C., Judge, M.D. and Merkel, R.A. 1994. *Principles of Meat Science*, Third Edition. Kendall/Hunt Publishing Company, Dubuque, IA.

Hoagland, R. 1908. The action of saltpeter upon the color of meat. In *25th Annual Report of the Bureau of Animal Industry, U.S. Department of Agriculture*. Government Printing Office, Washington, DC, pp. 301–314.

Holland, G.E. 1983. Curing of ham and smoked meats — the state of the art. *National Provisioner*, March 5, 6–13.

Hollenbeck, C.M. 1956. Curing of meat. U.S. Patent 2,739,899.

Jensen, L.B. 1953. Early preparations of foods. In *Man's Foods. Nutrition and Environments in Food Gathering Times and Food Producing Times*. The Garrard Press, Champaign, IL, pp. 159–170.

Jensen, L.B. 1954. Introduction and history. In *Microbiology of Meats, Third Edition*. The Garrard Press, Champaign, IL, pp. 1-11.

Karmas, E. 1977. Color and flavor. In *Sausage Products Technology*, Food Technology Review No. 39. Noyes Data Corporation, Park Ridge, NJ, pp. 4-58.

Karrer, P. and Bendas, H. 1934. Das Verhalten der Ascorbinsäure gegen Nitrate und Nitrite. *Helv. Chim. Acta*, **17**, 743-745.

Kerr, R.H., Marsh, C.T.N., Schroeder, W.F. and Boyer, E.A. 1926. The use of sodium nitrite in the curing of meat. *J. Agric. Res.*, **33**, 541-551.

Kisskalt, K. 1899. Beiträge zur Kenntnis der Ursachen des Rothwerdens des Fleisches beim Kochen, nebst einigen Versuchen über die Wirkung der schwefligen Säure auf die Fleischfarbe. *Arch. Hyg.* **35**, 11-18.

Lehmann, K.B. 1899. Über das Haemorrhodin, ein neues weitverbreitetes Blutfarbstoffderivat. *Sitzung. phys.-med. Ges. in Würzb.* **48**, 57-61.

Lewis, W.L. 1937. The use of nitrite of soda in curing meat. Institute of American Meat Packers, Chicago, IL.

López, M.O., de la Hoz, L., Cambero, M.I., Gallardo, E., Reglero, G. and Ordóñez, J.A. 1992. Volatile compounds of dry hams from Iberian pigs. *Meat Sci.* **31**, 267-277.

Mahon, J.H., Schlamb, K. and Brotsky, E. 1971. General concepts applicable to the use of polyphosphates in red meat, poultry and seafood processing. In *Phosphates in Food Processing*, ed. J.M. DeMan and P. Melynchyn. The AVI Publishing Co., Inc., Westport, CT, pp. 158-181.

Mighton, C.J. 1936. An analytical survey of cured meats. Publication 33. Institute of American Meat Packers, Chicago, IL, p. 22.

Nakatani, N. 1997. Antioxidants from spices and herbs. In *Natural Antioxidants. Chemistry, Health Effects, and Applications*, ed. F. Shahidi. AOCS Press, Champaign, IL, pp. 64-75.

Polenske, E. 1891. Über den Verlust, welchen das Rindfleisch an Nährwerth durch das Pökeln erleidet, sowie über die Veränderungen Salpeter-haltiger Pökellaken. Mittheilungen aus dem chemischen Laboratorium des Kaiserlichen Gesundheitsamtes, 13. *Arb. K. GesundhAmt.* **7**, 471-474.

Sabio, E., Vidal-Aragón, M.C., Bernalte, M.J. and Gata, J.L. 1998. Volatile compounds present in six types of dry-cured ham from south European countries. *Food Chem.* **61**, 493-503.

Savich, A.L. and Jansen, C.E. 1957. Improving the color of meat. U.S. Patent 2,798,812.

Sebranek, J.G. 1979. Advances in the technology of nitrite use and consideration of alternatives. *Food Technol.* **33**(7), 58-62, 93.

Shand, P.J. and Prefontaine, D.J. 1995. Guide to ingredients for sausage and processed meats. Saskatchewan Food Product Innovation Program — Product Development and Research for Meat Processors, University of Saskatchewan, Saskatoon, SK, Canada.

Shank, J.L. 1965. Meat-curing process. U.S. Patent 3,220,855.

Smith, L.A., Simmons, S.L., McKeith, F.J., Bechtel, P.I. and Brady, P.L. 1984. Effects of sodium tripolyphosphate on physical and sensory properties of beef and pork roasts. *J. Food Sci.* **49**, 1636–1637, 1641.

Toldrá, F. 1992. The enzymology of dry-curing of meat products. In *New Technologies for Meat and Meat Products*, ed. F.J.M. Smulders, F. Toldrá, J. Flores and M. Prieto. Audet, Nijmegen, The Netherlands, pp. 209–231.

Toldrá, F. and Flores, M. 1998. The role of muscle proteases and lipases in flavor development during the processing of dry-cured ham. *CRC Crit. Rev. Food Sci. Nutr.* **38**, 331–352.

Toldrá, F., Flores, M., Navarro, J.-L., Aristoy, M.-C. and Flores, J. 1997*a*. New developments in dry-cured ham. In *Chemistry of Novel Foods*, ed. A.M. Spanier, M. Tamura, H. Okai and O. Mills. Allured Publishing Corporation, Carol Stream, IL, pp. 259–272.

Toldrá, F., Flores, M. and Sanz, Y. (1997*b*). Dry-cured ham flavour: enzymatic generation and process influence. *Food Chem.* **59**, 523–530.

Townsend, W.E. and Olson, D.G. 1987. Cured meats and cured meat products processing. In *The Science of Meat and Meat Products, Third Edition*, ed. J.F. Price and B.S. Schweigert. Food & Nutrition Press, Trumbull, CT, pp. 431–456.

United States Department of Agriculture, Bureau of Animal Industry. 1926. USDA, BAI order 211, Amendment 4, pp. 1–67.

CHAPTER 3

THE COLOR OF MEAT

Assessing meat by its aesthetic appeal is something consumers do every time they walk up to the counter in a butcher's shop. The four main determinants of meat quality at the purchaser level are color, juiciness, flavor and toughness/tenderness. Of these attributes, color is the most important one because it is the first impression consumers have of any meat product and often is their basis for product selection or rejection (Hood and Riordan 1973); it's generally believed that a meat cut with an aesthetically appealing color will yield a high quality product (Cassens *et al.* 1988). Those meats that have become discolored, more often than not, have to be ground and marketed in a reduced-value form.

The color of fresh meat may range from the deep purplish-red of freshly cut beef to a delicate pink of chicken breast to a greenish tinge from improperly-stored pork bellies. Deterioration of meat color has long been used as an early warning of meat going-off and avoiding this poses a challenge to the meat industry. Both the United States Department of Agriculture (USDA) and Canadian Food and Drugs Act Regulations prohibit the use of chemical substances, such as ascorbic acid, its derivatives, or nicotinic acid to artificially prolong fresh meat color. With preserved meats this is not so, and the bright pink color of nitrite-cured bacon and ham has long been used as a selling point, particularly since the development of transparent film vacuum packaging. Yet, the fading of the color of cooked cured-ham under fluorescent lighting in supermarkets has long been recognized as a problem (Pate *et al.* 1971). Fortunately, the color of meat can be controlled somewhat if the many factors that influence it are adequately maintained.

The term "meat" signifies the edible flesh or muscle of animals that is acceptable for consumption by man. All processed or restructured products that might be prepared from these tissues are included in the definition. On the other hand, edible and acceptable have different interpretations depending upon one's cultural background and religion. While nearly every species of animal can be used as meat, most meat consumed by man comes from domesticated animals and aquatic species.

Lean meat is a complex biological system and its composition is relatively constant over a wide range of animals. Variation is most marked in the lipid content, which may be evident from different degrees of marbling (Varnam and Sutherland 1995). Lean meat contains approximately 75% water, 19% protein,

2.5% lipid, 1.2% carbohydrate, 2.3% inorganics, non-protein compounds containing nitrogen and trace amounts of vitamins (Lawrie 1991). The color of fresh meat is governed largely by the concentration and chemical nature of its hemoproteins, especially myoglobin (Mb), and the temperature/pH history of the post-slaughtered muscle (Ledward 1992).

The native pigment in muscle tissue is Mb. In living tissue, Mb is the storehouse of oxygen that is used in the normal biochemical processes of the muscle. Because activity of muscles differs greatly and oxygen demands vary, different Mb concentrations are found in various muscles of the animal. For example, the back muscles of hogs (*i.e.*, loin) are used primarily for support and posture and, therefore, have a much lower oxygen requirement than a leg muscle (*i.e.*, ham or shoulder) which is used for movement. Besides muscle type, Mb levels are influenced by many intrinsic factors related to function. The most important ones include species (*e.g.*, the muscles of the hare are richer in Mb than those of the rabbit), breed, sex (*e.g.*, the muscles of the bull contain more Mb than those of the cow), age (*e.g.*, the muscles of the steer are richer in Mb than those of the calf), anatomical location of the muscle (*e.g.*, the leg muscles in the chicken are richer in Mb than those of the breast), training or exercise (*e.g.*, the muscles of stall-fed animals generally contain less Mb than those of their free-range counterparts), and nutrition (Lawrie 1991; Ledward 1992). Species is perhaps the most easily appreciated factor affecting Mb content. Typical Mb concentrations of *longissimus dorsi* muscle in mature meat animals are 0.02% in rabbit, 0.06% in pig, 0.25% in sheep, 0.50% in ox and 0.91% in blue whale (Lawrie 1991). Synowiecki *et al.* (1992) reported that the hemoprotein content of seal muscle tissue was 5.9%, of which 82% was Mb.

The overall redness of fresh meat is largely governed by the concentration of Mb (molecular weight *ca.* 16,700 Da) and to a lesser extent by hemoglobin (Hb; molecular weight *ca.* 67,000 Da), as well as the forms in which they exist. In the living animal, Mb accounts for only 10% of the total iron, but during slaughter, bleeding removes most of the iron as Hb. In a well-bled piece of bovine skeletal muscle, as much as 95% or more of the remaining iron is accounted for as Mb (Francis 1985). The greater the Mb level, the more intense the color of the meat. Besides Mb, the remaining tissue pigments include Hb (especially if bleeding of the carcass has been inefficient), cytochromes, vitamin B_{12} and the flavins. These pigments generally contribute little to the color of meat.

Myoglobin and Fresh Meat Color

Myoglobin is a globular protein that is made up of a single polypeptide chain (*i.e.*, globin) consisting of *ca.* 153 amino acids and a prosthetic heme group, an iron(II) protoporphyrin-IX complex (Fig. 3.1). It is extremely

compact with dimensions *ca.* 45 × 35 × 25 Å (Stryer 1981). In simplified terms, the globin is folded around the iron of the heme group in 8 α-helical segments ranging in length from 7 to 24 residues, separated by non-helical regions. The helical regions make up nearly 80% of the molecule (Ledward 1992). The well-defined, three-dimensional structure of globin is stable over a fairly wide range of external conditions, but can be disrupted by sufficiently drastic changes in physical or chemical environments. This process, known as denaturation, has important consequences regarding structural and functional aspects of the protein. The heme moiety is held in a cleft of the globin by a coordinate bond between the imidazole nitrogen of the proximal histidine residue and the ferrous ion, and by several non-polar and hydrogen-bonding interactions

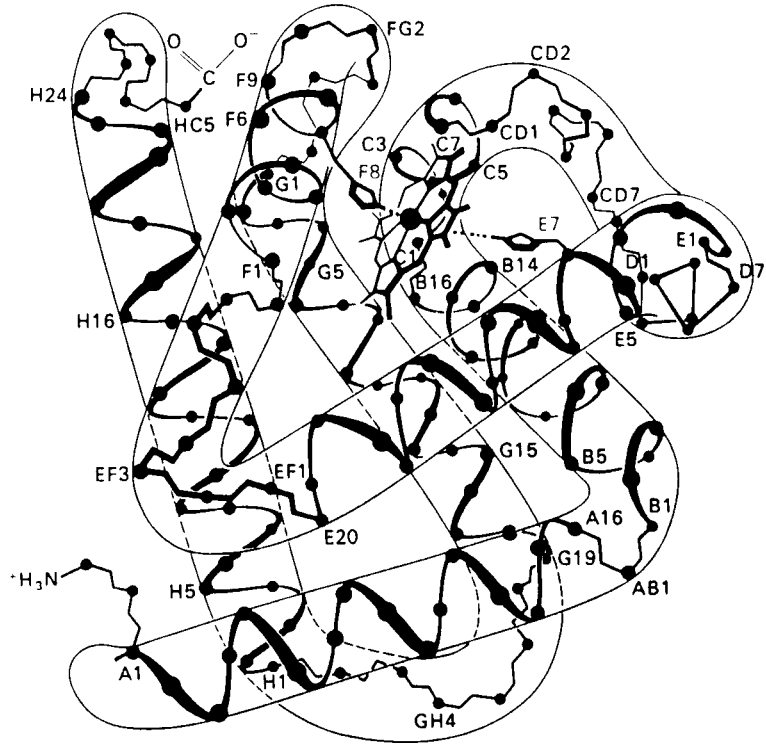


FIG. 3.1. THREE-DIMENSIONAL STRUCTURE OF MYOGLOBIN SHOWING THE LOCATION OF HEME WITH IRON ATOM COORDINATED TO HISTIDINE GROUPS LABELED F8 AND E7. AMINO ACID RESIDUES IN HELICAL REGIONS ARE LABELED WITH A SINGLE LETTER AND NUMBER WHILE RESIDUES IN NON-HELICAL REGIONS ARE LABELED WITH TWO LETTERS AND A NUMBER
(From Dickerson 1964).

at the porphyrin periphery. It is this heme group that gives Mb and its derivatives their distinctive color, and is the principal site for meat curing as it relates to color development. However, slight variations in the amino acid composition and isoelectric point of Mb from different species may account for observed differences in visual appearance and color stability of meats.

The heme molecule is an organometallic compound. The organic portion consists of four pyrrole groups linked by methine bridges forming a tetrapyrrole ring. Four methyl, two vinyl, and two propionate side chains are attached to the ring yielding the protoporphyrin-IX molecule. The iron atom is bonded to the four nitrogens in the center of a near-planar ring. Whether ferrous or ferric, the iron is coordinated in an octahedral environment, such that it can further accept two ligands normal to the heme plane. These sites are occupied by an imidazole group of a histidine residue of globin and an atom possessing a free electron pair. These bonding sites are called the fifth and sixth coordination positions, respectively. As mentioned above, the heme iron atom may exist in the ferrous (2+) or the ferric (3+) state, depending on the presence of reductants or oxidants in the medium. Lacking a covalent complex, either state can coordinate water. The structure and chemistry of the iron atom are keys to understanding the reactions and color changes that Mb undergoes (Livingston and Brown 1981).

The electron distribution of iron consists of an argon core surrounded by six 3d electrons and two 4s electrons. The atom (*i.e.*, $1s^2 2s^2 2p^6 3s^2 3p^6 3d^6 4s^2$) is easily ionized to the ferrous state by loss of the two 4s electrons. The ferrous ion can donate one of its 3d electrons to an oxidant giving rise to the electronically more stable ferric ion. The electron distribution of these ions is provided in Fig. 3.2, and in both cases the 3d orbitals are 5-fold-degenerate. Although unoccupied in the free ionic forms, the 4s and three 4p orbitals are involved in coordination complexes.

The ferrous ion can complex with up to a maximum of six donor ligands by both electrostatic and covalent interactions, depending upon the electron-donating potential and spatial arrangement of the ligand. Because these potentials vary, the energy of the 3d orbitals is split according to the ligand field theory. In most biological complexes, the orbitals occupy an octahedral orientation in which the five 3d orbitals of the metal divide into two sets, namely the t_{2g} set of d_{xy}, d_{xz}, d_{yz} (triply degenerate) and the e_g set of $d_{x^2-y^2}$, d_{z^2} (doubly degenerate). The iron 3d orbitals directed toward the ligands (*i.e.*, the e_g set) are of higher energy resulting from electrostatic repulsion than the 3d orbitals of the t_{2g} set whose energy remains relatively undisturbed. In a ligand field having tetragonal or rhombic symmetry, there is a further loss of degeneracy in the t_{2g} and e_g orbitals as illustrated in Fig. 3.3.

According to Hund's first rule, valence electrons continue to enter different orbitals of the same energy as long as possible. Furthermore, the electrons

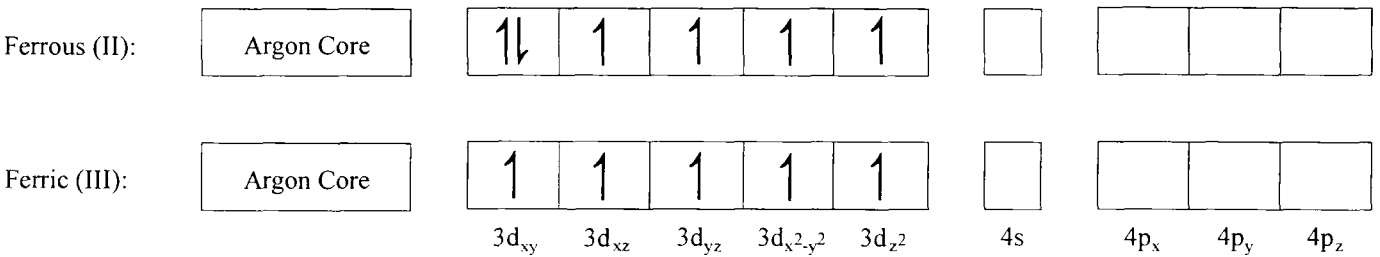


FIG. 3.2. ELECTRON DISTRIBUTION OF FERROUS AND FERRIC ION
(Adapted from Giddings 1977).

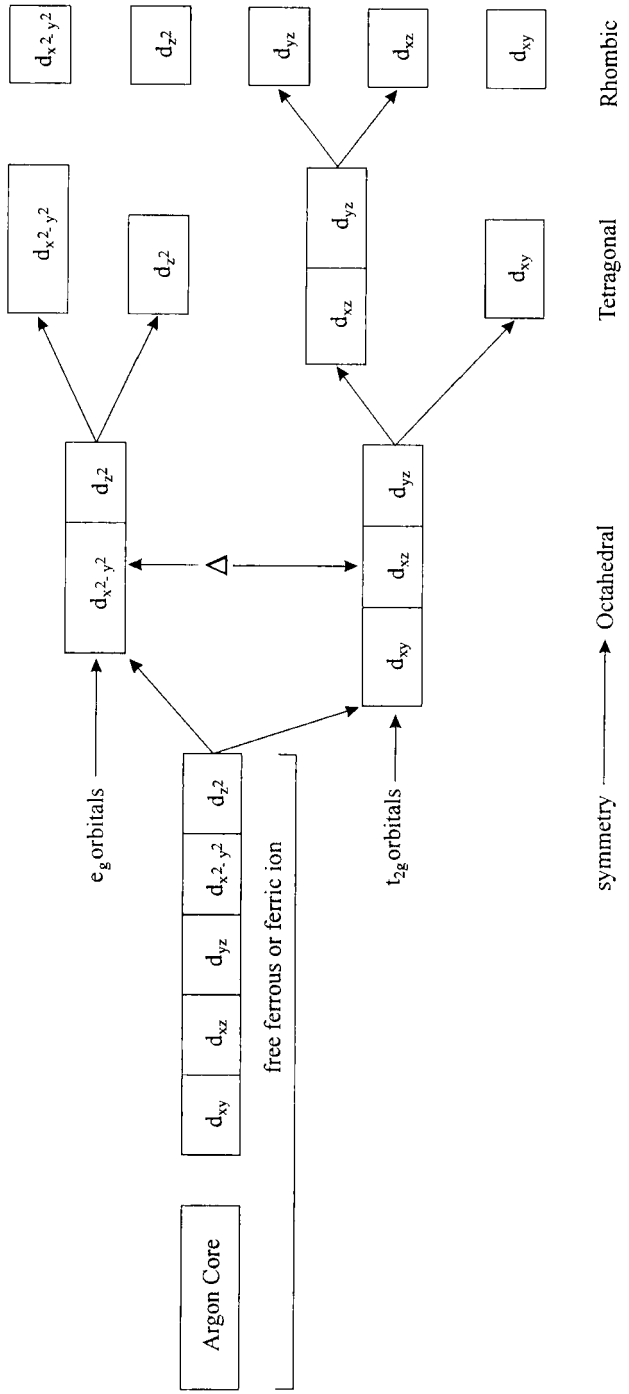


FIG. 3.3. SPLITTING OF THE 3D ORBITALS IN LIGAND FIELDS HAVING OCTAHEDRAL, TETRAGONAL OR RHOMBIC SYMMETRY
(Adapted from Giddings 1977)

occupying degenerate orbitals will have parallel spins (*i.e.*, Pauli exclusion principle). When two electrons are placed in the same orbital, their spins are paired and there is an unfavorable energy contribution resulting from increased electrostatic repulsion between electrons compelled to occupy the same region of space. Because the energy of the 3d-orbitals is split in an octahedral complex, the question of whether electrons continue entering the t_{2g} orbitals before that of the e_g set is dependent upon the energy required to cause pairing of two electrons in the same orbital and the energy difference between the t_{2g} and e_g orbitals. Configurations with the maximum number of unpaired electrons are called high-spin configurations, and those with the minimum number are called low-spin or spin-paired configurations (Cotton *et al.* 1995). Figure 3.4 depicts the possible configurations for the ferric and ferrous ions.

In the purple-red deoxy-form, the pentacoordinate heme Fe(II) compound is high spin (t_{2g}^4 e_g^2, $S=2$) with an ionic radius of 78 picometers (pm), which is too large to fit into the porphyrin plane. Consequently, the ferrous ion projects greater than 25 pm above the porphyrin ring plane toward the proximal histidine giving the molecule a square pyramidal configuration (Kendrew 1963; Thompson 1988). A vacant binding site for ligands lies on the side of the porphyrin away from the proximal histidine. Cherry-red oxymyoglobin (MbO_2) is formed upon oxygenation. Oxygen molecules diffuse through the aqueous environment of meat and enter the hydrophobic hematin cleft of Mb to occupy the sixth coordination site. This hexacoordinate low-spin (t_{2g}^6, $S=0$) Fe(II) complex is diamagnetic and is believed to lie in the plane of the porphyrin ring giving the molecule an octahedral configuration (Thompson 1988). Two types of bonding occur between the metal ion and the O_2 molecule. A σ bond is formed by donation of an unpaired electron of O_2 in the π^* orbital to the d_{z^2} orbital of the iron cation. Most of the ligands which bind to Mb have this type of bonding (*i.e.*, an electronegative atom, N or O, donates electrons to the metal). The other type of bonding occurring is π bonding, in which the iron donates electrons back to the ligand *via* the ligand's π or π^* orbitals. This type of bonding is sometimes called back-bonding and is very important in MbO_2. Because the O_2 molecule is a weak σ-electron donor, a histidine residue of globin opposite to the O_2 molecule in the heme cavity (*i.e.*, the so-called distal histidine) participates in forming a strong bond between the iron and the O_2 molecule. The histidine, bonded through a nitrogen atom, feeds extra electron density into the iron and subsequently the O_2 molecule. This results in a strengthening of the π bonding and the stability of the MbO_2 complex. For π bonding to occur, the metal must have sufficient electron density for donation to its ligand. In the case of ferrous iron this is so, and the relatively low charge on the nucleus leads to expanded *d*-orbitals (Livingston and Brown 1981). Besides O_2, Mb can complex with other ligands in its vacant sixth coordinate position such as NO and CO. According to Giddings (1977), six-coordinate Mb

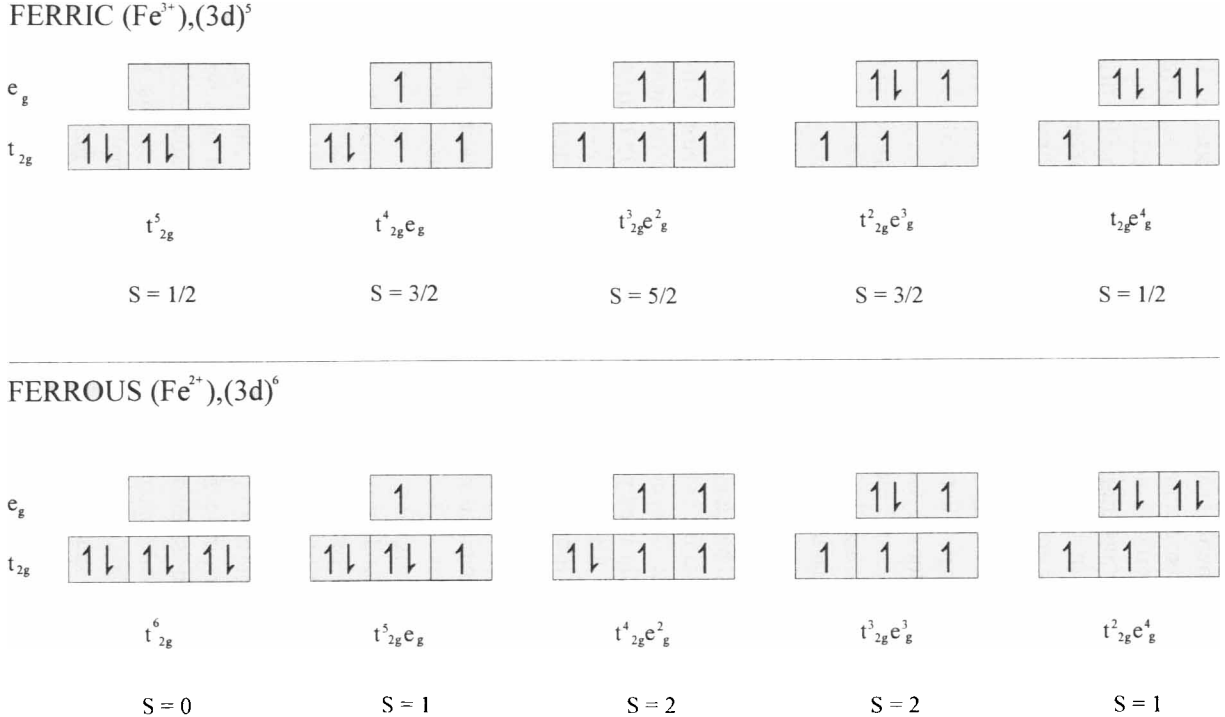


FIG. 3.4. THE DISTRIBUTION OF FERRIC AND FERROUS IRON 3D ELECTRONS IN D ORBITALS OF SIX-COORDINATE OCTAHEDRAL COMPLEXES. THE SPIN ALIGNMENTS ARE THOSE THAT HAVE MAXIMUM TOTAL MAGNETIC SPIN (S) FOR EACH CASE. THE HIGH-SPIN METMYOGLOBIN COMPLEX, HAVING A WATER MOLECULE AS THE SIXTH LIGAND, HAS S = 5/2. LOW-SPIN FERROMYOGLOBIN COMPLEXES, SUCH AS NITROSYLMYOGLOBIN, HAVE S = 0, WHILE DEOXYFERROUS MYOGLOBIN, WITH A FIVE COORDINATE SQUARE PYRAMIDAL SYMMETRY, IS THE ONLY HIGH-SPIN FERROMYOGLOBIN COMPLEX HAVING S = 2 (Adapted from Giddings 1977).

complexes are octahedral only to a first approximation as they generally exhibit tetragonal and rhombic distortion resulting in additional loss of degeneracy. The energy differential (*i.e.*, Δ) between the t_{2g} and e_g orbitals, as depicted in Fig. 3.3, depends largely on the sixth coordination site ligand, and is greater for strong field ligands such as O_2 and NO than for weak-field ligands.

The bright red color of fresh meat, due to MbO_2, results from Mb's great affinity for O_2, and it is this color that the consumer associates with freshness. Myoglobin reacts rapidly and reversibly with O_2 and consequently, the surface of comminuted meat blooms to a bright red color within minutes upon exposure to air. However, MbO_2's stability depends on a continuing supply of O_2 because the enzymes involved in oxidative metabolism rapidly use the available O_2 (Hedrick *et al.* 1994). With time, the small layer of MbO_2 present on the surface of the meat propagates downward, but the depth to which O_2 diffuses depends on several factors, such as activity of oxygen-utilizing enzymes (*i.e.*, O_2 consumption rate of the meat), temperature, pH, and external O_2 pressure (Ledward 1992). In other words, as air diffuses inward, an O_2 and a color gradient are established throughout the meat. Muscles differ in their rates of enzyme activity which, in turn, regulate the amount of O_2 available in the outermost layers of tissue. As the pH and temperature of the tissue increase, enzymes become more active and the O_2 content is reduced. Consequently, maintaining the temperature of meat near the freezing point minimizes the rate of enzyme activity and O_2 utilization and helps maintain a bright red color for the maximum possible time (Hedrick *et al.* 1994). O'Keeffe and Hood (1982) reported that after 2 hours of exposure to air at 0°C, the MbO_2 layer in different beef muscles was 1-3 mm thick, but increased to 7-10 mm after 7 days of storage. For comminuted meats where a larger surface area is exposed to air, a greater MbO_2 thickness is observed after a shorter period of time.

In contrast, the interior tissue of meat is purple-red in color. This is the color of Mb, sometimes called deoxy-Mb, and the color persists as long as reductants generated within the cells by enzyme activity are available. When these substances are depleted, the heme iron is oxidized to the ferric state. The brown pigment formed, which is characteristic of the color of meat left standing for a period of time, is called metmyoglobin (metMb). It is generated by the removal of a superoxide anion from the hematin and its replacement by a water molecule gives a high-spin ferric hematin ($t_{2g}^3\ e_g^2$, $S=5/2$). The ferric ion, unlike its ferrous counterpart, has a high nuclear charge and does not engage in strong π bonding. Therefore, metMb is unable to form an oxygen adduct. In fresh meat there is a dynamic cycle such that in the presence of O_2, the three pigments Mb, MbO_2, and metMb are constantly interconverted; all forms are in equilibrium with one another. Their respective absorption spectra are presented in Fig. 3.5. Care is exercised by the retailer to reduce the likelihood of metMb formation, as its presence downgrades the quality of fresh meats. When metMb is denatured

by thermal processing, meat remains brown in color, but this denatured pigment can be oxidized further to form yellow, green or colorless porphyrin-derived substances by bacterial action or photochemical oxidation. The interrelationship between fresh meat pigments is illustrated in Fig. 3.6 and outlined more extensively in Table 3.1.

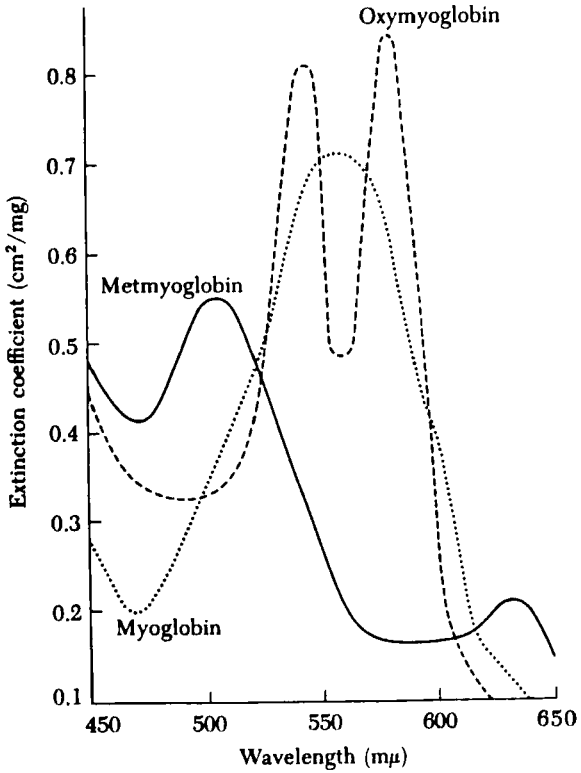


FIG. 3.5. ABSORPTION SPECTRA OF THE THREE MOST COMMON FORMS OF MYOGLOBIN IN FRESH MUSCLE TISSUE. THE SPECTRUM FOR OXYMYOGLOBIN IS TYPICAL OF ALL COORDINATE-COVALENT COMPLEXES OF HEME PIGMENTS (From Bandman 1987).

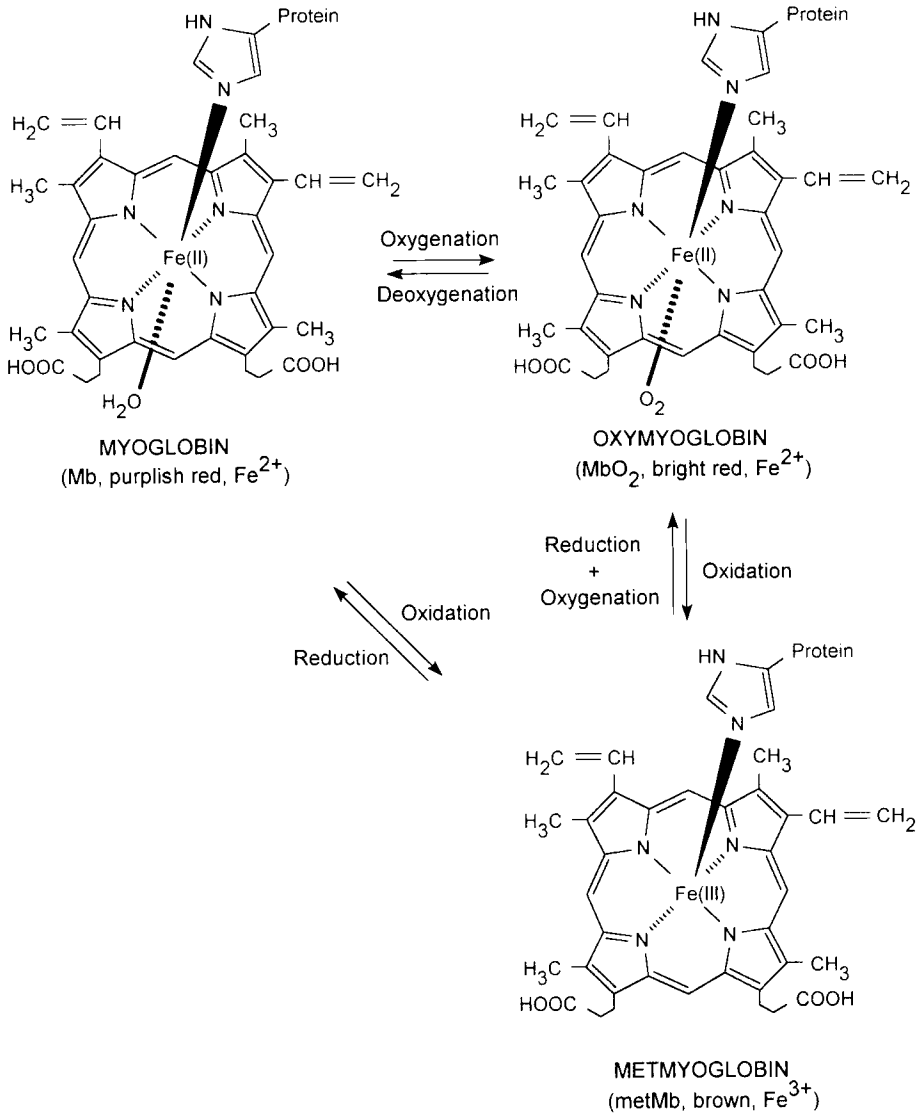


FIG. 3.6. INTERRELATIONSHIP BETWEEN PIGMENTS OF FRESH MEAT
(Adapted from Bard and Townsend 1971).

TABLE 3.1
THE COLORS OF MEAT[a]

Pigment	Color	Source	Comments
Endogenous pigments of fresh meats, raw, normal			
Hemes			
Myoglobin Hemoglobin	Purple	Reducing conditions	Interior of fresh meats. Exclusion of air
Oxymyoglobin	Red	Oxygenation	Surface of fresh meat exposed to air, "bloom"
Metmyoglobin	Brown	Oxidation	Old meat, low oxygen partial pressures as under liquid nitrogen
Cytochromes	Red		Very low concentrations, do not contribute to meat color directly
Flavins	Yellow		
Vitamin B_{12}	Red		
		–, discolored	
Nitric oxide heme pigments	Red	Cross contamination	Nitrite from cutting blocks
Carbon monoxy heme pigments	Red	Air	Semi-stable color, possible but not too likely
Oxymyoglobin	Red	Sulfite Niacinamide	Fresh meat color produced in old meats by the unscrupulous
		–, cooked, normal	
Denatured globin			
Hemichromes	Brown	Heating	Fe^{+++}, ligands in doubt
Hemochromes	Red	Heating	Fe^{++}, pink pigment of fresh meat cooked in the absence of air

Pigment	Color	Source	Comments
Endogenous pigments of cured meats, normal			
Nitrosylmyoglobin	Red	Nitric oxide	Uncooked cured meats
Nitrosyl hemochrome (cooked cured-meat pigment, CCMP)	Red	Nitric oxide	Cooked cured meats — one nitric oxide molecule per heme
		–, discolored	
Metmyoglobin	Brown	Nitrite	Under- or overture, too little or too much nitrite
Nitrimetmyoglobin	Green	Nitrite	Large excess of nitrite, acid conditions
Hemichromes	Brown	Age, acid	Old, faded cured meats
Endogenous pigments of fresh and cured meats, discolored			
Hemoglobin	Red	Capillaries	Rupture, "blood splash"
Choleglobin	Greenish	Oxidation	Oxidation under mild reducing conditions
Peroxymetmyoglobin	Red	Peroxides	Catalase negative bacteria, radiation
Hydroperoxy-metmyoglobin	Green	Peroxides	Peroxides in acid
Sulfmetmyoglobin	Green	Sulfides	Bacteria, radiation
Bile pigments	Brown	Oxidation	Ultimate degradation products
Endogenous pigments of lipid, normal			
Carotenes	Yellow		Grass-fed animals
Catecholamines	Brown		"Bronze" shoulder fat
Ceroid pigments	Brown	Lipids/proteins	Aged animals
Lipofuscin	Yellow	Lipids/proteins	Fluorescent Schiff base

Pigment	Color	Source	Comments
Endogenous pigments of lipids, discolored			
Hemoglobin	Red	Capillaries	Rupture, "fiery," "speckle"
Carbonyl/amino	Brown	Lipids/proteins	Maillard reaction
Pigments of meats produced from colorless, compounds, normal			
Carbonyl/amino	Brown	Lipids/proteins	
Caramels	Brown	Lipids	Thermal decomposition
		–, discolored	
Xanthoproteins	Green	Nitrite/gelatin	Acid conditions in channels next to bones
Exogenous pigments of cured meats, natural			
Carotenoids	Red Rose Red-orange Yellow	Alkanet Annatto Paprika Carotene Saffron Turmeric	Some added primarily for color, some are colors of added spices. Oleoresins of some added solely for color (*e.g.*, paprika)
Carminic acid	Red	Cochineal	
Chlorophyll	Green	Various spices	
		–, synthetic	
FD&C dyes	Orange		For surface color only
		–, abnormal	
Dyes	Any	Wrapping materials	Transfer to surface of meat
Dye	Purple	Grade stamp	Edible
Compounds proposed as substitutes for nitrite in cured meats			
Pyridine hemochromes	Pink, orange, purple	Pyridine derivatives	Form colored complexes with the endogenous heme pigments of meat

Pigment	Color	Source	Comments
Betaines	Red, purple	Beets	Dyes which do not diffuse well into muscle fibers
Various	Red	Bacteria	Color produced *in situ*
Lakes	Any		Dyes rendered insoluble by precipitation on inert materials
Compounds in cured meats that are initially colorless, normal or natural			
Polyphenols	Brown	Smoke	Phenolics from smoke interact on meat surface with each other and meat components
Carbonyl/amino	Brown	Smoke, meat	Maillard reaction
		–, discolorations	
Iron, copper salts	Black, green	Can seams, equipment	Corrosion resulting from meat in contact with metal surfaces
Muroxides	Purple	Ammonia	Bacterial action
Sulfides	Black	Metal	Bacteria produce H_2S
(Phosphates)	Brown		Phosphates sometimes cause meat to turn brown
Pigments of microbial origin in fresh meats, bacterial. Often cured meats.			
Phosphors	Green, blue, silver	*Pseudomonas* spp. *Achromobacter* spp. *Micrococcus* spp. *Photobacterium* spp. *Bacillus* spp. *Vibrio* spp. *Microspire* spp.	Usually not noted in well-lighted refrigerators
Fluorescent	Blue-green	*Pseudomonas* spp.	
	Cream, yellow, green	Halophiles	Lipid of hams
	Red	*Serratia* spp.	

Pigment	Color	Source	Comments
Pyrroles	Blue-purple	*S. violascieans*	"Purple stamp" in fat, "ink spot" in fat
	Blue-black	*Chromobacterium* spp. ·	
		–, *yeast*	
	Red	*Rhodotorula* spp.	
		T. rosea	Fat of meats, lard
	Purple	*Chromatorula* spp.	Muscle & fascia
Methoxydihydroxy-toluquinone	Purple	*P. spinulosus*	Old beef
		–, *molds, normal*	
Colonies	White		Surface of dry cured sausages
		–, *discolored*	
	Black	*Cladosporium* spp.	
		A. niger	
	Red	*A. candidus*	
Physical phenomena producing color in meats			
Hemes	Red & Green	Dichroism (Metamerism)	Transmission and/or reflectance of light
None	Blue, green	Iridescence	Blue-green colors playing over cut surfaces
Heme	Reddish colors	Visual perception	Redder reds due to proximity to contrasting colors, particularly green
	Pale colors	Opaque tissues	Intensity of pigment less because of white background
	Dark colors	Translucent tissues	Deep penetration of light increases saturation of reflected light

[a]Table from Fox 1987.

Cured Meat Color

In cured meats, it is the muscle pigments, not nitrite, which cause a reflectance of light characteristic of cured meat color. Nitrite merely acts to stabilize Mb through a reversible chemical bond in the same manner that the muscle pigment is stabilized by molecular oxygen in the live animal biological system or the oxygenated postmortem meat system. In other words, nitrites fix rather than impart color (Dryden and Birdsall 1980). The chemistry of nitrite curing is complex beginning with the generation of a nitrosating species and finally ending with nitrosylmyochromogen. The discussion of nitrite chemistry below has been restricted to its reaction in meat systems (Sebranek and Fox 1985; Skibsted 1992; Cornforth *et al.* 1998).

Nitrite itself is not the main nitrosating species; it's one of nitrite's derivatives. Nitrite is the conjugate base of a weak acid, namely nitrous acid (*i.e.*, HNO_2), and it has a pKa of 3.36.

$$HNO_2 \rightleftharpoons H^+ + NO_2^- \quad (1)$$

Because the pH of meat (*i.e.*, usually 5.5-6.5) is well above the pKa of HNO_2, its concentration in cured meat is very low, somewhere between 0.1 and 1.0% of the added nitrite. In meat, it's believed that the principal reactive species is the anhydride of HNO_2, dinitrogen trioxide, N_2O_3.

$$2\ HNO_2 \rightleftharpoons N_2O_3 + H_2O \quad (2)$$

Presence of endogenous reductants in muscle tissue and added ones (HRd), notably ascorbate, play an important role in kinetic aspects of the curing reaction. Dinitrogen trioxide reacts with both ascorbic acid and ascorbate, but the latter form is more reactive (Izumi *et al.* 1989).

$$N_2O_3 + HRd \rightleftharpoons RdNO + HNO_2 \quad (3)$$

$$RdNO \rightleftharpoons Rd + NO \quad (4)$$

The pH-dependence of the reaction between nitrite and ascorbate is complex owing to the different reactivities of the species. The reactivity of the nitrite/nitrous acid system increases with decreasing pH, whereas the reactivity of ascorbic acid/ascorbate as a reductant increases with increasing pH. Thus, in meat the reaction between nitrite and ascorbate is zero order with respect to

ascorbate, and therefore the rate-determining step is the dehydration of HNO_2 to N_2O_3. The paramagnetic NO molecule generated is an electron-pair donor and it forms very stable complexes with transition metal ions, such as iron.

When nitrite is added to comminuted meat, the meat turns brown because nitrite acts as a strong heme oxidant. The oxidizing capacity of nitrite increases as the pH of meat decreases, but nitrite itself may also partly be oxidized to nitrate during curing or storage. Myoglobin and MbO_2 are oxidized to metMb by nitrite. The ion itself can be reduced to NO. These products can combine with one another to form an intermediate pigment, nitrosylmetmyoglobin ($MbFe^{III}NO$).

$$MbFe^{III} + NO \rightleftharpoons MbFe^{III}NO \quad (5)$$

Nitrosylmetmyoglobin is unstable. It autoreduces with time and in the presence of endogenous and exogenous reductants in the postmortem muscle tissue to the corresponding relatively stable Fe(II) form, nitrosylmyoglobin (NOMb) (Yonetani *et al.* 1972). Killday *et al.* (1988) proposed a new mechanism for the meat curing process, as outlined in Fig. 3.7. They suggested that $MbFe^{III}NO$ is more adequately described as an imidazole-centered protein radical. This radical undergoes autoreduction yielding NOMb, and lacking exogenous reductants, reducing groups within the protein can donate electrons to the imidazole radical. Loew *et al.* (1991) reported, using peroxidase model systems, that quantum mechanical calculations show the existence of such low-lying imidazolate π-cation radical states. Similar effects of transient deproteinization of the imidazole moiety of the proximal histidine may stabilize the $MbFe^{III}NO$ radical and simplify its reduction in meat by electron transfer from added reductants.

Recently, a study carried out by Cornforth *et al.* (1998) strengthened the mechanism posed by Killday *et al.* (1988). Cornforth and co-workers examined the relative contribution of CO and NO_x towards pink ring formation in gas oven cooked beef roasts and turkey rolls. Data showed that pinking was not evident with up to 149 ppm of CO or 5 ppm of NO present in the burner gases; however, as little as 0.4 and 2.5 ppm of NO_2 was sufficient to cause pinking of the turkey and beef products, respectively. Cornforth *et al.* (1998) proposed that pinking previously attributed to CO and NO in gas ovens is instead due to NO_2, which has much greater reactivity than NO with moisture at the surface of meats. Their argument was predicated on the fact that NO has a low water solubility unlike that of NO_2. Therefore on the basis of this consideration, NO would be an unlikely candidate to cause pink ring, since at the low levels typical of gas ovens or smokehouses, NO would be unable to enter the aqueous meat system in sufficient quantity to cause pink ring at depths up to 1 cm from the

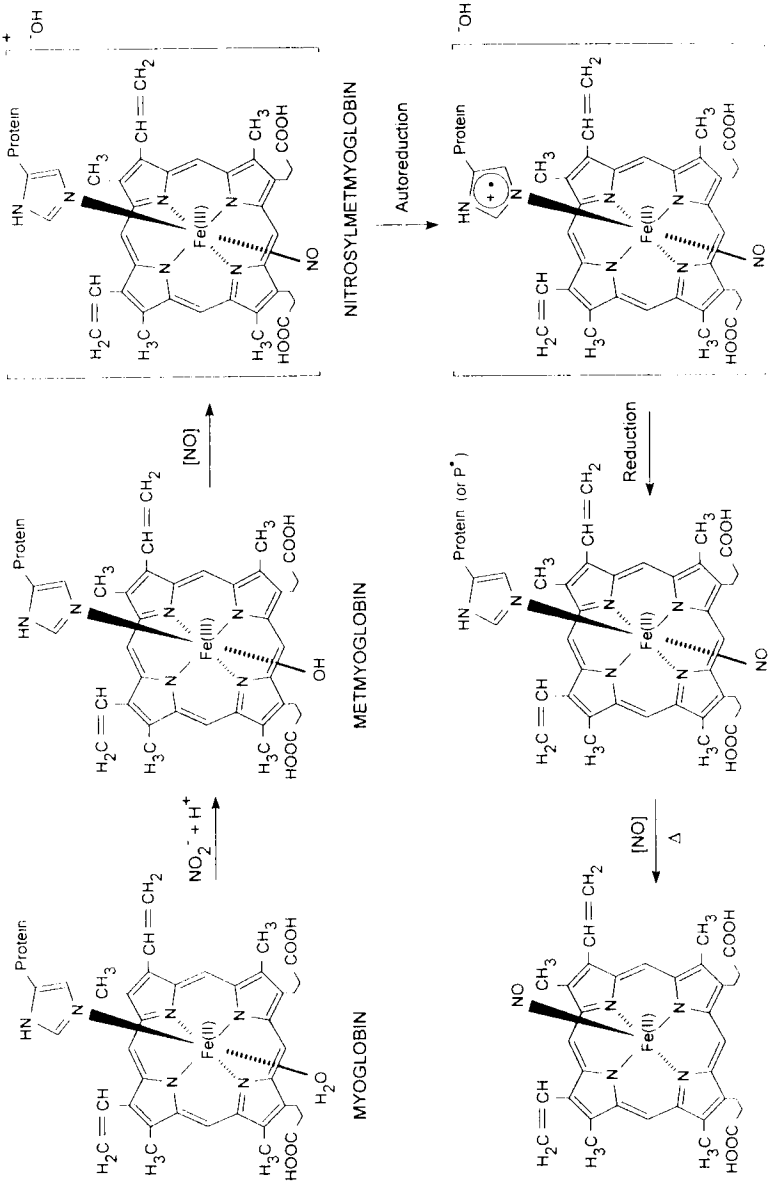


FIG. 3.7. A NEW MECHANISM FOR THE MEAT CURING PROCESS
(Adapted from Killday *et al.* 1988).

surface. On the other hand, NO_2 reacts readily with water to produce nitrous and nitric acid (Shank *et al.* 1962).

$$2\ NO_2 + H_2O \rightleftharpoons HNO_2 + NNO_3 \quad (6)$$

Nitrous acid produced at meat surfaces (Eq. 6) would be free to diffuse inward, where endogenous or exogenous meat reductants, including Mb itself may regenerate NO (Eq. 7). Nitric oxide binds to metMb followed by rapid autoreduction to NOMb as suggested by Killday *et al.* (1988) (Fig. 3.7).

$$Mb + HNO_2 \rightleftharpoons metMb + NO + H_2O \quad (7)$$

$$metMb + NO \rightleftharpoons NO - metMb \quad (5)$$

$$NO - metMb \underset{\text{autoreduction}}{\rightleftharpoons} NO - Mb \quad (8)$$

The characteristic red color of fresh cured meats (*i.e.*, before thermal processing) is due to NOMb. Nitrosylmyoglobin is a ferrous mononitrosylheme complex in which the reduced iron atom is coordinated to four nitrogen atoms of the protoporphyrin-IX plane, one nitrogen atom of the proximal histidine residue of globin (fifth coordinate position) and a NO group (sixth coordinate position). The NOMb pigment can be produced by the direct action of NO on a deoxygenated solution of Mb, but in conventional curing, it arises from the action of nitrite, as stated above. Upon thermal processing, globin denatures and detaches itself from the iron atom, and surrounds the heme moiety. Nitrosylmyochromogen or nitrosylprotoheme is the pigment formed upon cooking, and it confers the characteristic pink color to cooked cured meats. Table 3.1 lists the pigments of cured meat and Fig. 3.8 illustrates specifically the formation of the cooked cured-meat pigment (CCMP) from NOMb and its possible side reactions.

It should be noted that a direct relationship exists between the concentration of NOMb in the muscle (not the nitrite level) and the intensity of the cured color, *e.g.*, cured ham versus corned beef. When these muscle tissues are cured with equivalent amounts of nitrite, a more intense cured meat color is produced in the resulting corned beef than in the ham. Furthermore, addition of excess nitrite to that required to fix the pigment does not increase the intensity of the cured meat color in the cured ham, corned beef or other meat products (Dryden and Birdsall 1980).

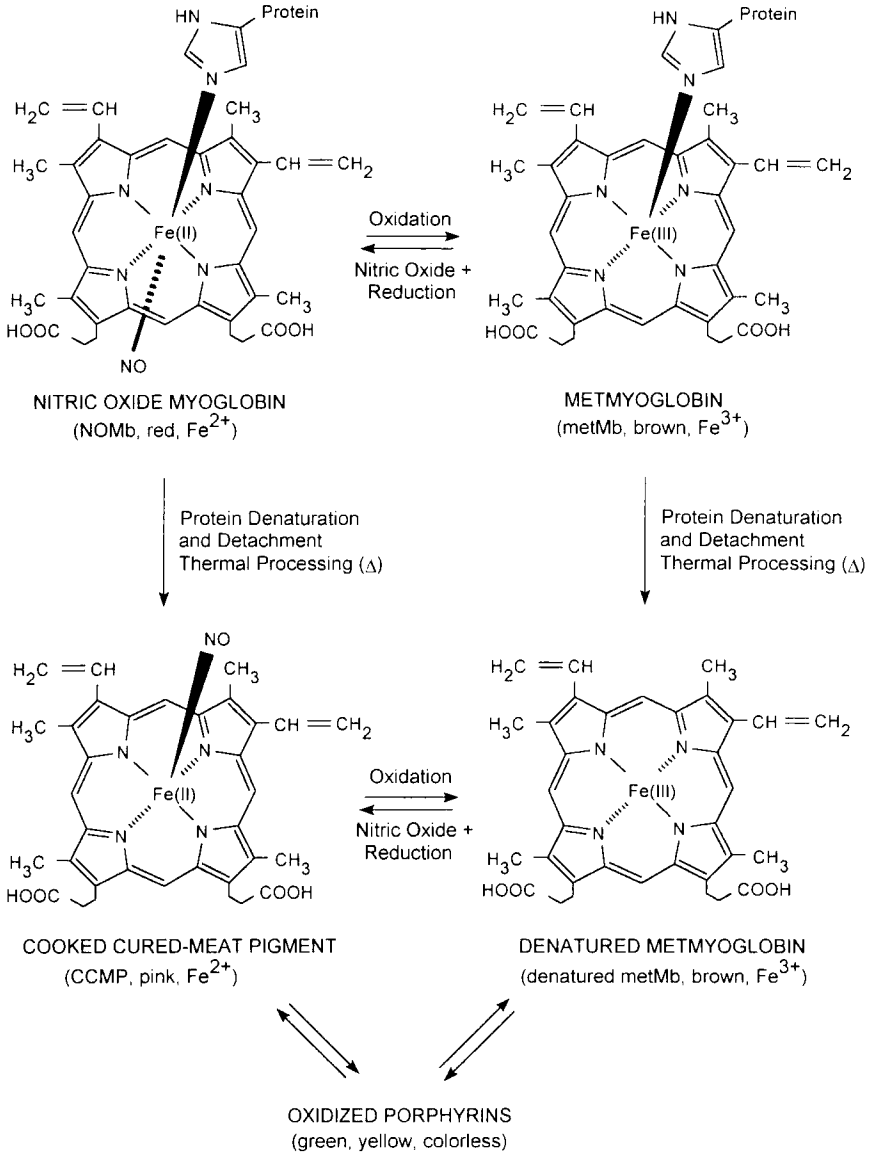


FIG. 3.8. SOME OF THE POSSIBLE CURING REACTIONS THAT RESULT FROM THE ADDITION OF NITRITE TO MEAT
(Adapted from Bard and Townsend 1971).

Haldane in 1901 was the first to recognize that CCMP was a nitrosylheme complex. The pigment contains at least one NO moiety in either the fifth or sixth coordinate position and a molecule, such as water in the other, but the possibility of a dinitrosylheme complex in which NO groups are bound in both axial positions has been suggested (Tarladgis 1962; Lee and Cassens 1976; Renerre and Rougie 1979). The controversy surrounding whether CCMP is mono- or dinitrosyl-containing compound will be discussed in detail below. Although the CCMP is a heat-stable NO hemochrome as evident by the fact that it doesn't undergo further color change upon additional thermal processing, it is susceptible to photodissociation. Furthermore in the presence of oxygen, CCMP's stability is limited by the rate of loss of NO.

$$2\ NO + O_2 \rightleftharpoons 2\ NO_2 \tag{9}$$

This effect is important if cured meats are displayed under strong fluorescent lighting while they are also exposed to air. Under these conditions, the surface color of cured meat will fade in a few hours, whereas under identical conditions, fresh meat will hold its color for a few days. A two-step process involving light-accelerated dissociation of NO from the heme followed by oxidation of both the NO moiety and the ferrous heme iron has been suggested as the probable mechanism for color fading of cured meats (Fox 1966). A brownish-gray color develops on the exposed meat surface during color fading; this pigment, sometimes called a hemichrome, has its heme group in the ferric state. The most effective way to prevent light fading is to exclude O_2 contact with cured meat surfaces. This is routinely accomplished by vacuum packaging of the meat in O_2 impermeable films. Consequently, clear films and illuminated display cabinets can be used to present cured meats without significant color fading. If O_2 is absent from the package, NO cleaved from heme moieties by light cannot be oxidized and can recombine with the heme (Hedrick *et al.* 1994).

Specific biochemical reducing systems that may be important in the development of cured meat color have been the subject of intensive investigation (Walters and Taylor 1965; Walters *et al.* 1967, 1975; Möhler 1974). Endogenous compounds such as cysteine, reduced nicotinamide adenine dinucleotide, cytochromes and quinones are capable of acting as reductants for NOMb formation (Fox 1987). These reductants form nitroso-reductant intermediates with NO and then release the NO to Mb, forming a NOmetMb complex that is then reduced to NOMb. In model systems, the rate limiting step in the production of NOMb was the release of NO from the reducant-NO complex (Fox and Ackerman 1968). Several researchers have investigated the effects of endogenous muscle metabolites including peptides, amino acids, and carbohydrates on the formation of NOMb. Tinbergen (1974) concluded that low-

molecular-weight peptides such as glutathione and amino acids with free sulfhydryl groups were responsible for the reduction of nitrite to NO, which is subsequently complexed with Mb to produce NOMb. Similar work by Ando (1974) also suggested that glutathione and glutamate are involved in cured-meat color formation. Depletion of these compounds in meat *via* oxidation occurs with time, but reductants such as sodium ascorbate or erythorbate are added to nitrite-cured meats before processing to ensure good color development (Alley *et al.* 1992). The role of reductants in heme-pigment chemistry is somewhat ambiguous, but they can promote oxidation and even porphyrin ring rupture under certain conditions. Thus to form the cured meat pigment, two reduction steps are necessary. The first is the reduction of nitrite to NO and the second is conversion of NOmetMb to NOMb.

Characterization of Nitrosylheme Pigments

Anderton and Locke (1955) first reported a simple technique whereby the pigment of cooked cured-meat products was rendered soluble in either acetone or an acetone-diethyl ether solvent system. The absorption spectrum of the extract revealed absorption maxima at 480, 537-542 and 563 nm, but the position of the maximum extinction of the β-band varied somewhat with the species and solvent used. Hornsey (1956) then described a simple and rapid method for extracting and quantitating the content of NO-heme pigment present in thermally processed nitrite-cured meat. The author stated that selective extraction of NO-heme pigments was achieved using a 4:1 (v/v) acetone-water mixture, and that no other meat pigments were extracted under these conditions. The nitrosoheme pigment complex exhibited absorption maxima at 476, 535 and 563 nm. A similar spectrum was obtained when hematin was reduced with sodium dithionite and a trace of nitrite added, followed by dilution with four parts of acetone. For quantitative purposes, the absorbance of the extract was measured spectrophotometrically at 540 nm, and based on a known molar extinction coefficient (*i.e.*, 11.3 $mM^{-1} \cdot cm^{-1}$), the concentration of the NO-heme pigment was determined. Because the extract was sensitive to light and oxygen, reductants were added to improve its stability. However, Hornsey (1956) did not characterize the number of NO ligands or sites of their attachment to the denatured hemoprotein. Möhler (1974) noted that the extracted nitrosylheme complex was of the porphyrin moiety alone and that no globin was attached to it. Sakata and Nagata (1983) reported that using the method described by Hornsey (1956), both the native NOMb pigment and its heat denatured counterpart (*i.e.*, CCMP) are extracted. Thus, acetone extraction cannot be used to fractionate NOMb and CCMP (Skibsted 1992).

Evidence for a Dinitrosylheme Complex

Based on spectral studies using acetone extracts from Hornsey's method (1956), Tarladgis (1962) concluded that the pigment of cooked nitrite-cured meat was a low-spin ferrous-porphyrin coordination complex. The author observed from visible (VIS) absorption spectra that the α band at 563 nm was more intense than the β band at 535 nm, suggesting a strong donation of electrons from the ferrous ion to the unsaturated NO ligand for the formation of a π-d covalent bond. Nitrosylheme can be extracted selectively from NOMb and nitrosylhemoglobin (NOHb) with aqueous acetone. Complexation of the metalloporphyrin with the strongly *trans*-directing nitrosyl ligand is believed to weaken the coordinate bond linking the heme and the globin. The protein is denatured upon acetone addition and the nitrosylheme enters solution. Fresh cured-meat pigments (*i.e.*, NOMb and NOHb) are cleaved from the protein and dissolved in acetone, while other hemoprotein derivatives such as MbO_2 and metMb are denatured as such. Hornsey's method (1956) is not specific for extraction of CCMP as Tarladgis (1962) has implied, although extraction is more efficient when the iron-imidazole bond has been cleaved after thermal processing. Although the pigment was easily extracted into acetone, no infrared (IR) spectra for these extracts were presented. The identity of the ligand in the vacant coordination position of the CCMP is uncertain. Tarladgis (1962), using optical and electron paramagnetic resonance (EPR) spectroscopies, has suggested that both axial coordinate positions of the iron in CCMP are occupied by NO groups. The author proposed that the two unpaired electrons from the NO moieties should have their spins coupled, thus rendering the molecule diamagnetic. Because no EPR signals were observed, Tarladgis (1962) concluded that the pigment was a dinitrosyl ferrohemochrome or dinitrosylprotoheme indicating the absence of an unpaired electron. However, lack of an EPR spectrum, based upon the experimental conditions employed, suggests only a diamagnetic nature for the extracted pigment and is not proof of dinitrosyl ligation.

Further evidence for the dinitrosyl form of CCMP is available from studies by Lee and Cassens (1976) and Renerre and Rougie (1979) in which $Na^{15}NO_2$ was used to determine the amount of ^{15}NO bound to unheated as compared to heated solutions of Mb. These authors found, using a modified Kjeldahl analysis, that heated samples contained twice as much ^{15}NO as their unheated counterparts. It was postulated that when the protein globin detached itself from NOMb during thermal processing, the protoheme was complexed with a second NO molecule. Yet, the possibility that NO can form $^{15}N_2O_3$ or bind with other constituents of the hemoprotein to account for the "consumption" of labeled ^{15}NO was not considered. In a series of papers by Bonnett and co-workers, these authors reported that the reaction of $NaNO_2$ with hemoproteins under mildly acidic conditions can occur at the ferrous ion to give the nitrosylheme pigment

(Bonnett *et al.* 1980*a*), in the porphyrin ring (Bonnett *et al.* 1978, 1980*b*) or in the protein (Bonnett and Nicolaidou 1979). Furthermore, these authors were first to hypothesize that the pigment of nitrite-cured meat is a mononitrosylheme complex.

The existence of a dinitrosylheme complex has also received support from studies of Wayland and Olson (1973, 1974). These authors showed that toluene solutions of tetraphenylporphyrin (TPP^{-2}) iron(III) chloride, a high-spin Fe(III) complex (S=5/2), reacted reversibly with NO to form a diamagnetic $Fe^{II}TPP(Cl^-)(NO^+)$ complex, but in the presence of methanol, $Fe^{III}TPPCl$ reacted with excess NO to produce $Fe^{II}TPP(NO)$. $Fe^{II}TPP(NO)$ was characterized as a low-spin (S=1/2) ferrous porphyrin complex with the odd electron in a molecular orbital with Fe (d_{z^2}) and NO (σ_N) character. The IR spectrum of $Fe^{II}TPP(NO)$ in Nujol mull had a strong ν_{NO} at 1700 cm^{-1}, and the EPR spectrum in a toluene glass showed 3 g values with ^{14}N hyperfine splitting of NO characteristic of a pentacoordinate heme complex. Additionally, $Fe^{II}TPP(NO)$ formed 1:1 adducts with nitrogenous donors such as pyridine (Pyr) and piperidine, but no adducts of phosphorous donors were observed. In a toluene glass, a rhombic g tensor was noted as well as ^{14}N hyperfine splitting in the g_3 region from both NO and the ring N donor. These spectra are typical of a hexacoordinate species and are similar to those of NO complexes of Fe(II) Mb and Hb in which iron is axially coordinated by a NO moiety and a histidine residue. Wayland and Olson (1974) also reported that in the presence of excess NO, $Fe^{II}TPP(NO)_2$ may form. Two IR N-O stretching frequencies were observed at 1870 cm^{-1} and 1690 cm^{-1} [*i.e.*, $Fe^{II}TPP(NO^-)(NO^+)$]. The band at 1870 cm^{-1} is in the range expected of a linear $Fe^{II}NO^+$ moiety and the 1690 cm^{-1} band is consistent with a bent $Fe^{II}NO^-$ group. Moreover, the intensity of the 1870 cm^{-1} band decreased and eventually disappeared upon evacuation of excess NO from the system. While CCMP prepared from hemin and NO could possibly have such a structure (*n.b.*, this issue will be discussed in detail later), there is no evidence for the formation of a dinitrosylprotoheme complex in the meat matrix.

Burge and Smith (1992) attempted to characterize the structure of the organic-soluble pigment from thermally processed nitrite-cured ham. They prepared the CCMP from hemin and $Na^{15}NO_2$ according to Shahidi *et al.* (1985), and then analyzed its structure by ^{15}N NMR and IR spectroscopies. Although IR spectroscopy for identification of functional groups in molecules is commonplace, overlapping of symmetric stretch ranges for bent (1725-1525 cm^{-1}) and linear (2000-1600 cm^{-1}) nitrosyl moieties limits the usefulness of this technique in identifying nitrosyl coordinate geometry (Bell *et al.* 1983). ^{15}N NMR is a more precise technique for studying nitrosyl ligands of nitrosyl-metal complexes because the number of NO moieties and their coordinate geometry can be determined. Burge and Smith (1992) assumed that CCMP contained two NO groups, because the pigment was then referred to as dinitrosyl ferrohemo-

chrome. However, this fact had not been satisfactorily proven by the aforementioned authors. Shahidi *et al.* (1985), based on literature reports and erroneous Kjeldahl use, concluded that the preformed CCMP was dinitrosyl ferrohemochrome. Based on the chemistry of reactions involved in the digestion step, nitrogen of NO groups cannot be accounted for because the Kjeldahl method only detects the quantity of reduced N, such as that found in $-NH_2$ groups, and not its various oxides or azides. The ^{15}N NMR studies of Burge and Smith (1992) revealed that the preformed pigment contained only one NO group, and the IR spectrum (Nujol mull) exhibited a single NO symmetric stretch at 1801 cm^{-1}. Because uncertainty in VIS absorption spectra existed, Burge and Smith (1992) never ruled out the possibility of initially having two NO groups in the molecule. Comparing acetone extracts of nitrite-cured ham, CCMP, and pigment whose volume was reduced under a stream of nitrogen, these authors concluded that disappearance of the 557-nm band in absorption spectra was due to loss of the second NO moiety attached to heme.

Evidence for a Mononitrosylheme Complex

Bonnett *et al.* (1978, 1980*a*) attempted to characterize the pigment of cooked cured meat, nitrosylprotoheme, as its dimethyl ester, which was obtained by the reaction of NO with methoxyiron(III) protoporphyrin dimethyl ester. A strong IR band at *ca.* 1660 cm^{-1} was diagnostic of the stretching mode of a bent Fe-NO moiety and a pentacoordinate complex. Scheidt and Frisse (1975) had reported a ν_{max} of 1670 cm^{-1} and an Fe-N-O angle of 149.2° for nitrosyl-$\alpha,\beta,\gamma,\delta$-tetraphenylporphinatoiron(II). Although the VIS spectra of these compounds were similar to one another and resembled that of nitrite-cured meat, Bonnett *et al.* (1978) suggested that use of VIS spectroscopy may be complicated by aerial oxidation of nitrosylheme, which becomes especially important at the dilution required to observe the Soret band (480 nm). Therefore, EPR spectroscopy using more concentrated samples in closed, oxygen-free systems at a low temperature was considered. The EPR spectrum of nitrosylprotoheme dimethyl ester in an acetone glass showed a triplet signal due to hyperfine splitting by a single axial nitrogenous ligand of NO indicative of a pentacoordinate system (*i.e.*, $g_1 = 2.102$, $g_2 = 2.064$, $g_3 = 2.010$, $a_3 = 1.63$ mT, Bonnett *et al.* 1980*a*). When this sample was kept in the sealed, oxygen-free EPR tube at room temperature and in the dark, the signal remained virtually unchanged over a two-year period reflecting the considerable thermodynamic stability of this compound. Nitrosylprotoheme extracted with acetone from thermally processed nitrite-cured meat also had an EPR signal expected of a pentacoordinate nitrosylheme complex. When the pigment was dissolved in piperidine, a solvent providing a second nitrogenous ligand, the EPR signal changed and the resulting spectrum was characteristic of a hexacoordinate system with a g_3 value

of 1.98. Moreover, the g_1, g_2 and the hyperfine structure at g_3 were no longer resolved unlike the hexacoordinate $Fe^{II}TPP(NO)(Pyr)$ system reported by Wayland and Olson (1974). Nitrosylmyoglobin showed a similar EPR spectrum, indicating that it was a hexacoordinate complex, where the fifth coordinate position was occupied by the imidazole group of the globin. Identical EPR characteristics of nitrosylprotoheme can be obtained from solutions of NOHb treated with acetone. The protoheme groups of Hb cannot be extracted with acetone, suggesting that there is a structural *trans*-effect created by the nitrosyl ligand which results in the weakening and lengthening of the bond between iron and the coordinated N atom of the imidazole group. Similarly, nitrosylprotoheme extracted with acetone from cured meats showed an EPR spectrum expected for a pentacoordinate nitrosylheme. While these extraction experiments provided confirmation of the general chemical nature of the chromophore of cured meat, they did not reveal the coordination sphere *in situ*.

Bonnett *et al.* (1980*a*) examined various cured meat samples directly, as opposed to their extracts, using EPR spectroscopy. An EPR signal with hyperfine splitting characteristic of a pentacoordinate nitrosylheme complex was observed, thus indicating that the iron-imidazole bond was effectively broken. These authors suggested that the color of cooked cured meat was due to nitrosylprotoheme, which was physically trapped within a matrix of denatured globin. This situation affords some protection to the nitrosyl pigment against aerial oxidation, which is reinforced by the presence of natural or added reductants. Further support for this view comes from the EPR spectrum of uncooked bacon, which showed the presence of both penta- and hexacoordinate nitrosylhemes. After gentle heating of the sample in the EPR tube, the broad high-field feature at an effective g value of 1.99, characteristic of a hexacoordinate species, disappeared and the features characteristic of a pentacoordinate species became sharper and more intense. In other words, thermal processing of nitrite-cured meat breaks the imidazole-iron(II) bond without a second NO molecule coordinating to the pigment.

Killday *et al.* (1988) reported that sodium dithionite reduction of chlorohemin [iron(III)] in dimethyl sulfoxide (DMSO), followed by exposure to NO gas in argon formed a red pigment [nitrosyl iron(II)protoporphyrin] that was identical to the pigment extracted in acetone from thermally processed cured corned beef. Both compounds had an R_f of 0.55 on TLC plates developed with ethyl acetate - methanol (1:1, v/v). The infrared spectrum of the synthesized pigment exhibited a nitrosyl stretch at 1656 cm^{-1}, consistent with a bent NO-ligand state. No band in the 1900 cm^{-1} region corresponding to a second coordinated nitrosyl ligand was evident. Fast atom bombardment mass spectrometry for the synthetic pigment revealed fragments consistent with a molecular ion of 646 Da, corresponding to a mononitrosyl heme complex, as opposed to a dinitrosyl one, which would have shown clusters at 30 amu

greater. Based on these observations, the authors concluded that the pigment was a mononitrosyl ferrous protoporphyrin.

Killday *et al.* (1988) further suggested a pathway for the meat-curing reaction involving a $MbFe^{III}NO$ intermediate and a nitrite-protein complex (Fig. 3.7). After heating chlorohemin [iron(III)] dimethyl ester in DMSO with imidazole and then introducing NO, the resulting product exhibited a nitrosyl stretching band identical to that of nitrosyl iron(II)protoporphyrin dimethyl ester prepared by dithionite reduction. Thus, they provided evidence for the internal autoreduction of ferric nitrosylheme complexes, as previously proposed by Giddings (1977). As aforementioned, Killday *et al.* (1988) suggested that $MbFe^{III}NO$ is autoreduced by globin imidazole residues and that a protein radical of globin reacts with a second molecule of nitrite, resulting in the stoichiometry found in the labeling experiments of Lee and Cassens (1976) and Renerre and Rougie (1979).

Using labeled ^{15}NO in aqueous model systems, Andersen *et al.* (1990) reported that only one NO molecule coordinated to Fe(II) of purified equine Mb. However, heating the reaction mixture to temperatures in excess of 70°C increased the ^{15}NO content of the product without resulting in any significant changes in the visible (VIS) absorption spectrum (λ_{max}, ϵ_{max}; 546 nm, 12 500 L $\cdot$ mol^{-1} $\cdot$ cm^{-1}; 578 nm, 11 200 L $\cdot$ mol^{-1} $\cdot$ cm^{-1}). The authors stated that the increased labeling probably indicated nitrosation of the globin chain, since such a reaction is expected to have little influence on the spectral properties of the iron(II) chromophore, in contrast to the coordination of two NO molecules directly to iron(II). In fact, as a result of prolonged heating, Andersen *et al.* (1990) found that it was possible to increase the labeling of ^{15}NO to a level higher than the 2:1 stoichiometry required for dinitrosylation, thereby suggesting that further nitrosation reactions with the globin are of importance. Recently, Jankiewicz *et al.* (1994) provided further support to the view that the CCMP is mononitrosylprotoheme from IR and VIS absorption studies.

Maxwell and Caughey (1976) reported the preparation of a solid pentacoordinate nitrosylheme ester of pyridine using protoporphyrin-IX dimethyl ester iron(II). The heme pigment was heated at 80°C under vacuum until all ligated pyridine, which was detected quantitatively, had been removed. Upon exposure of the solid to NO, an uptake of 1.0 mol of NO per mole of Fe was observed, consistent with the formation of a nitrosylheme with one NO ligand. Infrared spectra of nitrosylheme complexes prepared in solution revealed stretching of a single ν_{NO} at 1670 cm^{-1}, indicating a pentacoordinate complex when non-nitrogenous solvents were used. Hexacoordinated species with one NO moiety were observed in solutions when either the solid pentacoordinate NO compound had been added to solvent containing a nitrogenous base or the heme had been exposed to NO gas in a solution with excess nitrogenous base present. A single ν_{NO} value at 1620 cm^{-1} was observed which is consistent with bent-end-on

bonding (*i.e.*, Fe-N-O) with iron(II) serving as π donor and the N of NO as σ donor with an overall shift of electron density from iron to NO upon bonding. This interpretation is not consistent with conclusions drawn from EPR studies by Wayland and Olson (1974), to the effect that the electron density shift was in the opposite direction, namely from NO to iron(II) to give a partially positive NO ligand (Yonetani *et al.* 1972). Because EPR data gave evidence of spin density, but did not indicate the charge distribution, it need not be considered inconsistent with the conclusions drawn from IR data. Maxwell and Caughey (1976) also showed that the ν_{NO} in the IR spectrum of NOHb exhibited the hexacoordinate configuration similar to a 1-methylimidazole protoheme nitrosyl compound. Upon addition of inositol hexaphosphate (IHP) to the system, a wavenumber shift of 50 cm^{-1} to approximately 1670 cm^{-1}, characteristic of the pentacoordinate structure of nitrosylprotoheme, was observed. These authors suggested that the IHP-induced frequency shift provides strong evidence for loss of the *trans*-histidine ligand, because this shift is precisely of the same magnitude as that measured upon loss of imidazole in protein-free hemes. Electron paramagnetic resonance spectra of NOHb in which $^{14}N^{16}O$ and $^{15}N^{16}O$ were used with and without IHP were quite striking.

The EPR spectra of frozen solutions of native bovine NOMb (0.05 M phosphate buffer, pH=5.5) with strongly resolved hyperfine splitting were recorded by Kamarei and Karel (1983), and they resembled the EPR spectrum of NOHb in the presence of IHP. These authors stated that IHP converts NOHb from a relaxed to a tense quaternary state. In the tense state, the bond between the proximal histidine and iron is ruptured in the α-chains of NOHb. The observed hyperfine splitting was consistent with coupling of the ^{14}N nucleus of the proximal histidine. This was explained by assuming that the *trans*-effect of the NO ligand results in such a dramatic stretching of the Fe-N_{HIS} bond that no spin transfer from iron to N_{HIS} occurs. Thus, native bovine NOMb (uncooked) behaves as a pentacoordinate complex, but Dickinson and Chien (1971), who measured the EPR spectra of single crystals of sperm whale NOMb, observed for the first time clear splitting of resonance lines due to the imidazole nitrogen of the proximal histidine, thereby providing definite proof of hexacoordination. Clearly pH and other factors are important in determining whether native NOMb or NOHb exist as penta- or hexa-coordinated species.

Trittelvitz *et al.* (1972) stated that analysis of NOHb offers interesting aspects, because NO is both a spin label for EPR studies and a strong ligand at the sixth coordination site of the iron. Lang and Marshall (1966) noted that the unpaired electron of the paramagnetic NOHb occupies a *d*-orbital of the iron atom. Trittelvitz *et al.* (1972) stated that the hyperfine structure resulting from the interaction of the unpaired electron with the N-nucleus of NO should bring evidence of a conformationally induced change of the binding properties of the

sixth ligand. These authors indicated that the g value from the EPR spectrum of ^{14}NOHb showed a hyperfine structure of three lines similar to that observed by Maxwell and Caughey (1976) and Bonnett *et al.* (1980*a*). Using ^{15}NO, the three-line spectrum became a two-line spectrum, and by comparing these spectra with theoretical energy level splitting diagrams, the hyperfine structure was ascribed to the sixth ligand of the heme iron. The EPR spectrum of ^{14}NO-Mb did not show any hyperfine structure, similar to that of Bonnett *et al.* (1980*a*).

Evidence that the Chemical Structure of the Preformed CCMP Is Identical to that of the Pigment Prepared *in situ* upon Thermal Processing of Nitrite-Cured Meat

Our interest in the chemical nature of the CCMP stems from our investigations of nitrite-free meat curing systems. We have reported the preparation of CCMP from hemin in the presence of reductants and NO (Shahidi *et al.* 1994). This pigment, preformed outside the meat matrix, is used as part of a composite mixture for nitrite-free curing of meat products, but its chemical nature has not been adequately elucidated. As aforementioned, the chemical structure of the nitrosylhemochromogen pigment of cooked nitrite-cured meat is either a five-coordinate mononitrosyliron(II) or a six-coordinate dinitrosyliron(II) protoporphyrin complex. If one concludes from the evidence that the structure of the pigment of thermally processed nitrite-cured meat is a mononitrosylheme complex, it is still another matter to prove that the CCMP prepared by the aforementioned process also has a mononitrosylheme structure. Therefore, the chemical structure of the preformed CCMP was elucidated using VIS, IR as well as EPR spectroscopies (Pegg *et al.* 1996; Pegg and Shahidi 1996).

Visible Absorption Spectroscopy

The VIS absorption spectrum of the pigment extracted from cooked nitrite-cured ham with a 4:1 (v/v) acetone-water mixture (Hornsey 1956) exhibited an absorption pattern characteristic of iron-porphyrin compounds with a red color and had maxima at 563 (α), 540 (β) and 480 nm (Soret), $A_\beta/A_\alpha = 0.93$. Absorption characteristics of the preformed CCMP prepared from hemin and nitric oxide (*i.e.*, before application to meat) in a 4:1 (v/v) acetone-water mixture were compared with those of the pigment extracted from nitrite-cured ham. Similar maxima were apparent in all cases (Fig. 3.9) (Pegg 1993). The VIS absorption spectra of pigments extracted from CCMP-treated pork systems after thermal processing were also qualitatively similar and had absorption maxima and shoulders at the same wavelengths as those of the nitrite-cured ham.

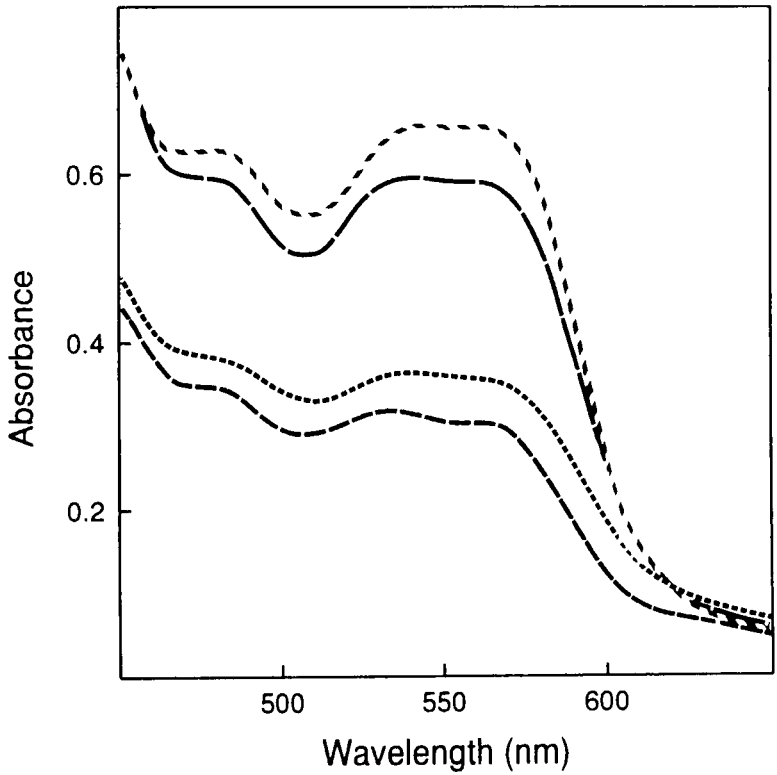


FIG. 3.9. ABSORPTION SPECTRA IN ACETONE-WATER (4:1, V/V) OF THE COOKED CURED-MEAT PIGMENT (CCMP) PREPARED FROM BOVINE RED BLOOD CELLS AND SODIUM NITRITE, ~~~ ; CCMP PREPARED FROM HEMIN AND NITRIC OXIDE, —; PIGMENTS EXTRACTED FROM NITRITE-CURED HAM, ---; AND PIGMENTS EXTRACTED FROM CCMP-TREATED PORK, ---. (From Pegg 1993).

A novel titration methodology was used to investigate the chemical nature of CCMP. Cooked cured-meat pigment was formed by titrating a reduced hemin solution against a saturated solution of NO using the sensitivity of a spectrophotometer to detect the end point (Pegg and Shahidi 1996). Jankiewicz *et al.* (1994) prepared the nitrosyl derivative of heme and heme dimethyl ester in an 80% (v/v) DMSO model system using an aqueous solution of sodium nitrite as the nitrosating agent. These authors used 80% (v/v) DMSO as their solvent of choice because they noted that hemin and its nitrosyl derivative were insoluble in water. However, both reduced hemin and its nitrosyl analog are soluble at the

alkaline pH of a dilute carbonate solution (0.04M, pH = 11.0). The characteristics of the pigment were investigated in both an aqueous and a DMSO model system using NO rather than sodium nitrite as the nitrosating agent.

The visible absorption spectra of reduced hemin and its nitrosyl derivative in the carbonate buffer were recorded, and maximum change in absorbance between spectra of equal concentration of the two occurred at 486 nm (Pegg and Shahidi 1996). Rather than bubbling NO directly into a fresh preparation of reduced hemin to obtain the nitrosylheme complex, aliquots of a saturated solution of NO were transferred to an air-tight cuvette *via* a Hamilton syringe. The change in absorbance at the 486 nm band was monitored. The basic equation for the reaction of hemin ($ClFe^{III}Hem$) with NO in a protic solvent such as water or methanol, and in the presence of a reductant, is as follows (Bonnett *et al.* 1980*a*):

$$ClFe^{III}Hem + 2\ NO \xrightarrow{H_2O} ONFe^{II}Hem + HONO + HCl, \quad \text{where Hem = porphyrin dianion} \tag{10}$$

Data provided by Young (1981) indicated that the concentration of a saturated solution of NO in water at standard pressure and a temperature of 293°K is 2.02×10^{-3} M. Based on the amount of hemin present in the model system (*ca.* 0.40 μmol in 3075 μL) and the stoichiometry of the above reaction, it was determined that *ca.* 400 μL of the saturated solution of NO would be needed to reach the end point of the titration if CCMP were a mononitrosylheme complex; 800 μL would be required if the pigment were dinitrosylprotoheme. For the assay, 350-400 μL of the saturated solution of NO were used to reach the end point of the titration, suggesting that only one nitrosyl group is ligated to the reduced heme. These results are in agreement with those of Killday *et al.* (1988) and Jankiewicz *et al.* (1994), who suggested that a 1:1 complex between NO and reduced heme is formed.

The absorption spectrum of reduced hemin in the 80% (v/v) DMSO system exhibited 2 absorption maxima at 524 and 554 nm and a minimum at 538 nm. This is consistent with absorption characteristics reported by Jankiewicz *et al.* (1994). As aliquots of the saturated solution of NO were added to the reduced hemin, a nitrosylheme complex formed as evidenced by change in absorption spectra. A marked reduction in absorbance at the 524 and 554 nm bands was apparent for equal concentrations of reduced heme and its nitrosyl counterpart. Due to the formation of a new compound as increments of NO were added, derivative spectroscopy was employed. Often derivative spectra reveal details that are lost in the original spectrum. Furthermore, concentration measurements of an analyte in the presence of an interfering substance can sometimes be made more easily or more accurately (Skoog and Leary 1992). Both first and second derivative spectra of the absorbance were recorded, but only the second

derivative spectra are presented in Fig. 3.10. Minima in these spectra at 524 and 556 nm correspond with the local maxima of the absorbance spectra, and the maximum at 538 nm conforms to the local minimum of the absorbance spectra. As aliquots of the saturated solution of NO were added to the reduced hemin system, the nitrosylheme derivative formed and a decrease in absorbance occurred at the 556 nm maximum. This translates to an increase in the second derivative of absorbance at 556 nm. Because the medium consists primarily of DMSO, the stoichiometry between the added nitrosating species and reduced hemin should be *ca.* 1:1. Based on the amount of hemin present in the model system (*ca.* 0.85 μmol), it was calculated that *ca.* 450 μL of the saturated solution of NO would be needed to form a mononitrosylheme complex. By comparing the second derivative absorption spectrum of nitrosylprotoheme (*i.e.*, reduced hemin system into which NO had been bubbled) and that of reduced hemin, after 475 μL of the nitrosating agent had been added, over 96% of the reduced hemin was converted to its nitrosylheme derivative.

Infrared (IR) Spectroscopy

Cooked cured-meat pigment, prepared according to Shahidi *et al.* (1994), was dissolved in DMSO and then transferred to matching sodium chloride IR cells. Pegg and Shahidi (1996) reported that the IR spectrum revealed a strong absorption at ν = 1659 cm^{-1}, which corresponds to the first nitrosyl group bound with the ferrous atom of the heme molecule. A similar finding was reported by Jankiewicz *et al.* (1994) and Bonnett *et al.* (1980*a*). The latter authors further noted that the stretching frequency at ν = 1659 cm^{-1} is consistent with the presence of a bent Fe-NO moiety and a pentacoordinate complex. More importantly, there was no stretching band at ν = *ca.* 1900 cm^{-1}, which according to Killday *et al.* (1988) would indicate the presence of a second nitrosyl group in CCMP.

Electron Paramagnetic Resonance (EPR) Spectroscopy

Further evidence supporting the view that the CCMP is a mononitrosylheme complex comes from EPR studies of Pegg *et al.* (1996). The EPR spectral parameters of CCMP in an acetone glass (Fig. 3.11A) were compared to those of nitrosylprotoheme dimethyl ester investigated by Bonnett *et al.* (1980*a*) and to Fe^{II}TPP(NO) reported by Wayland and Olson (1974). In all cases, the EPR parameters of these systems were similar and possessed characteristics recognized as those of the pentacoordinate nitrosylheme system. EPR spectra of the nitrosylprotoheme dimethyl ester, Fe^{II}TPP(NO) and the preformed CCMP in a solvent glass (*i.e.*, one not providing a nitrogenous ligand) showed g_1, g_2 and g_3 values characteristic of a rhombic g tensor due to the anisotropic nature of the system. Bonnett *et al.* (1980*a*) reported a solvent effect on g_3, noting that

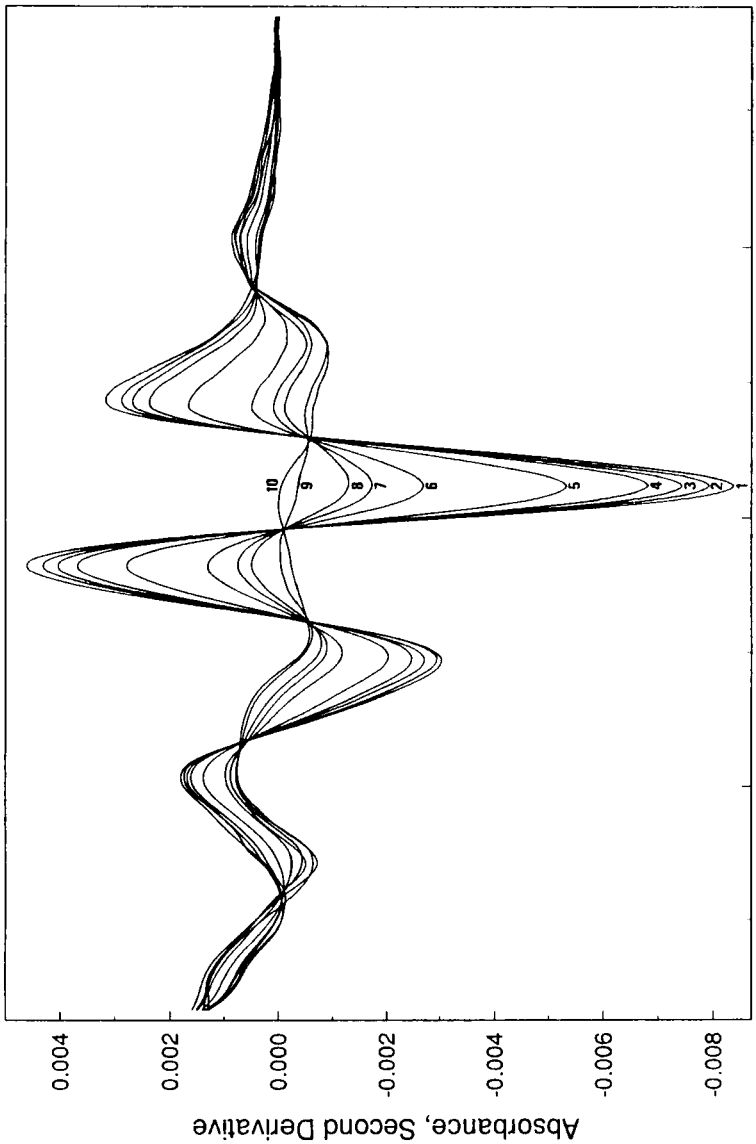


FIG. 3.10. SECOND DERIVATIVE SPECTRA OF ABSORBANCE OF REDUCED HEMIN IN 80% (V/V) DIMETHYL SULFOXIDE AND THE CHANGE THEREOF AS THE NITROSYLHEME COMPLEX FORMS AFTER ADDITION OF ALIQUOTS OF A SATURATED SOLUTION OF NO. (1) 0 μl; (2) 25 μl; (3) 50 μl; (4) 75 μl; (5) 125 μl; (6) 200 μl; (7) 275 μl; (8) 350 μl; (9) 475 μl; AND (10) NO GAS (From Pegg and Shahidi 1996).

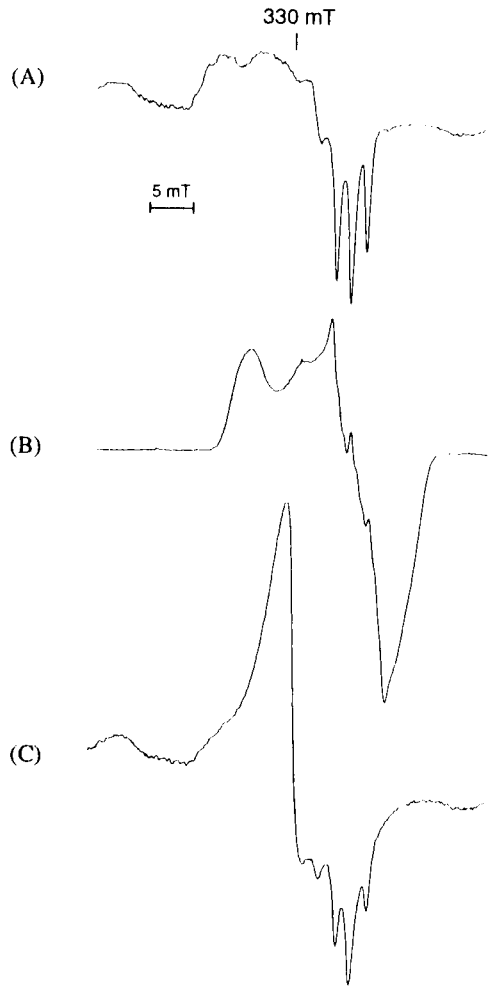


FIG. 3.11. EPR SPECTRA OF SOLVENT GLASSES AT 77 K SHOWING EITHER PENTA- OR HEXA-COORDINATION. (A) PREFORMED COOKED CURED-MEAT PIGMENT (CCMP) IN ACETONE (*I.E.*, AFTER CENTRIFUGATION TO REMOVE FINES), (B) CCMP IN PYRIDINE, C) CCMP IN ACETONE (*I.E.*, NO CENTRIFUGATION, FINES PRESENT) (From Pegg *et al.* 1996).

less polar aromatic solvents gave slightly lower g values than aprotic polar solvents. EPR spectra also exhibited hyperfine splitting in the g_3 region from NO ligation. Hyperfine coupling by the single nitrogenous ligand (I = 1) produced a

signal split into a triplet of equal line intensities (Fig. 3.11A). According to Wayland and Olson (1974), this ^{14}N hyperfine coupling provides evidence for placing the odd electron in a molecular orbital with substantial iron d_{z^2} character. The odd electron of NO becomes highly delocalized onto the iron atom. Neto *et al.* (1988) investigated the appearance of pentacoordinate symmetry of myoglobin from various species after treatment with NO in both solution and powder form by EPR spectroscopy. They noted a significant temperature effect with regard to resolution of EPR spectra (289-112K). This is routine, particularly when examining changes in the physical state of a paramagnetic sample (*i.e.*, solution to solvent glass), but there was virtually no change in the EPR spectra at the lower temperatures examined (132 and 112K); perhaps only a slight improvement in the signal-to-noise ratio. Although the EPR spectrum of $Fe^{II}TPP(NO)$ reported by Wayland and Olson (1974) was measured at 120K, while that of nitrosylprotoheme dimethyl ester and CCMP was measured at 77K, the temperature effect at this range should not have significantly influenced the EPR signal with respect to the pentacoordinate symmetry of the molecule and the g values obtained.

Dissolution of CCMP in pyridine (*i.e.*, a solvent providing a nitrogenous ligand) resulted in a dramatic change in its EPR spectrum. There was a difference in g_1 and g_2 values, and the hyperfine structure at g_3 was no longer resolved. The resulting signal from the solvent glass was similar to that observed for nitrosylprotoheme dimethyl ester in piperidine reported by Bonnett *et al.* (1980*a*). Loss of resolution of the hyperfine splitting in the EPR spectrum indicated that the N of pyridine occupied the sixth coordinate position on the central ferrous ion of the nitrosylheme complex. Nitrosylhemoglobin and NOMb also showed these characteristics in their EPR spectrum (Maxwell and Caughey 1976; Morse and Chan 1980). A comparison between the EPR spectra of CCMP in an acetone glass, showing evidence of pentacoordination, and a pyridine glass, showing characteristics of hexacoordination, is provided in Fig. 3.11.

The EPR spectrum of CCMP in an acetone glass was also compared to those spectra of lyophilized CCMP and PCCMP (Fig. 3.12A,B,C). Again in all cases, spectra were similar and EPR parameters of these systems possessed characteristics recognized as those of the pentacoordinate nitrosylheme system as described above. However it should be noted that, in preliminary investigations, the EPR spectrum of CCMP in an acetone glass was somewhat different from that observed in Fig. 3.11A. The characteristic ^{14}N hyperfine splitting was evident in the g_3 region with an a_3 of 1.71 mT, but a small shoulder with two other splittings of low intensity and a single line with a prominent positive feature in the g = *ca.* 2 region were also observed (Fig. 3.11C). Existence of dinitrosylprotoheme complexes has been reported in the literature (Wayland and Olson 1974; Olson *et al.* 1982; Lançon and Kadish 1983), but such complexes do not correspond with the observed EPR spectrum of CCMP. Wayland and

Olson (1974) reported that $Fe^{II}TPP(NO)$ can reversibly coordinate with a second NO moiety when $Fe^{II}TPP(NO)$ is exposed to a positive pressure of NO. No EPR transitions were detected in the toluene glass medium (77 K) of such a system [*i.e.*, $Fe^{II}TPP(NO)_2$], which is consistent with the formation of an even-electron species. Solution magnetic susceptibility measurements, as a function of temperature, also concurred with this finding. In other words, $Fe^{II}TPP(NO)_2$ is diamagnetic. Furthermore, if a second NO coordinated to nitrosylprotoheme and the free electrons in the π^* orbitals of each NO molecule occupied separate orbitals (*i.e.*, no pairing in a d_{z^2} orbital), the hyperfine structure at g_3 should reveal a quintet (for $I=1$ and $n=2$) with line intensities in the ratios of 1:2:3:2:1, but this was not observed. It is conceivable that the system was

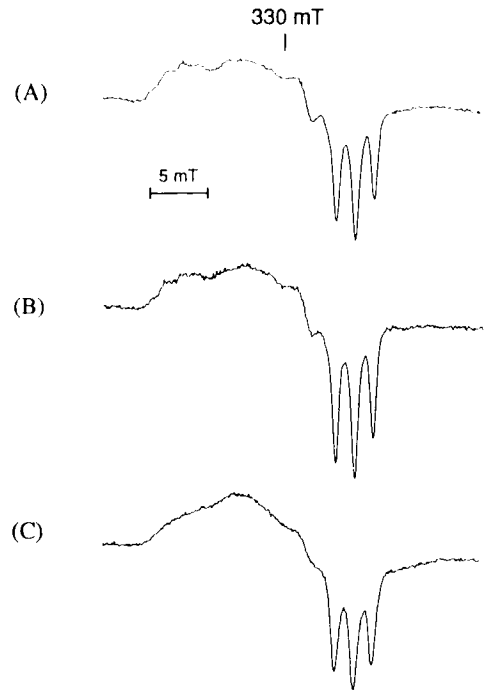


FIG. 3.12. CHARACTERISTIC EPR SPECTRA OF PENTACOORDINATE NITROSYL-PROTOHEME IN ACETONE AT 77K. (A) COOKED CURED-MEAT PIGMENT (CCMP); (B) LYOPHILIZED CCMP; AND (C) POWDERED COOKED CURED-MEAT PIGMENT (PCCMP)
(From Pegg *et al.* 1996).

behaving as a triplet state, but when the extract was centrifuged to remove any fines and the EPR spectrum then rerun, these extra bands disappeared and only hyperfine splitting by a single ^{14}N nuclei was evident (Fig. 3.12A). The hyperfine feature in the EPR spectrum of the sample that had not been centrifuged was believed to be a combination of the EPR of the solid-state and dissolved sample. The intensity of the line at g = 2.03 also decreased. Interestingly enough, Bonnett *et al.* (1980*a*) also observed this signal in their EPR spectra of nitrite-cured meat samples. These authors noted that the species responsible for the signal was only evident in meat, and was not extracted from the tissue by acetone. They could not explain the signal, but suggested that it may be a radical arising from components of bound redox systems or from products of various oxidation pathways. Perhaps the meat matrix behaves as a diluter to spread out each signal arising from nitrosylprotoheme, but the line signal at g = 2.03 arises from an incomplete segregation of signals. Bonnett *et al.* (1980*a*) suggested that thermal processing of nitrite-cured meat effectively breaks the iron-imidazole bond leaving the pentacoordinate nitrosylprotoheme physically trapped in a matrix of denatured globin. If one compares the EPR spectrum of uncooked bacon to that of its heat processed counterpart, presence of the hexacoordinate signal of NOMb and the pentacoordinate signal of nitrosylprotoheme, resulting from some denaturation, is evident. However after thermal processing, only the EPR signal of a pentacoordinate system is observed, and the intensity of the g = 2.03 signal decreases compared to its unpasteurized analog. In other words, the EPR signals arising from the nitrite-cured meat system may be occurring by a similar mechanism responsible for the signals occurring in the acetone extract of CCMP that had not been centrifuged.

Finally, the EPR spectrum of CCMP was compared to those of nitrite-cured and CCMP-treated cooked beef systems *in situ*, as well as their respective acetone extracts. Although EPR is restricted to paramagnetic systems, it has the advantage of not being perturbed by protein denaturation, which makes the use of optical techniques difficult (Alves and Wajnberg 1993). Tarladgis (1962) reported that no EPR signal was obtainable from acetone extracts of cured meat. He proposed that the CCMP was dinitrosylprotoheme, and therefore diamagnetic. In this study, narrow EPR tubes and cryogenic temperatures (77 K) were employed to compensate for the high dielectric loss of acetone. Qualitatively, EPR parameters observed for these nitrosylhemes were similar in all cases, but no attempt was made to quantitate the extracts. The position of the g_1 and g_2 signals (approximate) and the g_3 signal, as well as the associated hyperfine splitting, a_3, are provided in Table 3.2. As Bonnett *et al.* (1980*a*) noted, the observed triplet in nitrite-cured meat systems, due to the hyperfine splitting of NO in the g_3 region, suggests that the iron-imidazole bond is effectively cleaved from NOMb during thermal processing. If this was not so, a second nitrogenous ligand (*i.e.*, from imidazole) bound to the iron would provide EPR characteris-

tics of a hexacoordinate system with a shift in the g_3 value. The spectra obtained, however, clearly indicated that the pigment of cured meat is mononitrosylprotoheme.

TABLE 3.2
EPR SPECTRAL PARAMETERS FOR NITRITE-CURED AND COOKED CURED-MEAT PIGMENT (CCMP) TREATED BEEF SYSTEMS AND THEIR ACETONE EXTRACTS AT 77 K[a]

System[b]	g_1	g_2	g_3	a_3 (mT)
Nitrite-cured beef	2.112	2.070	2.009	1.69
Extract of nitrite-cured beef	2.110	2.070	2.005	1.68
CCMP-treated beef	2.113	2.072	2.009	1.69
Extract of CCMP-treated beef	2.107	2.061	2.006	1.70
Extract of nitrosylprotoheme from ham[c]	2.102	2.064	2.008	1.66

[a] Table from Pegg *et al.* 1996
[b] All beef samples contained 20% (w/w) distilled water and 550 $mg \cdot kg^{-1}$ sodium ascorbate and were thermal processed until an internal temperature of $72 \pm 2°C$ was reached.
[c] EPR data reported from Bonnett *et al.* 1980*a*.

REFERENCES

Alley, G., Cours, D. and Demeyer, D. 1992. Effect of nitrate, nitrite and ascorbate on colour and colour stability of dry, fermented sausage prepared using "back slopping". *Meat Sci.* **32**, 279–287.

Alves, O.C. and Wajnberg, E. 1993. Heat denaturation of metHb and HbNO: e.p.r. evidence for the existence of a new hemichrome. *Intern. J. Biol. Macromol.* **15**, 273–279.

Andersen, H.J., Johansen, H.S., Shek, C.K. and Skibsted, L.H. 1990. Nitric oxide exchange in nitrosylmyoglobin. *Z. Lebensm. Unters. Forsch.* **191**, 293–298.

Anderton, J.I. and Locke, D.J. 1955. Extraction of pigment from cooked cured-meat products. *Nature* **175**, 818–819.

Ando, N. 1974. Some compounds influencing colour formation. In *Proceedings of the International Symposium on Nitrite in Meat Products*, Zeist, The Netherlands, September 10-14, 1973, ed. B. Krol and B.J. Tinbergen. Wageningen Centre for Agricultural Publishing and Documentation, Wageningen, The Netherlands, pp. 149–160.

Bandman, E. 1987. Chemistry of animal tissues. Part 1 — Proteins. In *The Science of Meat and Meat Products, Third Edition*, ed. J.F. Price and B.S. Schweigert. Food & Nutrition Press, Trumbull, CT, pp. 61–101.

Bard, J. and Townsend, W.E. 1971. Cured meats: Meat curing. In *The Science of Meat and Meat Products, Second Edition*, ed. J.F. Price and B.S. Schweigert. W.H. Freeman and Company, San Francisco, CA, pp. 452–470.

Bell, L.K., Mason, J., Mingos, D.M.P. and Tew, D.G. 1983. ^{15}N NMR studies of nitrosyl (bent and linear), nitro, and nitrito ligands in 4-, 5-, and 6-coordinate complexes of the platinum metals. *Inorg. Chem.* **22**, 3497–3502.

Bonnett, R., Chandra, S., Charalambides, A.A., Sales, K.D. and Scourides, P.A. 1980*a*. Nitrosation and nitrosylation of haemoproteins and related compounds. Part 4. Pentaco-ordinate nitrosylprotohaem as the pigment of cooked cured meat. Direct evidence from E.S.R. spectroscopy. *J.C.S. Perkin I*, 1706–1710.

Bonnett, R., Charalambides, A.A. and Martin, R.A. 1978. Nitrosation and nitrosylation of haemoproteins and related compounds. Part 1. Porphyrins and metalloporphyrins. *J.C.S. Perkin I*, 974–980.

Bonnett, R., Hursthouse, M.B., Scourides, P.A. and Trotter, J. 1980*b*. Nitrosation and nitrosylation of haemoproteins and related compounds. Part 3. Attack at the vinyl groups of protoporphyrin dimethyl ester. X-ray analysis of 8^1(E)-8^2-nitroprotoporphyrin dimethyl ester. *J.C.S. Perkin I*, 490–494.

Bonnett, R. and Nicolaidou, P. 1979. Nitrosation and nitrosylation of haemoproteins and related compounds. Part 2. The reaction of nitrous acid with the side chains of α-acylamino-acid esters. *J.C.S. Perkin I*, 1969–1974.

Burge, D.L. Jr. and Smith, J.S. 1992. Characterization of model nitrosylheme pigments with visible, infrared and ^{15}N Fourier transform nuclear magnetic resonance spectroscopy. *J. Muscle Foods* **3**, 123–131.

Cassens, R.G., Faustman, C. and Jiménez-Colmenero, F. 1988. Modern developments in research on color of meat. In *Trends in Modern Meat Technology 2. Proceedings of the International Symposium*, Den Dolder, The Netherlands, November 23-25, 1987. ed. B. Krol, P.S. van Roon and J.H. Houben. Wageningen Centre for Agricultural Publishing and Documentation, Wageningen, The Netherlands, pp. 5–11.

Cornforth, D.P., Rabovitser, J.K., Ahuja, S., Wagner, J.C., Hanson, R., Cummings, B. and Chudnovsky, Y. 1998. Carbon monoxide, nitric oxide, and nitrogen dioxide levels in gas ovens related to surface pinking of cooked beef and turkey. *J. Agric. Food Chem.* **46**, 255–261.

Cotton, F.A., Wilkinson, G. and Gaus, P.L. 1995. Introduction to the transition elements: Ligand field theory. In *Basic Inorganic Chemistry, 3rd Ed.*, John Wiley & Sons, New York, pp. 503–544.

Dickerson, R.E. 1964. X-ray analysis and protein structure. In *The Proteins. Composition, Structure, and Function, 2nd Ed., Vol. 2*, ed. H. Neurath. Academic Press, New York, pp. 603–778.

Dickinson, L.C. and Chien, J.C.W. 1971. An electron paramagnetic resonance study of nitrosylmyoglobin. *J. Am. Chem. Soc.* **93**, 5036–5040.

Dryden, F.D. and Birdsall, J.J. 1980. Why nitrite does not impart color. *Food Technol.* **34**(7), 29–31, 42.

Fox, J.B. Jr. 1966. The chemistry of meat pigments. *J. Agric. Food Chem.* **14**, 207–210.

Fox, J.B. Jr. 1987. The pigments of meat. In *The Science of Meat and Meat Products, Third Edition*, ed. J.F. Price and B.S. Schweigert. Food & Nutrition Press, Trumbull, CT, pp. 193–216.

Fox, J.B. Jr. and Ackerman, S.A. 1968. Formation of nitric oxide myoglobin: Mechanisms of the reaction with various reductants. *J. Food Sci.* **33**, 364–370.

Francis, F.J. 1985. Pigments and other colorants. In *Food Chemistry, Second Edition, Revised and Expanded*, ed. O.R. Fennema. Marcel Dekker, New York, pp. 545–584.

Giddings, G.C. 1977. The basis of color in muscle foods. *CRC Crit. Rev. Food Sci. Nutr.* **9**, 81–114.

Haldane, J. 1901. The red colour of salted meat. *J. Hygiene* **1**, 115–122.

Hedrick, H.B., Aberle, E.D., Forrest, J.C., Judge, M.D. and Merkel, R.A. 1994. Principles of Meat Science, Third Edition. Kendall/Hunt Publishing Company, Dubuque, IA.

Hood, D.E. and Riordan, E.B. 1973. Discolouration in pre-packaged beef: measurement by reflectance spectrophotometry and shopper discrimination. *J. Food Technol.* **8,** 333–343.

Hornsey, H.C. 1956. The colour of cooked cured pork. I.Estimation of the nitric oxide-haem pigments. *J. Sci. Food Agric.* **7**, 534–540.

Izumi, K., Cassens, R.G. and Greaser, M.L. 1989. Reaction of nitrite with ascorbic acid and its significant role in nitrite-cured food. *Meat Sci.* **26**, 141–153.

Jankiewicz, L., Kwaśny, M., Wasylik, K. and Graczyk, A. 1994. Structure studies on the nitrosyl derivative of heme. *J. Food Sci.* **59**, 57–59.

Kamarei, A.R. and Karel, M. 1983. Effects of ionizing radiation on nitric oxide myoglobin. Part 1. Effects on the NO-haem moiety. *Intern. J. Radiat. Biol.* **44**, 123–134.

Kendrew, J.C. 1963. Myoglobin and the structure of proteins. *Science* **139**, 1259–1266.

Killday, K.B., Tempesta, M.S., Bailey, M.E. and Metral, C.J. 1988. Structural characterization of nitrosylhemochromogen of cooked cured meat: Implications in the meat-curing reaction. *J. Agric. Food Chem.* **36**, 909–914.

Lançon, D. and Kadish, K.M. 1983. Electrochemical and spectral characterization of iron mono- and dinitrosyl porphyrins. *J. Am. Chem. Soc.* **105**, 5610–5617.

Lang, G. and Marshall, W. 1966. Mössbauer effect in some haemoglobin compounds. *J. Mol. Biol.* **18**, 385–404.

Lawrie, R.A. 1991. Chemical and biochemical constitution of muscle. In *Meat Science, Fifth Edition*. Pergamon Press, Oxford, UK, pp. 48–81.

Ledward, D.A. 1992. Colour of raw and cooked meat. In *The Chemistry of Muscle-based Foods*, ed. D.E. Johnston, M.K. Knight and D.A. Ledward. Special Publication 106, The Royal Society of Chemistry, Cambridge, UK, pp. 128–144.

Lee, S.H. and Cassens, R.G. 1976. Nitrite binding sites on myoglobin. *J. Food Sci.* **41**, 969–970.

Livingston, D.J. and Brown, W.D. 1981. The chemistry of myoglobin and its reactions. *Food Technol.* **35**(5), 244–252.

Loew, G.H., Axe, F.U., Collins, J.R. and Du, P. 1991. Quantum-mechanical studies of model peroxidase compound I complexes. *Inorg. Chem.* **30**, 2291–2294.

Maxwell, J.C. and Caughey, W.S. 1976. An infrared study of NO bonding to heme B and hemoglobin A. Evidence for inositol hexaphosphate induced cleavage of proximal histidine to iron bonds. *Biochemistry* **15**, 388–396.

Möhler, K. 1974. Formation of curing pigments by chemical, biochemical or enzymatic reactions. In *Proceedings of the International Symposium on Nitrite in Meat Products*, Zeist, The Netherlands, September 10-14, 1973, ed. B. Krol and B.J. Tinbergen. Wageningen Centre for Agricultural Publishing and Documentation, Wageningen, The Netherlands, pp. 13–19.

Morse, R.H. and Chan, S.I. 1980. Electron paramagnetic resonance studies of nitrosyl ferrous heme complexes. *J. Biol. Chem.* **255**, 7876–7882.

Neto, L.M., Nascimento, O.R., Tabak, M. and Caracelli, I. 1988. The mechanism of reaction of nitrosyl with met- and oxymyoglobin: an ESR study. *Biochim. Biophys. Acta* **956**, 189–196.

O'Keeffe, M. and Hood, D.E. 1982. Biochemical factors influencing metmyoglobin formation on beef from muscles of differing colour stability. *Meat Sci.* **7**, 209–228.

Olson, L.W., Schaeper, D., Lançon, D. and Kadish, K.M. 1982. Characterization of several novel iron nitrosyl porphyrins. *J. Am. Chem. Soc.* **104**, 2042–2044.

Pate, T.D., Shuler, R.O. and Mandigo, R.W. 1971. The influence of glucono delta lactone on cured ham color and color stability. *J. Food Sci.* **36**, 48–50.

Pegg, R.B. 1993. Development of Nitrite-free Meat Curing Systems. Ph.D. Thesis, Memorial University, St. John's, NF, Canada.

Pegg, R.B. and Shahidi, F. 1996. A novel titration methodology for elucidation of the structure of preformed cooked cured-meat pigment by visible spectroscopy. *Food Chem.* **56**, 105–110.

Pegg, R.B., Shahidi, F., Gogan, N.J. and DeSilva, S.I. 1996. Elucidation of the chemical structure of preformed cooked cured-meat pigment by electron paramagnetic resonance spectroscopy. *J. Agric. Food Chem.* **44**, 416–421.

Renerre, M. and Rougie, P. 1979. Influence du chauffage sur la fixation du nitrite à la myoglobine. *Ann. Technol. Agric.* **28**, 423–431.

Sakata, R. and Nagata, Y. 1983. A method for measuring the color forming ability of processed meat products: Extractability of the native nitroso heme pigments. *Jap. J. Zootech. Sci.* **54**, 667–670.

Scheidt, W.R. and Frisse, M.E. 1975. Nitrosylmetalloporphyrins. II. Synthesis and molecular stereochemistry of nitrosyl-$\alpha,\beta,\gamma,\delta$-tetraphenylporphinato-iron(II). *J. Am. Chem. Soc.* **97**, 17–21.

Sebranek, J.G. and Fox, J.B. Jr. 1985. A review of nitrite and chloride chemistry: Interactions and implications for cured meats. *J. Sci. Food Agric.* **36**, 1169–1182.

Shahidi, F., Pegg, R.B. and Sen, N.P. 1994. Absence of volatile *N*-nitrosamines in cooked nitrite-free cured muscle foods. *Meat Sci.* **37**, 327–336.

Shahidi, F., Rubin, L.J., Diosady, L.L. and Wood, D.F. 1985. Preparation of the cooked cured-meat pigment, dinitrosyl ferrohemochrome, from hemin and nitric oxide. *J. Food Sci.* **50**, 272–273.

Shank, J.L., Silliker, J.H. and Harper, R.H. 1962. The effect of nitric oxide on bacteria. *Appl. Microbiol.* **10**, 185–189.

Skibsted, L.H. 1992. Cured meat products and their oxidative stability. In *The Chemistry of Muscle-based Foods*, ed. D.E. Johnston, M.K. Knight and D.A. Ledward. Special Publication 106, The Royal Society of Chemistry, Cambridge, UK, 266–286.

Skoog, D.A. and Leary, J.J. 1992. Application of molecular ultraviolet/visible absorption spectroscopy. In *Principles of Instrumental Analysis, 4th Ed.* Saunders College Publishing, Fort Worth, TX, pp. 150–173.

Stryer, L. 1981. Oxygen transporters: Myoglobin and hemoglobin. In *Biochemistry*. W.H. Freeman and Company, New York, pp. 43–64.

Synowiecki, J., Heeley, D.H. and Shahidi, F. 1992. Purification and characterization of myoglobin from harp seal (*Phoca groenlandica*). *J. Agric. Food Chem.* **40**, 714–718.

Tarladgis, B.G. 1962. Interpretation of the spectra of meat pigments. II. Cured meats. The mechanism of colour fading. *J. Sci. Food Agric.* **13**, 485–491.

Thompson, R.M. 1988. Nitrosylmetalloporphyrin complexes as models for cured meat pigments. Ph.D. Thesis, University of Surrey, UK.

Tinbergen, B.J. 1974. Low-molecular meat fractions active in nitrite reduction. In *Proceedings of the International Symposium on Nitrite in Meat Products*, Zeist, The Netherlands, September 10-14, 1973, ed. B. Krol and B.J. Tinbergen. Wageningen Centre for Agricultural Publishing and Documentation, Wageningen, The Netherlands, pp. 29–36.

Trittelvitz, E., Sick, H. and Gersonde, K. 1972. Conformational isomers of nitrosyl-haemoglobin. An electron-spin-resonance study. *Eur. J. Biochem.* **31**, 578–584.

Varnam, A.H. and Sutherland, J.P. 1995. Meat and Meat Products. Technology, Chemistry and Microbiology. Chapman & Hall, London, UK.

Walters, C.L., Burger, I.H., Jewell, G.G., Lewis, D.F. and Parke, D.V. 1975. Mitochondrial enzyme pathways and their possible role during curing. *Z. Lebensm. Unters. Forsch.* **158**, 193–203.

Walters, C.L., Casselden, R.J. and Taylor, A.McM. 1967. Nitrite metabolism by skeletal muscle mitochondria in relation to haem pigments. *Biochim. Biophys. Acta* **143**, 310–318.

Walters, C.L. and Taylor, A.McM. 1965. The reduction of nitrite by skeletal-muscle mitochondria. *Biochim. Biophys. Acta* **96**, 522–524.

Wayland, B.B. and Olson, L.W. 1973. Nitric oxide complexes of iron(II) and iron(III) porphyrins. *J.C.S. Chem. Comm.*, 897–898.

Wayland, B.B. and Olson, L.W. 1974. Spectroscopic studies and bonding model for nitric oxide complexes of iron porphyrins. *J. Am. Chem. Soc.* **96**, 6037–6041.

Yonetani, T., Yamamoto, H., Erman, J.E., Leigh, J.S. Jr. and Reed, G.H. 1972. Electromagnetic properties of hemoproteins. V. Optical and electron paramagnetic resonance characteristics of nitric oxide derivatives of metalloporphyrin-apohemoprotein complexes. *J. Biol. Chem.* **247**, 2447–2455.

Young, C.L. 1981. Nitric oxide. In *Solubility Data Series. Volume 8. Oxides of Nitrogen,* International Union of Pure and Applied Chemistry. Pergamon Press, Oxford, UK, pp. 260–351.

CHAPTER 4

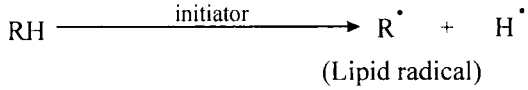
OXIDATIVE STABILITY OF MEAT LIPIDS

Lipids are an integral part of foodstuffs. In meat, the two main classes of lipids are adipose and intramuscular tissues. Adipose tissue consists primarily of triacylglycerols while intramuscular tissue is composed of both triacylglycerols and membrane-bound fats, such as phospholipids and lipoproteins. The fatty acids associated with these tissues are either saturated or unsaturated. Oxidation of unsaturated lipids has been studied extensively since it relates to deterioration of muscle foods, production of both desirable and undesirable breakdown products and numerous reactions associated with other food constituents (Wong 1989). The changes in the quality of meat are manifested by changes in flavor (Morrissey and Apte 1988), color (Faustman *et al.* 1989), nutritive value (Kanner 1994), as well as loss of protein functionality and possible generation of toxic compounds (Addis 1986; Addis and Park 1989). These days one can find an increasing assortment of precooked and restructured meat products in the marketplace as a result of consumer demand for more convenience foods. These products are quite susceptible to lipid oxidation and formation of warmed-over-flavor (WOF), thus presenting the meat processing industry with some serious challenges (Gray *et al.* 1996). Apart from microbial spoilage, lipid oxidation is generally accepted as being the primary process by which loss in quality of meat and meat products occurs (Buckley *et al.* 1995).

Autoxidation is the main pathway of oxidative deterioration of meat lipids. The process proceeds via a free-radical mechanism involving initiation, propagation, and termination steps as illustrated in Fig. 4.1 (Hamilton 1989; Raharjo and Sofos 1993). An initiator causes homolytic cleavage of the lipid-hydrogen covalent bond adjacent, that is α, to the site of unsaturation in fatty acid molecules. It has been postulated that singlet oxygen is the active species involved in free radical formation in the initiation step, with tissue pigments such as myoglobin (Mb) acting as sensitizers. A lipid free radical ($R^{\bullet}$) reacts with molecular oxygen forming a peroxy radical ($ROO^{\bullet}$). This radical in turn abstracts a hydrogen atom from a second unsaturated fatty acid molecule producing a hydroperoxide (ROOH) and a new lipid radical which may also react with oxygen. This chain reaction appears to be self-sustaining as long as oxygen and unoxidized lipids are present. Due to resonance stabilization of lipid radical species, a shift in the position of double bonds results in the formation of hydroperoxide positional- and geometric-isomers, but intermolecular reactions of radicals may result in the formation of non-radical species (*i.e.*, termination

products) such as dimers, polymers, cyclic peroxides, and hydroperoxy compounds.

Initiation



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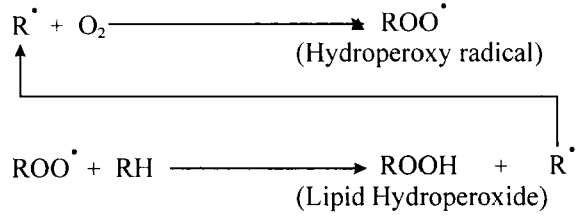


Termination

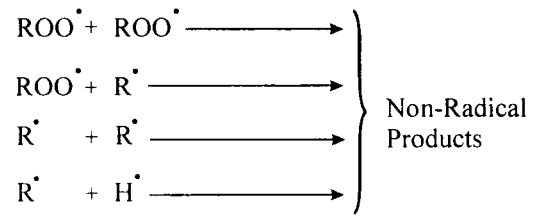


FIG. 4.1. MECHANISM OF AUTOXIDATION

Hydroperoxides, the primary products of lipid oxidation, are considered to be the most important initial reaction products obtained from autoxidation. Yet, they do not contribute to off-flavors of oxidized fats. Monohydroperoxides are colorless and odorless, but of a very transitory nature and decompose via an

intermediate alkoxy radical forming a prolific number of volatiles. Figures 4.2 and 4.3 summarize this decomposition process, which accounts for the generation of the numerous aldehydes, ketones, alcohols, hydrocarbons, lactones and 2-alkylfurans. Secondary products of lipid oxidation are the major contributors to off-flavors in meat. These compounds do not contribute to desirable meaty flavor, but rather impart green, rancid, fatty, pungent and other off-flavor characteristics to meat (Chang and Peterson 1977). The extent of autoxidation depends on many factors including oxygen partial pressure, the degree of unsaturation of lipids, the presence and concentration of antioxidants and/or prooxidants, packaging materials, exposure to light, and temperature of storage. The nature of fatty acids in meat and their concentrations have a very pronounced effect on the rate of autoxidation.

Lipid Oxidation of Uncured Meats

Tims and Watts (1958) observed that lipid oxidation in refrigerated cooked meats was more pronounced than that in fresh or frozen uncooked meat. To describe this rapid development of lipid-derived oxidized flavor, they coined the term "warmed-over flavor" (WOF). The rancid or stale flavor becomes readily apparent within 48 hours in cooked meats, as opposed to the more slowly developing rancidity encountered in fresh meats, which becomes evident only after prolonged freezer storage (Pearson *et al.* 1977; Spanier *et al.* 1992*a*). Although WOF was first recognized in cooked meat, it develops in fresh meat that has been comminuted and exposed to air, and in unheated products such as mechanically separated and restructured meats in which the muscle tissue is disrupted and air is incorporated (Greene 1969; Sato and Hegarty 1971; Gray and Pearson 1987). It is now generally accepted that any process involving disruption of the integrity of muscles, such as cooking, grinding or restructuring, enhances the development of WOF (Spanier *et al.* 1992*b*). In recent years, demand has grown for pre-cooked, ready-to-eat meat products in the marketplace *i.e.*, home meal replacement, and in fast food franchises, thereby providing expanding potential for consumer exposure to WOF (Stoick *et al.* 1991). Because WOF development is a dynamic process of flavor change, due principally to a cascade of oxidative events (Asghar *et al.* 1988), an understanding of the mechanism and prevention of its occurrence in meat and meat products is important to the food scientist.

In the late 1980s, several researchers questioned the term WOF on the basis that it did not adequately describe the flavor changes in cooked meat during storage (Vercellotti *et al.* 1987, 1989; St. Angelo *et al.* 1988; Spanier *et al.* 1988). These scientists suggested that WOF development was not solely a consequence of lipid oxidation; strong evidence of protein degradation reactions was reported. Furthermore, it was believed that heteroatomic compounds formed

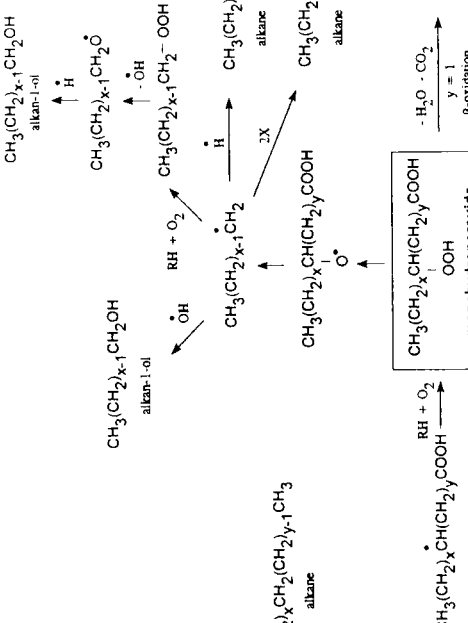


FIG. 4.2. SCHEMATIC REPRESENTATION OF SOME POSSIBLE REACTION PATHWAYS IN THE THERMAL OXIDATION OF SATURATED FATTY ACIDS
(From MacLeod and Ames 1988).

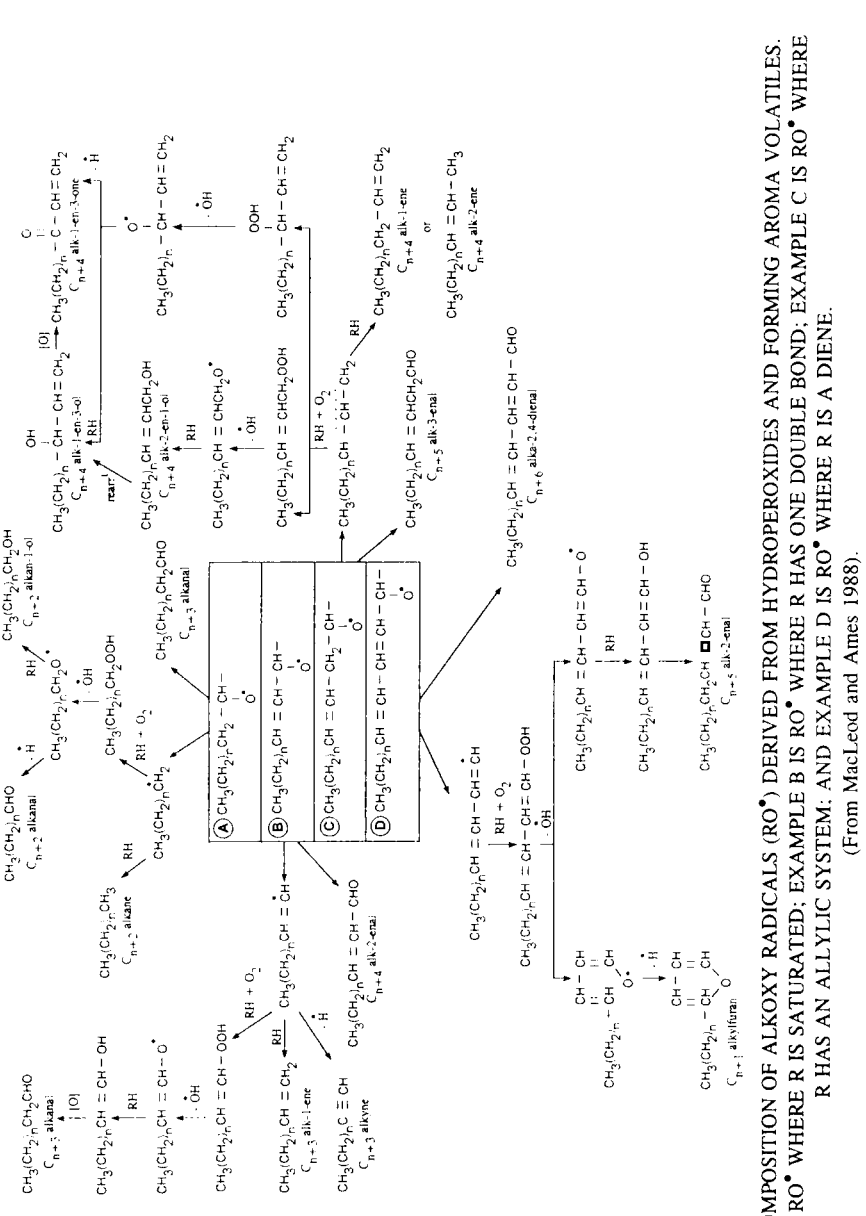


FIG. 4.3. DECOMPOSITION OF ALKOXY RADICALS ($RO^{\bullet}$) DERIVED FROM HYDROPEROXIDES AND FORMING AROMA VOLATILES. EXAMPLE A IS $RO^{\bullet}$ WHERE R IS SATURATED; EXAMPLE B IS $RO^{\bullet}$ WHERE R HAS ONE DOUBLE BOND; EXAMPLE C IS $RO^{\bullet}$ WHERE R HAS AN ALLYLIC SYSTEM; AND EXAMPLE D IS $RO^{\bullet}$ WHERE R IS A DIENE.
(From MacLeod and Ames 1988).

from these reactions could be implicated with the phenomenon of WOF, particularly with the deterioration of desirable meaty flavor notes. In an effort to better describe the complex series of chemical reactions that contribute to an overall increase in off-flavor notes and a loss in desirable meaty ones, the term meat flavor deterioration (MFD) was proposed. From a sensory point of view, these chemical reactions caused increases in undesirable cardboard and painty notes and decreases in desirable cooked meaty notes (St. Angelo *et al.* 1987; Love 1988). The intensity of these undesirable sensory notes has been positively correlated with the content of carbonyl compounds formed via lipid autoxidation reactions (Drumm and Spanier 1991). Moreover, the decrease in intensity of desirable sensory notes may be due to either a reduction in the content of those compounds that contribute to desirable meat flavor or a masking of the desirable flavor volatiles by an increase in the content of undesirable flavor compounds (St. Angelo *et al.* 1987). Johnson and Civille (1986) described the following general trend in off-flavor development in cooked, stored meat: the disappearance of the fresh flavors, the appearance then disappearance of the cardboard flavor, and the final dominance of other flavors by the oxidized/rancid note. They suggested that a descriptive vocabulary would benefit future studies that might examine causes and prevention of WOF by providing an established protocol so as to allow comparison and evaluation of data from different laboratories.

Of the lipids in meat, phospholipids are most susceptible to oxidation. Dupuy *et al.* (1987) demonstrated that subcutaneous fat produced about 50 volatile compounds during MFD development, whereas intramuscular lipids generated more than 200 volatile compounds. There seems to be no question that oxidation of phospholipids is the primary source of off-flavor notes produced during MFD (Spanier 1992). The tendency of phospholipids to undergo rapid oxidation is largely due to their high unsaturated fatty acid content, which is accelerated upon thermal processing (Igene *et al.* 1979). Oxidation of the unsaturated C_{18} fatty acids of meat, namely oleate, linoleate and linolenate, has been reported to produce low-molecular-weight aldehydes (C_3 — C_{12}) such as pentanal, hexanal and 2,4-decadienal which are believed to be partially responsible for WOF and rancidity development of cooked meats during storage.

The catalytic effect of iron porphyrins and metal ions during oxidation has been the subject of a great deal of study since Robinson (1924) first described that iron porphyrins could oxidize polyunsaturated fatty acids. Hemoproteins have been implicated as major prooxidants of lipid peroxidation in meats (Tappel 1952; Watts 1954; Greene *et al.* 1971; Love and Pearson 1974; Igene *et al.* 1979). Wills (1966) and Liu and Watts (1970) assessed the role of heme and non-heme iron as catalysts of lipid oxidation in various animal tissues and concluded that both heme and non-heme iron had catalytic activity in raw and cooked systems. Catalysis of lipid oxidation by heme pigments was an accepted

mechanism until the work of Sato and Hegarty (1971). These authors reported that non-heme iron, rather than Mb, was the active catalyst responsible for the rapid oxidation of cooked meat. Sato and Hegarty (1971) removed heme pigments from muscle tissue by dialysis, added Mb or hemoglobin (Hb) back to the dialyzed samples, cooked them and then stored the meats under refrigeration conditions. The 2-thiobarbituric acid (TBA) test revealed that heme pigments had no significant effect on the extent of lipid oxidation; Love (1972) further demonstrated that metMb did not influence the TBA scores in cooked meat systems. Love and Pearson (1974) and Igene *et al.* (1979) confirmed and extended the basic finding of Sato and Hegarty (1971). They reported that intact hemoproteins had little effect on the rate of oxidation in cooked meats, while non-heme Fe^{2+} at concentrations as low as 1 ppm resulted in enhanced oxidation in samples of water-extracted cooked meats. Ferrous iron is known to decompose lipid hydroperoxides forming very reactive alkoxy radicals for the propagation reactions, whereas ferric iron produces relatively less reactive peroxy radicals from fatty acid hydroperoxides.

$$ROOH + Fe^{2+} \rightarrow RO^{\bullet} + OH^{-} + Fe^{3+} \quad (1)$$

$$ROOH + Fe^{3+} \rightarrow ROO^{\bullet} + H^{+} + Fe^{2+} \quad (2)$$

Resolution of the roles played by heme and non-heme iron as catalysts of lipid oxidation in meat products is very important in understanding the factors responsible for the development of off-flavors. Igene *et al.* (1979) proposed that thermal processing releases a significant amount of non-heme iron from the native muscle pigments which then accelerates lipid oxidation in cooked meats. Studies by Schricker *et al.* (1982), Schricker and Miller (1983), Chen *et al.* (1984) and Tichivangana and Morrissey (1984) have concurred with this finding.

However, the debate continues. Several studies have cast some doubt as to whether non-heme iron, as opposed to heme iron, is the stronger prooxidant in muscle tissue (Verma *et al.* 1985; Johns *et al.* 1989; Monahan *et al.* 1993). Using emulsions of lard, egg albumin and water, Verma *et al.* (1985) reported that ferric heme pigments were more effective catalysts of lipid oxidation than their ferrous counterparts. When employed at concentrations similar to those present in meat products, ferrous and ferric salts were found to be very weak catalysts of lipid oxidation, when compared to ferric heme pigments. Emulsions prepared using meat of high and low metmyoglobin (metMb) contents supported these findings. In experiments with water-washed muscle residue, Johns *et al.* (1989) noted that heme iron, when used at levels similar to those present in muscle, was a powerful catalyst of lipid oxidation while inorganic iron appeared to have little prooxidant activity. These authors proposed that differences in (1)

the postmortem history of muscle samples used in the preparation of water-washed muscle residue, (2) the oxidation states of prooxidants, (3) the mode of addition of prooxidants to model systems, and (4) sample storage times following addition of prooxidants could explain why their results differed from those reported previously by Sato and Hegarty (1971), Love and Pearson (1974) and Tichivangana and Morrissey (1985). Monahan *et al.* (1993) compared the prooxidant effects of heme (Hb and Mb) and inorganic iron ($FeSO_4$) in the muscle model systems used by Love and Pearson (1974) and Johns *et al.* (1989). They found that heme proteins had a greater prooxidant effect than inorganic iron in raw and thermally processed pork muscle residue when these prooxidants were present at levels approaching those in red meats. The rate of lipid oxidation catalyzed by each prooxidant increased as the iron concentration increased over a range of 1-17 μg/g muscle tissue. Furthermore, the relative prooxidant effects of heme protein and inorganic iron were not affected by either the mode of addition of the prooxidants to the muscle residue (*i.e.*, addition in a concentrated form or dispersed in water), or by the method of cooking (*i.e.*, fast versus slow cooking). Monahan *et al.* (1993) commented that the prooxidant potential of non-heme iron bound to ferritin or present as insoluble hemosiderin, which accounts for a significant portion of the total iron in pork and chicken meat, needs to be addressed. Studies by Seman *et al.* (1991) and Kanner and Doll (1991) provided data which suggested that ferritin may be at least partially responsible for catalyzing lipid oxidation in muscle foods. Thus, further research is necessary to define the relative roles of heme proteins and ferritin in catalysis of lipid oxidation and to ascertain whether these proteins in their native state, or the inorganic iron that may be released from them, is the effective prooxidant in raw and cooked muscle. It is clear that many unanswered questions remain pertaining to the relative roles of heme and non-heme iron in the development of lipid oxidation in meats.

Lipid Oxidation of Nitrite-Cured Meats

In 1954, Watts noted that development of oxidative rancidity was delayed in nitrite-cured meats. Younathan and Watts (1959) investigated the extent of lipid oxidation in cured and uncured cooked pork stored at refrigeration temperatures over a two week period using the TBA test. Uncured samples yielded significantly ($P<0.05$) higher TBA values than their cured counterparts at all storage periods indicating that nitrite addition to meat suppresses oxidative deterioration of meat lipids. Zipser *et al.* (1964), Cho and Bratzler (1970) and Hadden *et al.* (1975) have all shown the inhibitory effect of nitrite against oxidation and WOF development in cooked cured-meat products.

Sato and Hegarty (1971) reported that nitrite inhibits WOF development even at levels as low as 50 ppm, and it could completely retard lipid oxidation

in ground beef when used at a concentration of 2000 ppm. Bailey and Swain (1973) confirmed the antioxidant role of nitrite in refrigerated cooked hams by correlating subjective taste-panel flavor scores with TBA values. MacDonald *et al.* (1980*a*,*b*,*c*) went a step further and studied the effects of various levels of nitrite (0, 50, 200, and 500 ppm) on the oxidative stability of cooked hams. A comparison of the data revealed a significant reduction ($P<0.05$) in TBA values of nitrite-cured ham, but no significant difference ($P>0.05$) in mean TBA values between hams cured with sodium nitrite at 200 and 500 ppm level.

Fooladi *et al.* (1979) investigated the role and function of nitrite in preventing the development of WOF in cooked beef, pork and chicken. Samples treated with nitrite at a fixed level of 156 ppm were evaluated against controls with no additives by the TBA test and by sensory panel scores before and after cooking on day 0 and again after 2 days of storage at 4°C (Table 4.1). For all three species, a significant difference ($P<0.01$) in TBA values between cured and uncured meats was observed. Added nitrite inhibited WOF development in cooked meat, resulting in a 2-fold reduction in TBA values for beef and chicken and a 5-fold reduction in pork. Sensory panel data were in agreement with findings of the TBA test. Differences in taste panel scores between cured and uncured samples were significant ($P<0.05$) for chicken and highly significant ($P<0.01$) for pork and beef. The effect of sodium nitrite on lipid oxidation in cooked, minced muscles from various species stored at 4°C for 24 h, as monitored by TBA values, is presented in Fig. 4.4 (Morrissey and Tichivangana 1985). At a level of 200 ppm, nitrite brought about a 17-fold reduction in TBA values of fish and a 12-fold reduction in TBA values of chicken, pork and beef compared to those of their uncured counterparts.

Mechanism of the Antioxidative Action of Nitrite

The mechanism(s) by which nitrite prevents or retards the peroxidation of meat lipids is still a matter of discussion (Fox and Benedict 1987). For example, is nitrite or nitric oxide per se the actual antioxidant in cured meat or is it a reaction product from nitrite's interaction with various constituents of the meat? From literature, four different mechanisms have been proposed for the antioxidative effect of nitrite in meats:

(1) formation of a stable complex between heme pigments and nitrite, thereby preventing the release of iron from the porphyrin molecule.
(2) stabilization of unsaturated lipids within tissue membranes against oxidation.
(3) interaction of nitrite as a metal chelator so that it ties up trace metals in meat as well as any liberated non-heme iron from denatured heme pigments.

(4) formation of nitroso and nitrosyl compounds in meat which possess antioxidative properties by acting as radical scavengers.

TABLE 4.1
THE EFFECT OF NITRITE ON OXIDATIVE STABILITY OF MEAT

Treatment	TBA Numbers (mg/kg)		
	Without nitrite	With nitrite	Mean difference
Chicken Samples			
Raw, 0 days	2.52	1.36	1.16
Cooked, 0 days	3.58	1.06	2.52[a]
Raw, 2 days at 4°C	5.52	1.47	4.05[a]
Cooked, 2 days at 4°C	6.98	3.05	3.93[a]
Pork Samples			
Raw, 0 days	1.52	0.85	0.67
Cooked, 0 days	1.83	0.72	1.11
Raw, 2 days at 4°C	2.48	1.42	1.06
Cooked, 2 days at 4°C	7.85	1.64	6.21[a]
Beef Samples			
Raw, 0 days	0.92	0.66	0.26[b]
Cooked, 0 days	1.07	0.75	0.32[a]
Raw, 2 days at 4°C	1.84	1.17	0.67[a]
Cooked, 2 days at 4°C	4.12	2.06	2.06[a]

(Adapted from Fooladi *et al.* 1979). [a] $p < 0.01$; [b] $p < 0.05$.

According to Gray and Pearson (1987), preventing the release of Fe^{2+} during thermal processing by stabilizing the porphyrin ring appears to be the most important of these proposed mechanisms. Igene *et al.* (1985) reported that cooking significantly ($P<0.05$) increased the proportion of non-heme iron in untreated beef from 6.6 to 10.8 μg Fe/g muscle tissue whereas the levels of non-heme iron remained unchanged in the nitrite-cured sample (*ca.* 6.8 μg Fe/g muscle tissue).

Sato and Hegarty (1971) and Goutefongea *et al.* (1977) support the view that nitrite reacts with lipids in tissue membranes, leading to a stabilization and retardation of lipid oxidation. Moreover, the extent of nitrite's interaction with lipids was related to their degree of unsaturation. The reaction of various nitrogen oxides with olefins has been reported to result in the formation of nitroso-nitrite derivatives (*i.e.*, nitrosites) and nitroso-nitro compounds (*i.e.*, pseudonitrosites). Walters *et al.* (1979) found evidence that nitrite added to the

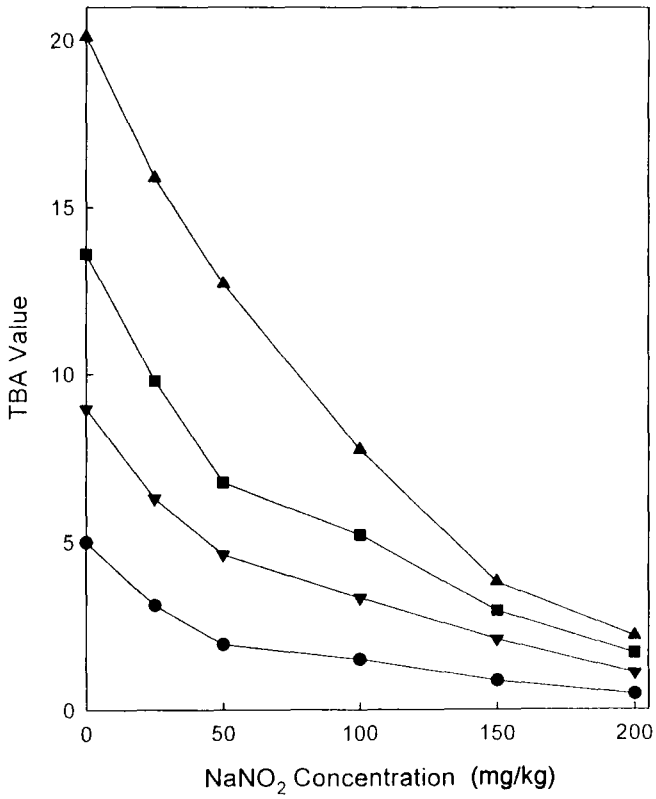


FIG. 4.4. EFFECT OF SODIUM NITRITE ON LIPID OXIDATION IN COOKED, MINCED MUSCLES FROM VARIOUS SPECIES STORED AT 4°C FOR 48 H: Δ, FISH; ■, CHICKEN; ▼, PORK; AND •, BEEF
(Adapted from Morrissey and Tichivangana 1985.)

double bonds of unsaturated fatty acids and formed pseudonitrosites, and suggested that these reaction products could act as transnitrosating agents. Liu *et al.* (1988) proposed a mechanism in which these nitro-nitroso derivatives of olefins nitrosated amines to form *N*-nitrosamines during the frying of nitrite-cured meats such as bacon. In a more recent study utilizing IR spectroscopy, Freybler *et al.* (1993) showed that nitrite or dinitrogen trioxide reacts with unsaturated lipids to form other nitro-nitroso derivatives which stabilize lipids toward peroxidative changes. Igene and Pearson (1979) studied the reaction of nitrite with purified unsaturated phospholipids and demonstrated that nitrite

significantly reduced TBA values while improving sensory scores. Furthermore, they suggested that nitrite functions as an antioxidant by forming a complex with the phospholipid components, thereby stabilizing the membranes, as well as by forming a chromogen with heme pigments. By analyzing the difference in the TBA values of cooked nitrite-cured beef surimi with its uncured counterpart, Igene *et al.* (1985) attributed the effective inhibition of lipid oxidation to nitrite by its stabilization of membrane lipids. More specifically, these researchers demonstrated that nitrite was an effective antioxidant against the degradation of phosphatidylethanolamine, the major phospholipid responsible for the development of MFD in cooked meat. Zubillaga *et al.* (1984) reported that the polar-lipid fraction of raw nitrite-cured beef and pork had sufficient activity in inhibiting the oxidation of linoleate as determined by a β-carotene bleaching method and by conjugated diene formation. From their data, these researchers concluded that residual nitrite, carbon- and nitrogen-nitroso compounds or products from the addition of nitrogen oxides to olefins did not account for the observed antioxidant activity.

Kanner *et al.* (1984) proposed that the antioxidant effects of nitrite in cured meat resulted from the formation of NO that can react with metals, heme pigments and other biomolecules in the meat matrix. Nitric oxide was also found to inhibit the Fenton reaction, the generation of ferryl ions (Kanner *et al.* 1991) and lipoxygenase and cyclooxygenase activities (Kanner *et al.* 1992). Kanner (1994) suggested that the antioxidative effects of NO seem to be derived from its capability to ligate to ferrous ion and to work as an electron donor and a scavenger of free radicals. Furthermore, it was found that NO prevented the release of iron from NOMb by H_2O_2 via a mechanism connected to its antioxidative effect (Kanner *et al.* 1991).

Kanner (1979), Kanner *et al.* (1980), Morrissey and Tichivangana (1985) and Shahidi *et al.* (1988) have all clearly demonstrated that some nitrosylheme compounds possess antioxidant effects. The preformed CCMP was found to act as a weak antioxidant in meat model systems (Shahidi *et al.* 1987*a*) and to exhibit a concentration-dependent antioxidative effect in a β-carotene/linoleate model system (Wettasinghe and Shahidi 1997). Wettasinghe and Shahidi (1997) examined the effect of CCMP at three levels and compared data obtained with that of a control and those of systems containing NOMb, metMb or BHA (Table 4.2). Depending upon the concentration, the behavior of CCMP in the β-carotene/linoleate model system changed from prooxidative to antioxidative. Kanner *et al.* (1979) made a similar observation for NOMb. At the 2.2μM concentration, CCMP's ability to quench free radicals in this system was inadequate; however, at the higher concentrations tested, a strong antioxidative effect was evident. Furthermore, this effect was enhanced by the addition of sodium ascorbate to the system. Suggestions have been made that nitrosylated iron porphyrin compounds act in the early stages of lipid autoxidation to quench

substrate-free radicals and thereby inhibit their propagation (Kanner *et al.* 1980). In the case of CCMP, the NO group might quench free radicals in the medium. Skibsted (1992) noted that peroxy radicals are the longer-lived among the free radical intermediates from autoxidation. In order to form non-radical products, the radicals involved must react with other free radicals. Skibsted singled out the results of Morrissey and Tichivangana (1985), which provided evidence that NOMb can act as a chain terminator. Although he was uncertain of the nature of the products, Skibsted proposed the following reaction,

$$ROO^{\bullet} + 3\ MbFe^{II}NO + 2\ H_2O \rightleftharpoons ROH + 3\ MbFe^{II} + 3\ NO_2^- + 3\ H^+ \quad (3)$$

and brought attention to the fact that Mb and NO_2^- were reaction products that are necessary for the regeneration of the antioxidant NOMb.

TABLE 4.2
EFFECT OF HEME PIGMENTS AND BHA ON β-CAROTENE BLEACHING IN A β-CAROTENE-LINOLEATE MODEL SYSTEM AS REFLECTED BY CUMULATIVE LOSS OF β-CAROTENE.

System	Reaction time (min)			
	1	2	3	4
Control (H_2O)	1.41 ± 0.02^{d}	1.80 ± 0.07^{de}	2.08 ± 0.11^{d}	2.41 ± 0.20^{d}
Concentration 2.2 μM				
BHA	0.20 ± 0.05^{fg}	0.27 ± 0.10^{f}	0.30 ± 0.07^{f}	0.34 ± 0.03^{h}
metMb	9.18 ± 0.07^{a}	10.7 ± 0.86^{a}	11.3 ± 0.90^{a}	11.5 ± 0.65^{a}
NOMb	1.09 ± 0.18^{de}	1.51 ± 0.13^{e}	1.82 ± 0.25^{de}	1.99 ± 0.35^{de}
CCMP	5.68 ± 0.45^{b}	7.05 ± 0.38^{b}	7.85 ± 0.35^{b}	8.40 ± 0.34^{b}
Concentration 6.2 μM				
BHA	0.18 ± 0.07^{fg}	0.25 ± 0.11^{f}	0.31 ± 0.10^{f}	0.36 ± 0.10^{h}
metMb	6.16 ± 0.64^{b}	7.35 ± 0.78^{b}	7.92 ± 0.80^{b}	8.20 ± 0.79^{b}
NOMb	0.82 ± 0.01^{def}	1.17 ± 0.13^{ef}	1.36 ± 0.11^{def}	1.49 ± 0.01^{defg}
CCMP	0.45 ± 0.08^{efg}	0.49 ± 0.01^{f}	0.88 ± 0.11^{ef}	1.02 ± 0.07^{efgh}
Concentration 10 μM				
BHA	0.11 ± 0.01^{g}	0.21 ± 0.02^{f}	0.31 ± 0.01^{f}	0.35 ± 0.08^{h}
metMb	4.74 ± 0.12^{c}	5.96 ± 0.11^{c}	6.54 ± 0.11^{c}	6.52 ± 0.35^{c}
NOMb	0.69 ± 0.14^{efg}	1.10 ± 0.32^{ef}	1.35 ± 0.24^{def}	1.52 ± 0.28^{def}
CCMP	0.41 ± 0.04^{efg}	0.49 ± 0.01^{f}	0.54 ± 0.03^{f}	0.57 ± 0.06^{fgh}

Results are mean values of 3 determinations ± standard deviation. Means sharing the same superscripts in a column are not significantly ($P > 0.05$) different from one another. Abbreviations are: butylated hydroxyanisole, BHA; metmyoglobin, metMb; nitrosylmyoglobin, NOMb; and cooked cured-meat pigment, CCMP. (From Wettasinghe and Shahidi 1997).

S-Nitrosocysteine (SNC), a possible reaction product generated during curing, has been shown to be a potent antioxidant (Kanner 1979). The inhibitory effect of SNC on lipid oxidation in a cooked turkey meat product was reported by Kanner and Juven (1980). Equimolar concentrations of SNC and nitrite imparted a similar inhibitory effect. It was also demonstrated that at room temperature, or upon cooking, SNC dissociates to form heme — NO complexes in meat. Since only 1-2 ppm of nitrite is sufficient for cured-meat color production (MacDougall and Hetherington 1992), the concentration of SNC, on a molar basis, may arguably be much smaller than that of the added nitrite. Consequently, comparison of the antioxidant activity of nitrite with SNC at equimolar concentrations may not be realistic (Shahidi 1992).

From a growing body of experimental evidence, Skibsted (1992) suggested that several mechanisms involving compounds derived from nitrite, and not just one, were responsible for the marked oxidative stability of cured products. This author also pointed out that nitrite-derived compounds could affect both the initiation and propagation steps of autoxidation. Moreover, the different pools of nitrite-modified compounds (*i.e.*, nitrosated cytochromes, nitrosated tryptophyl moieties on proteins, unidentified nitrosating derivatives of ascorbic acid, and the nitrosyl pigment) seem to be rapidly interchanging with each other and with residual nitrite and N_2O_3. Skibsted (1992) proposed that exhaustion of one group of compounds due to oxidation or participation in antioxidative reactions would therefore, under optimal conditions, be followed by transnitrosation reactions and reformation of the antioxidant. Results from the study of nitrosation of Mb by nitrosated lysozyme (Ito *et al.* 1983) and the reversible light-induced fading of the red color of vacuum packed sliced ham during storage, indicating reformation of NOMb initially photooxidized (Andersen *et al.* 1988; 1990*a*) and the low activation barrier for NO exchange in NOMb (Andersen *et al.* 1990*b*), were cited as evidence in support of this proposal.

Assessment of Lipid Oxidation in Meats by the 2-Thiobarbituric (TBA) Test

A relatively minor product of autoxidation of polyunsaturated fatty acids in meat is malonaldehyde. It is a three-carbon dialdehyde ($C_3H_4O_2$) with carbonyl groups at the C-1 and C-3 positions. Malonaldehyde tends to exist as a stable enol, which creates the possibility of *cis-trans* isomerism about the enolic double bond (Bertz and Dabbagh 1990). However, in the presence of water, it exists primarily as its non-volatile enolate anion.

This ubiquitous lipid oxidation degradation product has been investigated extensively due to its reactivity with biological molecules such as amino moieties of amino acids, proteins and nucleic acids as well as with sulfhydryl groups (Chio and Tappel 1969*a*, *b*; Draper *et al.* 1986). In muscle tissue, malonaldehyde is bound to various constituents of the meat and therefore, prior to

determination, it must be released from them. It is difficult to determine the optimal conditions for malonaldehyde's release from these bound forms as they differ from one material to another and require different conditions for hydrolysis. Yet, malonaldehyde's presence and concentration in meat and meat products is commonly monitored as a marker of lipid peroxidation by the TBA test (Shahidi and Hong 1991*a*). This spectrophotometric determination, first reported by Kohn and Liversedge (1944) and then described in detail by Tarladgis *et al.* (1960), involves the reaction of malonaldehyde in oxidized foods with the TBA reagent; a pink adduct forms with a distinctive absorption maximum at 532 nm. This classical test is one of the most frequently used methods for malonaldehyde quantitation in meat and meat products because of its simplicity and speed.

Various procedures have been employed for carrying out the TBA test. It can be performed: (1) on a meat sample directly by heating it with TBA solution and then extracting the red pigment produced from the reaction mixture with butanol or a butanol-pyridine mixture (Turner *et al.* 1954; Sinnhuber and Yu 1958; Placer *et al.* 1966; Uchiyama and Mihara 1978; Ohkawa *et al.* 1979; Pokorný and Dieffenbacher 1989); (2) on an aliquot of the steam distillate of meat samples (Tarladgis *et al.* 1960; Igene *et al.* 1979; Yamauchi *et al.* 1982; Ke *et al.* 1984); (3) on aqueous acid extracts of meat samples (Tarladgis *et al.* 1964; Witte *et al.* 1970; Siu and Draper 1978; Caldironi and Bazan 1982; Salih *et al.* 1987; Bedinghaus and Ockerman 1995); and (4) on the extracted lipid portion of meat samples (Younathan and Watts 1960; Pikul *et al.* 1983; 1989). The chromophore formed from the reaction of malonaldehyde with TBA is then quantified spectrophotometrically. Commonly, 1,1,3,3-tetramethoxypropane or its tetraethoxy analogue is used as a standard.

There are problems inherent in each aforementioned method of analysis. For example, direct heating of a meat sample with TBA reagent under acidic conditions (pH 3 or lower) enhances the degradation of existing lipid hydroperoxides, such as malonaldehyde precursors, and generates additional reactive radicals and scission products other than malonaldehyde (Raharjo and Sofos 1993). Thus, more malonaldehyde will be generated and the TBA values obtained will be inflated and not represent a true assessment of malonaldehyde content in the meat.

The distillation method is similar to that of the direct heating approach, except that TBA is reacted only with the meat distillate. Physical and chemical interference by extraneous meat constituents in the reaction with TBA reagent is minimized because the meat is never directly in contact with TBA. As was the case for the direct heating protocol, an overestimation of malonaldehyde content results.

The aqueous acid extraction TBA test may be considered as a better assay for estimating the malonaldehyde content in meat than both the direct heating

and distillation TBA methods because the meat itself is not exposed to heat treatment. In this way, the malonaldehyde content of the meat should not be overestimated; however, the problem comes from interfering substances extracted into solution. Presence of water soluble proteins, peptides, amino acids and fat droplets in the extract necessitates a filtration step that can be tedious and not completely effective. Turbidity of the medium containing the malonaldehyde-TBA adduct will inflate absorbance readings and consequently overestimate TBA values.

Reaction of TBA with the lipid portion of a meat sample generates the red colored malonaldehyde-TBA complex. The advantage of this procedure is that the presence of interfering substances, such as soluble proteins, peptides, amino acids and pigments, in meat samples is eliminated. However, due to the hydrophilic nature of malonaldehyde, only a minor portion of it will be present in the organic lipid extract from meat. Moreover, removal of the organic solvent necessitates heating which could result in further lipid oxidation and malonaldehyde formation. Therefore, when the lipid is used for TBA analysis, the malonaldehyde detected is produced mostly due to the assay conditions and data on the level of malonaldehyde present in the original meat sample is no longer available (Raharjo and Sofos 1993).

The TBA test was once believed to be specific for malonaldehyde (Tarladgis *et al.* 1960; 1964), but this is not so. In fact, the TBA method has been criticized as lacking specificity and adequate sensitivity towards malonaldehyde (Raharjo and Sofos 1993). A variety of aldehydes such as alkanals, alk-2-enal, alk-2,4-dienals and 4-hydroxyalkenal are generated as scission products of lipid hydroperoxides (Kosugi *et al.* 1988; 1989). Many of these can react with the TBA reagent to form a pink-chromogenic adduct with an identical absorption maximum as the TBA-malonaldehyde complex (Marcuse and Johansson 1973; Kosugi and Kikugawa 1986; Witz *et al.* 1986; Kosugi *et al.* 1987; 1988; 1989). Kosugi and co-workers (1986; 1987; 1988; 1989) found that condensation reactions between hexanal and heptanal and the TBA reagent yielded not only a red pigment, but also an unstable yellow one with an absorption maximum between 450-470 nm. The reaction of alk-2-enals such as 2-hexenal and 2-octenal with an excess of TBA reagent under acidic conditions produced yellow, orange and red chromophores.

Due to the uncertainty about the exact identity of compounds that can react with the TBA reagent, the ambiguous term "thiobarbituric acid-reactive substances" (TBARS) is now commonly used in lieu of TBA number or value (Ke *et al.* 1984; Gray and Pearson 1987; Guillén-Sans and Guzmán-Chozas 1998). Because the TBA reagent is not specific for malonaldehyde alone, certain limitations exist when performing the test for evaluation of the oxidative state of foods and biological materials due to the chemical complexity of these systems. For example, Dugan (1955) reported that sucrose and some constituents

of wood smoke react with the TBA reagent to give a red color. Baumgartner *et al.* (1975) found that a mixture of acetaldehyde and sucrose when subjected to the TBA test produced a 532-nm absorbing pigment identical to that produced by malonaldehyde and TBA. To compensate for these factors, numerous modifications of the original TBA test have been reported in the literature (Marcuse and Johansson 1973; Ke and Woyewoda 1979; Robles-Martinez *et al.* 1982; Pokorný *et al.* 1985; Tomás and Funes 1987; Schmedes and Hølmer 1989), but this raises a host of new problems. Sample preparation, types of acidulants and their concentration in the reaction mixture, pH of the reaction mixture, composition of the TBA reagent, length of the TBA reaction, and possible use of antioxidants and chelators in the systems are amongst the factors which may influence results reported by researchers from various laboratories. For example, Moerck and Ball (1974) suggested that Tenox II be added to the distillation mixture prior to heating in order to retard further oxidation and subsequently artifact formation during this step, whereas Ke *et al.* (1977) used propyl gallate (PG) and ethylenediaminetetraacetic acid (EDTA) during distillation for the same purpose. Rhee (1978) pointed out that some phenolic antioxidants such as butylated hydroxyanisole (BHA), used to retard further oxidation of samples, may in fact enhance the decomposition of lipid peroxides during distillation. Therefore, it is always preferable to quantitate the extent of lipid oxidation by a complementary analytical procedure to verify the results.

In addition to chemical reactivity of substances other than malonaldehyde with the TBA reagent, physical properties of the system may interfere with the test. The presence of colored additives such as the CCMP as well as turbidity of extracts due to solubilized proteins or fat droplets may interfere with accurate determination of the colored chromogen(s) of TBARS-TBA by the spectrophotometer. Conversely, a steam-distillation methodology may be used to recover malonaldehyde from the acidified food product. An aliquot of the distillate is reacted with the TBA reagent, and the intensity of the chromophore is again determined spectrophotometrically. Unfortunately, the distillation method generally affords higher values of TBARS due to artifact formation resulting from further breakdown of labile hydroperoxides. Ward (1986) suggested that without knowledge of the exact nature of the TBARS, which TBARS-adduct(s) are formed, the fatty acid profile of the lipids in question, the oxidative pathways taken by components of the lipid system leading to the formation of TBARS, and the relationship of the TBARS to flavor-producing molecules, the TBA assay is only of limited value for assessing the extent of oxidation or relating it to the sensory response. Shahidi and Hong (1991*a*, *b*) suggested that the relative, rather than the absolute, values of TBARS should be compared against one another in such determinations.

The pink pigment of the TBA-malonaldehyde reaction was first isolated and characterized by Sinnhuber *et al.* (1958), who showed it to be a condensation

product of one molecule of malonaldehyde with two molecules of TBA. Nair and Turner (1984) elucidated the structure of this complex, purified by crystallization, by IR, UV-VIS and NMR methodologies, and showed that it existed in two prominent tautomeric forms. The proposed mechanism for the formation of the complex is illustrated in Fig. 4.5.

Complications Raised by Nitrite in the TBA Test

Nitrite curing inhibits MFD and rancidity development in cooked meats, but determining analytically nitrite's efficacy as an antioxidant is difficult. Residual nitrite present in cured products interferes with the TBA test (Zipser and Watts 1962). This interference is believed to be due to the nitrosation of malonaldehyde, which renders all or a portion of it unreactive in the TBA test, thereby resulting in an underestimation of TBARS (Zipser and Watts 1962; Shahidi *et al.* 1985; Kolodziejska *et al.* 1990). For nitrite-cured products, Zipser and Watts (1962) modified the TBA test by adding sulfanilamide prior to the distillation step to scavenge residual nitrite and to hinder nitrosation of malonaldehyde. Sulfanilamide reacts with residual nitrite to yield a diazonium salt, and permits malonaldehyde to react quantitatively with the TBA reagent. These authors concluded that sulfanilamide addition allows accurate quantification of malonaldehyde in nitrite-cured meat products within the limits of precision of the TBA test.

Shahidi *et al.* (1985) suggested that sulfanilamide itself may give rise to the formation of condensation products with malonaldehyde in the form of a 1-amino-3-iminopropene derivative (Fig. 4.6). In 1991, Shahidi *et al.* reported that addition of as little as 50 ppm nitrite to a malonaldehyde solution, prior to its distillation for the TBA test, eliminated the formation of the malonaldehyde-TBA complex, thus indicating complete nitrosation of malonaldehyde. Results obtained by Kolodziejska *et al.* (1990) agreed with these findings. Addition of sulfanilamide to the system prior to distillation prevented this problem; however, the TBA values were still lower than when no nitrite or sulfanilamide were present in the system. Similar observations were made for meat cured with at least 100 ppm of nitrite to which sulfanilamide had been added. The latter results suffered from errors ranging between 6 and 20%. According to Kolodziejska *et al.* (1990), the reaction of malonaldehyde with sulfanilamide was reversible such that malonaldehyde became available to react with the TBA reagent. These authors based their argument on the fact that the absorption intensity of the chromophore(s) of possible adducts of sulfanilamide and TBA with malonaldehyde and the typical malonaldehyde-TBA system at the 532 nm band were equivalent. Although it is true that the absorption bands of the malonaldehyde-sulfanilamide model system in the UV region disappeared upon TBA addition and that the formation of Schiff bases is reversible, the above

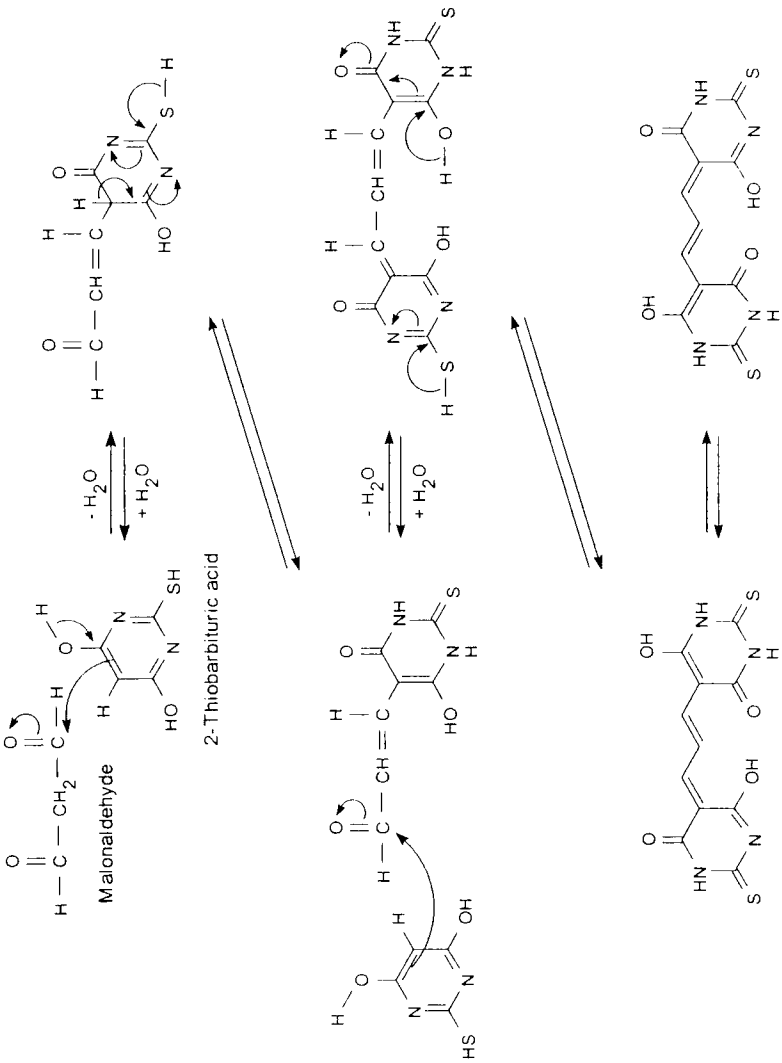


FIG. 4.5. POSSIBLE MECHANISM BETWEEN MALONALDEHYDE AND 2-THIOBARBITURIC ACID IN THE CLASSICAL TBA TEST FOR MONITORING LIPID OXIDATION
(From Pegg and Shahidi 1991).

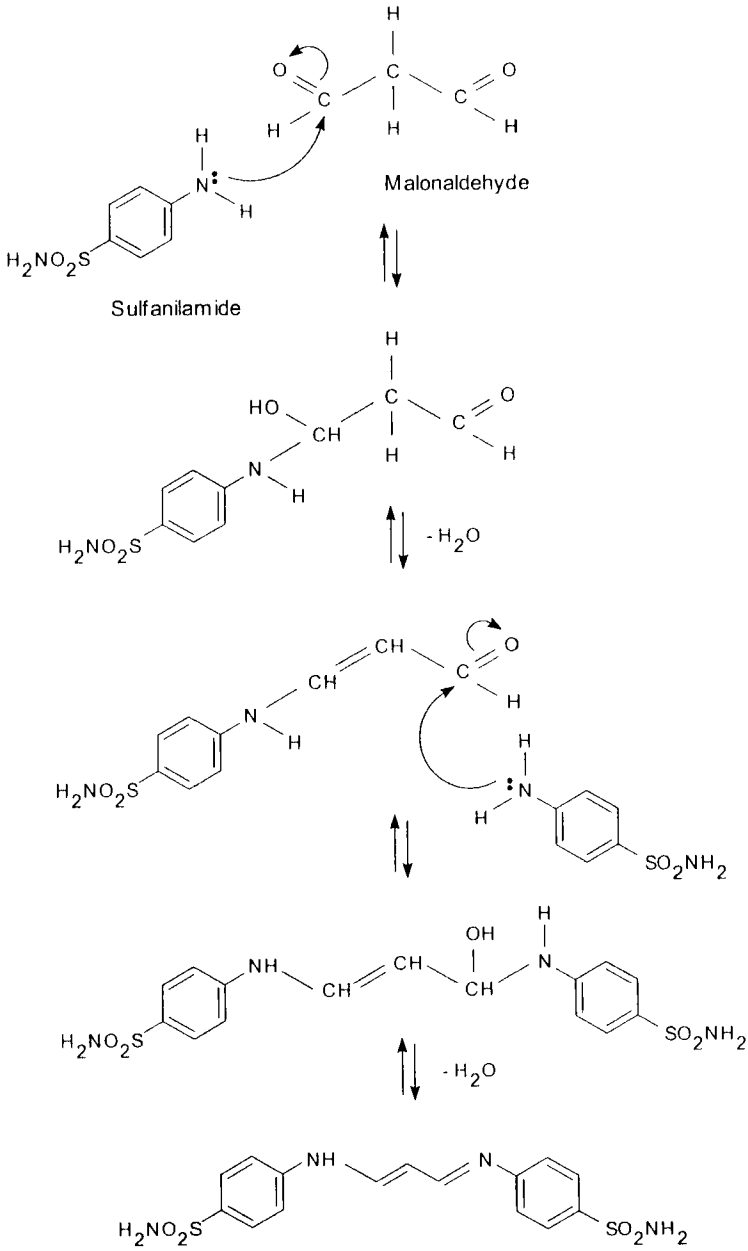


FIG. 4.6. PROPOSED MECHANISM FOR THE FORMATION OF A 1-AMINO-3-IMINOPROPENE DERIVATIVE OF SULFANILAMIDE AND MALONALDEHYDE (From Pegg 1993).

authors failed to acknowledge that the visible absorption spectrum of the ternary system was quite different from that of its counterpart devoid of sulfanilamide. Likewise, sulfanilamide addition to the prepared malonaldehyde-TBA system did not shift the already-observed absorption bands at 372 and 532 nm, but produced a new band at 472 nm (Shahidi and Pegg 1990). The multiple interactions between malonaldehyde with sulfanilamide and TBA are discussed in greater detail by Pegg *et al.* (1992).

Hexanal Analysis as an Alternative to the TBA Test for the Oxidative Stability of Meat Lipids

An alternative approach for assessing lipid oxidation in meat products is to measure the carbonyl compounds formed upon degradation of fatty acid hydroperoxides. Carbonyl compounds have been identified as significant contributors to the flavor of uncured meats (Shahidi *et al.* 1986; Shahidi 1989*b*, Ramarathnam *et al.* 1991*a*,*b*). Some have exceptionally strong aromas and can be detected during autoxidation of fatty acids, even if they are present at low levels. The concentration of some of these aldehydes has been shown to correlate with MFD. In particular, the concentration of hexanal has been suggested to be a useful primary marker of MFD (Bailey *et al.* 1980; Dupuy *et al.* 1987; Shahidi *et al.* 1987*b*; Shahidi 1989).

Hexanal is a seemingly ubiquitous component of food, both fresh and stored. This stems from the fact that practically all foods have some linoleate (18:2ω6), the fatty acid from which hexanal is derived. A profile of the fatty acids found in muscle tissue of various animals is presented in Table 4.3.

Linoleate plays a significant role in the oxidized flavor of all meats, especially pork. Initial products of autoxidized linoleate consist predominately of the 9- and 13- hydroperoxides (46.5 and 49.5%, respectively) because the reactivity of the diallylic system favors attack of oxygen at carbon positions 9 and 13 (Belitz and Grosch 1999). The 9-, 10-, 12- and 13-hydroperoxides at 32, 17, 17 and 34%, respectively, are products of photosensitized oxidation of linoleate (Belitz and Grosch 1999). These hydroperoxides are unstable, and their fragmentation occurs by homolytic and heterolytic cleavage mechanisms (Frankel *et al.* 1984). Homolytic β-scission of 13-hydroperoxyoctadeca-9,11-dienoic acid produces an alkoxy radical intermediate. This undergoes carbon-carbon splitting forming either pentane and 13-oxo-9,11-tridecadienoic acid, or hexanal and an unsaturated C_{12} fatty acid (Frankel 1991). Products of the homolytic β-scission of 9-hydroperoxyoctadeca-10,12-dienoic acid include octanoic acid and 2,4-decadienal, or 9-oxo-nonanoic acid and a C_9 unsaturated hydrocarbon (Fig. 4.7). Autoxidation of methyl linoleate in model systems has been reported to produce many aldehydes as shown in Table 4.4.

TABLE 4.3
UNSATURATED FATTY ACID CONTENT OF LIPIDS IN VARIOUS MUSCLE FOODS

Fatty Acid	Content (%)					
	Beef [a]	Chicken[b]	Fish[c]	Lamb[d]	Pork[e]	Seal[f]
18:1ω9	33.44	46.02	19.59	19.51	12.78	29.7
18:2ω6	10.52	12.55	5.88	18.79	35.08	4.1
18:3ω3	1.66	1.86	8.07	0.44	0.33	0.8
20:1ω9	—	—	—	—	—	15.9
20:2ω6	0.69	0.34	0.20	0.35	—	—
20:3ω6	2.77	0.16	0.36	0.62	1.31	1.3
20:4ω6	8.51	0.84	3.75	13.01	9.51	1.8
20:5ω3	0.76	tr	7.16	—	1.31	5.7
22:1ω9	—	—	—	—	—	6.3
22:4ω6	0.88	—	0.65	—	0.98	—
22:5ω3	0.92	tr	2.39	—	2.30	3.4
22:6ω3	—	tr	2.39	—	2.30	5.5
24:1ω9	—	—	—	—	—	1.3
Total	60.15	61.77	50.44	52.72	65.90	75.8

[a]Adapted from Igene *et al.* (1980); [b]Adapted from Onodenalore (1993); [c]Adapted from Mai and Kinsella (1979); [d]Adapted from Lazarus *et al.* (1977); [e]Adapted from Yamauchi *et al.* (1980); and [f]Adapted from Shahidi and Synowiecki (1996). tr, trace.

By far, hexanal predominates among these volatile aldehydes, but this is not surprising. Hexanal is the only aldehyde that arises from both the 9- and 13-hydroperoxides of linoleate, and from other unsaturated aldehydes formed during oxidation of linoleate (Schieberle and Grosch 1981). The production of 2,4-decadienal is always less than that of hexanal because this dienal can only arise through β-scission of 9-hydroperoxyoctadeca-10,12-dienoic acid. In the autoxidized linoleate model system containing both saturated and unsaturated

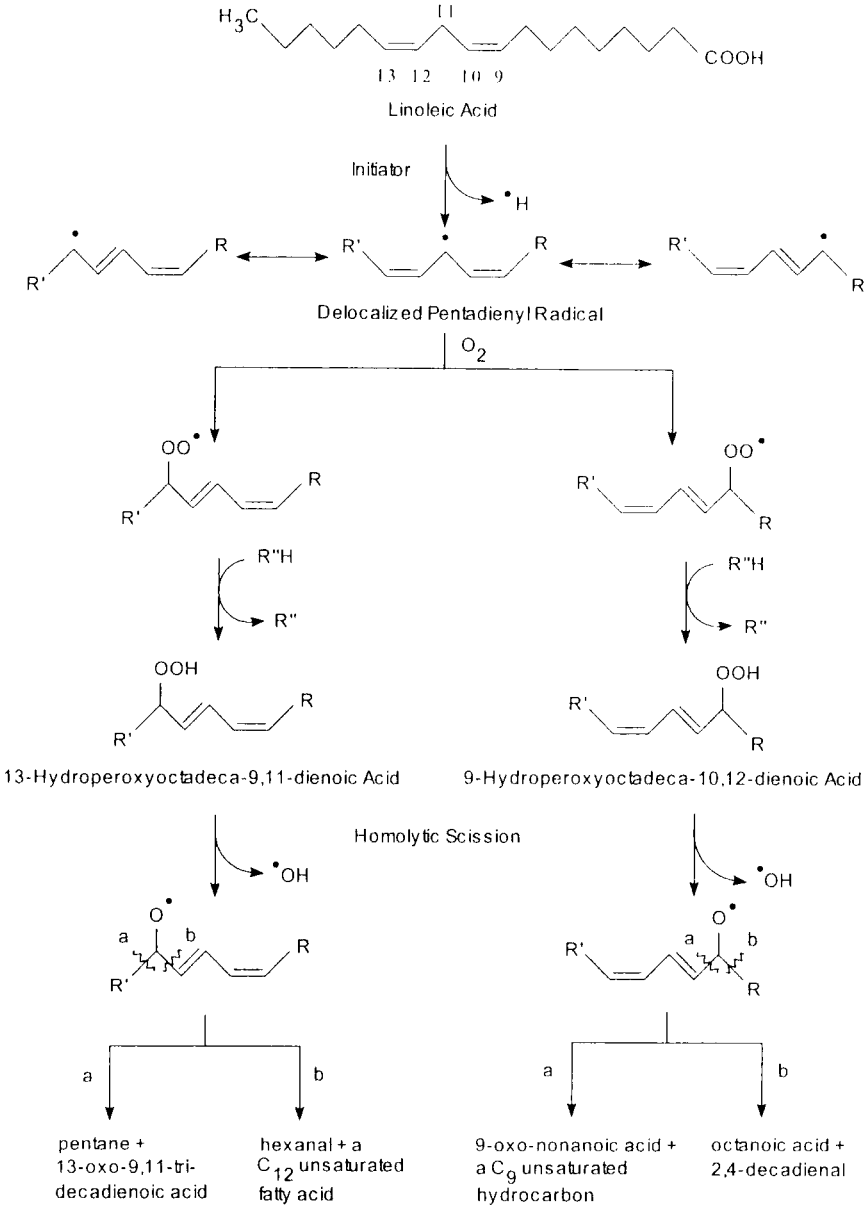


FIG. 4.7. AUTOXIDATION OF LINOLEIC ACID AND THE PRODUCTION OF HEXANAL (Adapted from Frankel *et al.* 1984).

TABLE 4.4.
VOLATILE DOMINANT ALDEHYDES DERIVED BY AUTOXIDATION OF LINOLEATE[a]

Aldehyde	Quantity[b] ($\mu g \cdot g^{-1}$)	Odor Threshold Value (ppb)	
		in Water	in Oil
pentanal	55	10	100
hexanal	5100	4.5	150
heptanal	50	30	45
trans-2-heptenal	450	50	14000
Octanal	45	40	50
cis-2-octenal	990	--	--
trans-2-octenal	420	4	7000
cis-3-nonenal	30	--	--
trans-3-nonenal	30	--	--
cis-2-decenal	20	--	--
trans-2,*trans*-4-nonadienal	30	90	460
trans-2,*cis*-4-decadienal	250	--	20
trans-2,*trans*-4-decadienal	150	0.1	200

[a] Adapted from Belitz and Grosch (1999).
[b] One gram of linoleate was autoxidized at 20°C by an uptake of 0.5 mole oxygen per mole of methyl linoleate.

aldehydes, 2,4-decadienal oxidized faster forming hexanal than the saturated aldehydes. Schieberle and Grosch (1981) suggested that attack of free peroxy radicals ($RO_2^{\bullet}$) on the unsaturated moieties of 2,4-decadienal produces peroxyl peroxides which are more labile than the primary hydroperoxides themselves. They decompose readily to hexanal, 2-butene-1,4-dial and other organic compounds. Matthews *et al.* (1971) identified pentane, furan, ethanal, hexanal, acrolein, butenal, 2-heptenal, 2-octenal, benzaldehyde, glyoxal, *trans*-2-butene-

1,4-dial, acetic acid, hexanoic acid, 2-octenoic acid, 2,4-decadienoic acid and benzene as the oxidation products of 2,4-decadienal in model systems.

Usefulness of Hexanal as a Primary Indicator of MFD

In the late 1970s and early 80s, reports appeared which noted the presence of hexanal in cooked muscle foods and its possible role as an indicator of lipid oxidation. Occurrence of hexanal and other aldehydic degradation products from autoxidation of edible oils had already been known for some time (Matthews *et al.* 1971; Warner *et al.* 1978; Henderson *et al.* 1980). Bailey *et al.* (1980) reported the formation of low-molecular-weight aldehydes in cooked roast beef upon storage, and commented that hexanal and 2-pentylfuran were good indicators of lipid autoxidation. They also found that there were little, if any, qualitative differences in the volatiles produced during storage of meat at 4°C over 3 days, but there were significant quantitative differences.

Dupuy *et al.* (1987) noted that in cooked ground roast beef, pentanal, hexanal, 2,3-octanedione, nonanal and the total volatiles increased appreciably during the storage period at 4°C as did the sensory scores and TBA numbers. Of all of these compounds, hexanal content increased most, from 0.05 to 35 ppm, after 5 days of storage. A similar trend was observed in cooked chicken and turkey meats. In the white and dark muscles of chicken, hexanal levels increased from 0.1 to 15 ppm and from 0.9 to 11 ppm, respectively, during the same period. Similar data were acquired for the white and dark muscles of turkey. The level of hexanal and total volatiles was approximately 3 times greater for cooked beef compared to chicken or turkey after 5 days of storage. It was concluded that since the concentration of hexanal increased more rapidly than any other aldehyde, it should be a useful primary marker of WOF development.

Dupuy *et al.* (1987) also noted that addition of sodium chloride to meats, prior to thermal processing, stimulated the formation of carbonyl compounds during storage, whereas the addition of sodium tripolyphosphate (STPP) in the presence of sodium chloride inhibited its formation at the levels tested. Love and Pearson (1976) had previously reported that the addition of STPP, which retards oxidation in meats by its chelating ability, caused a 50% decrease in hexanal production in a model system. Stoick *et al.* (1991), who examined the hexanal levels of cooked, restructured beef steaks, reported that STPP reduced hexanal levels to 50% of a sodium chloride control, whereas addition of the antioxidant, *t*-butylhydroquinone (TBHQ), provided more complete protection by keeping hexanal levels at 3% of the salt-containing control.

The addition of antioxidants to meat systems retards autoxidation and limits production of overtone carbonyl compounds. Barbut *et al.* (1985) showed that addition of rosemary oleoresin or a butylated hydroxyanisole/butylated

hydroxytoluene (BHA/BHT) antioxidant mixture to a cooked turkey sausage substantially reduced measurable TBARS as well as the content of oxidatively-derived carbonyls such as pentanal, hexanal, heptanal and 2,3-octanedione. Their results were in agreement with those of Shahidi *et al.* (1987*b*) who demonstrated that hexanal levels in cooked ground pork could be controlled by the addition of various antioxidants and chelating agents. Shahidi *et al.* (1987*b*) further showed that the hexanal content of meats treated with different antioxidants and chelating agents was linearly interrelated with corresponding TBA values and sensory scores. These authors noted that after storing the cooked pork control sample for 2 days, the TBA numbers were practically identical. It was suggested that the hexanal content would be a better indicator of the oxidative state of cooked meats than TBA values in the early stages of storage.

Morrissey and Apte (1988) examined the volatile constituents of cooked beef, pork and fish after 2 days of refrigerated storage and the role of heme and non-heme iron in hexanal production. The carbonyl compounds isolated were derivatized with 2,4-dinitrophenylhydrazine, and the resulting hydrazones were separated using reversed-phase high performance liquid chromatography and identified at 360 nm by a UV-VIS detector. Because preliminary studies had indicated that hexanal production continually increased during the early stages of storage of muscle foods, while other volatiles did not show a consistent pattern of increase during the same period, Morrissey and Apte (1988) focused their attention solely on hexanal generation. They ascribed the inconsistencies in the other volatiles to further oxidation or degradation resulting in new compounds. The hexanal concentration in fish muscle after 2 days of storage at 4°C was more than 2 times that of beef and 3 times that of pork. Noteworthy is the fact that these values correlated highly with TBA values. The influence of heme and non-heme iron in the systems showed that their stimulating effect on hexanal production was in the order of Fe^{2+} > Hb > ferritin. Hexanal formation is obviously a function of the lipid profile and the presence of prooxidants, antioxidants and chelators in the system.

Ang and Young (1989) investigated the flavor volatiles of cooked chicken during storage by a static headspace-gas chromatography (HS-GC) methodology. They reported that TBA values and hexanal levels increased in cooked chicken patties during a 5-day storage period at 4°C (correlation coefficient 0.95). These authors also observed that addition of STPP depressed TBARS and hexanal values accordingly. Su *et al.* (1991) showed that in cooked chicken breast patties during 3 days of refrigerated storage, significant correlations existed between values of TBARS, hexanal, and other HS volatiles, namely pentanal, heptanal and the total volatiles. These studies suggested that the rapid HS-GC technique may substitute for the TBA test.

Spanier *et al.* (1992*b*) went a step further and reported relationships among GC volatiles, TBARS markers, and descriptive sensory attributes of cooked beef

patties. They showed that during a 4 day storage period, significant correlations (correlation coefficients > 0.7) existed between pentanal, hexanal and TBARS values and desirable sensory descriptors (such as cooked beefy/brothy) and WOF descriptors (such as painty and card-boardy). Similar correlations have been reported by St. Angelo *et al.* (1987; 1988; 1990).

REFERENCES

Addis, P.B. 1986. Occurrence of lipid oxidation products in foods. *Food Chem. Toxicol.* **24**, 1021–1030.

Addis, P.B. and Park, S-W. 1989. Role of lipid oxidation products in atherosclerosis. In *Food Toxicology. A Perspective on the Relative Risks*, ed. S.L. Taylor and R.A. Scanlan. Marcel Dekker, New York, NY, pp. 297–330.

Andersen, H.J., Bertelsen, G., Boegh-Soerensen, L., Shek, C.K. and Skibsted, L.H. 1988. Effect of light and packaging conditions on the colour stability of sliced ham. *Meat Sci.* **22**, 283–292.

Andersen, H.J., Bertelsen, G., Ohlen, A. and Skibsted, L.H. 1990*a*. Modified packaging as protection against photodegradation of the colour of pasteurized, sliced ham. *Meat Sci.* **28**, 77–83.

Andersen, H.J., Johansen, H.S., Shek, C.K. and Skibsted, L.H. 1990*b*. Nitric oxide exchange in nitrosylmyoglobin. *Z. Lebensm. Unters. Forsch.* **191**, 293–298.

Ang, C.Y.W. and Young, L.L. 1989. Rapid headspace gas chromatographic method for assessment of oxidative stability of cooked chicken meat. *J. Assoc. Off. Anal. Chem.* **72**, 277–281.

Asghar, A., Gray, J.I., Buckley, D.J., Pearson, A.M. and Booren, A.M. 1988. Perspectives on warmed-over flavor. *Food Technol.* **42**(6), 102–108.

Bailey, M.E., Dupuy, H.P. and Legendre, M.G. 1980. Undesirable meat flavor and its control. In *The Analysis and Control of Less Desirable Flavors in Foods and Beverages*, ed. G. Charalambous. Academic Press, Inc., New York, NY, pp. 31–52.

Bailey, M.E. and Swain, J.W. 1973. Influence of nitrite on meat flavor. In *Proceedings of the Meat Industry Research Conference*. American Meat Institute Foundation, Arlington, VA, pp. 29–45.

Barbut, S., Josephson, D.B. and Maurer, A.J. 1985. Antioxidant properties of rosemary oleoresin in turkey sausage. *J. Food Sci.* **50**, 1356–1359, 1363.

Baumgartner, W.A., Baker, N., Hill, V.A. and Wright, E.T. 1975. Novel interference in thiobarbituric acid assay for lipid peroxidation. *Lipids* **10**, 309–311.

Bedinghaus, A.J. and Ockerman, H.W. 1995. Antioxidative Maillard reaction products from reducing sugars and free amino acids in cooked ground pork patties. *J. Food Sci.* **60**, 992–995.

Belitz, H.-D. and Grosch, W. 1999. Lipids. In *Food Chemistry, Second Edition*, Springer-Verlag, Berlin, Germany, pp. 152–236.

Bertz, S.H. and Dabbagh, G. 1990. NMR spectroscopy of malondialdehyde. *J. Org. Chem.* **55**, 5161–5165.

Buckley, D.J., Morrissey, P.A. and Gray, J.I. 1995. Influence of dietary vitamin E on the oxidative stability and quality of pig meat. *J. Anim. Sci.* **73**, 3122–3130.

Caldironi, H.A. and Bazan, N.G. 1982. Effect of antioxidants on malonaldehyde production and fatty acid composition in pieces of bovine muscle and adipose tissue stored fresh and frozen. *J. Food Sci.* **47**, 1329–1332, 1337.

Chang, S.S. and Peterson, R.J. 1977. Symposium: The basis of quality in muscle foods. Recent developments in the flavor of meat. *J. Food Sci.* **42**, 298–305.

Chen, C.C., Pearson, A.M., Gray, J.I., Fooladi, M.H. and Ku, P.K. 1984. Some factors influencing the nonheme iron content of meat and its implications in oxidation. *J. Food Sci.* **49**, 581–584.

Chio, K.S. and Tappel, A.L. 1969*a*. Synthesis and characterization of the fluorescent products derived from malonaldehyde and amino acids. *Biochem.* **8**, 2821–2827.

Chio, K.S. and Tappel, A.L. 1969*b*. Inactivation of ribonuclease and other enzymes by peroxidizing lipids and by malonaldehyde. *Biochem.* **8**, 2827–2832.

Cho, I.C. and Bratzler, L.J. 1970. Effect of sodium nitrite on flavor of cured pork. *J. Food Sci.* **35**, 668–670.

Draper, H.H., McGirr, L.G. and Hadley, M. 1986. The metabolism of malondialdehyde. *Lipids* **21**, 305–307.

Drumm, T.D. and Spanier, A.M. 1991. Changes in the content of lipid autoxidation and sulfur-containing compounds in cooked beef during storage. *J. Agric. Food Chem.* **39**, 336–343.

Dugan, L.R. Jr. 1955. Stability and rancidity. *J. Am. Oil Chem. Soc.* **32**, 605–609.

Dupuy, H.P., Bailey, M.E., St. Angelo, A.J., Vercellotti, J.R. and Legendre, M.G. 1987. Instrumental analyses of volatiles related to warmed-over flavor of cooked meats. In *Warmed-over Flavor of Meat*, ed. A.J. St. Angelo and M.E. Bailey. Academic Press, Inc., Orlando, FL, pp. 165–191.

Faustman, C., Cassens, R.G., Schaefer, D.M., Buege, D.R., Williams, S.N. and Scheller, K.K. 1989. Improvement of pigment and lipid stability in Holstein steer beef by dietary supplementation with vitamin E. *J. Food Sci.* **54**, 858–862.

Fooladi, M.H., Pearson, A.M., Coleman, T.H. and Merkel, R.A. 1979. The role of nitrite in preventing development of warmed-over flavour. *Food Chem.* **4**, 283–292.

Fox, J.B. Jr. and Benedict, R.C. 1987. The role of heme pigments and nitrite in oxidative processes in meat. In *Warmed-over Flavor of Meat*, ed. A.J. St. Angelo and M.E. Bailey. Academic Press, Inc., Orlando, FL, pp. 119–139.

Frankel, E.N. 1991. Recent advances in lipid oxidation. *J. Sci. Food Agric.* **54**, 495–511.

Frankel, E.N., Neff, W.E. and Selke, E. 1984. Analysis of autoxidized fats by gas chromatography-mass spectrometry. IX. Homolytic vs. heterolytic cleavage of primary and secondary oxidation products. *Lipids* **19**, 790–800.

Freybler, L.A., Gray, J.I., Asghar, A., Booren, A.M., Pearson, A.M. and Buckley, D.J. 1993. Nitrite stabilization of lipids in cured pork. *Meat Sci.* **33**, 85–96.

Goutefongea, R., Cassens, R.G. and Woolford, G. 1977. Distribution of sodium nitrite in adipose tissue during curing. *J. Food Sci.* **42**, 1637–1641.

Gray, J.I., Gomaa, E.A. and Buckley, D.J. 1996. Oxidative quality and shelf life of meats. *Meat Sci.* **43**, S111–S123.

Gray, J.I. and Pearson, A.M. 1987. Rancidity and warmed-over flavor. In *Advances in Meat Research. Vol 3: Restructured Meat and Poultry Products*, ed. A.M. Pearson and T.R. Dutson. Van Nostrand Reinhold Co., New York, NY, pp. 221–269.

Greene, B.E. 1969. Lipid oxidation and pigment changes in raw beef. *J. Food Sci.* **34**, 110–113.

Greene, B.E., Hsin, I-M. and Zipser, M.W. 1971. Retardation of oxidative color changes in raw ground beef. *J. Food Sci.* **36**, 940–942.

Guillén-Sans, R. and Guzmán-Chozas, M. 1998. The thiobarbituric acid (TBA) reaction in foods: A review. *CRC Crit. Rev. Food Sci. Nutr.* **38**, 315–330.

Hadden, J.P., Ockerman, H.W., Cahill, V.R., Parrett, N.A. and Borton, R.J. 1975. Influence of sodium nitrite on the chemical and organoleptic properties of comminuted pork. *J. Food Sci.* **40**, 626–630.

Hamilton, R.J. 1989. The chemistry of rancidity in foods. In *Rancidity in Foods, Second Edition*, ed. J.C. Allen and R.J. Hamilton. Elsevier Science Publishers Ltd., London, UK, pp. 1–21.

Henderson, S.K., Witchwoot, A. and Nawar, W.W. 1980. The autoxidation of linoleates at elevated temperatures. *J. Am. Oil Chem. Soc.* **57**, 409–413.

Igene, J.O., King, J.A., Pearson, A.M. and Gray, J.I. 1979. Influence of heme pigments, nitrite, and non-heme iron on development of warmed-over flavor (WOF) in cooked meat. *J. Agric. Food Chem.* **27**, 838–842.

Igene, J.O. and Pearson, A.M. 1979. Role of phospholipids and triglycerides in warmed-over flavor development in meat model systems. *J. Food Sci.* **44**, 1285–1290.

Igene, J.O., Pearson, A.M., Dugan, L.R. Jr. and Price, J.F. 1980. Role of triglycerides and phospholipids on development of rancidity in model meat systems during frozen storage. *Food Chem.* **5**, 263–276.

Igene, J.O., Yamauchi, K., Pearson, A.M. and Gray, J.I. 1985. Mechanisms by which nitrite inhibits the development of warmed-over flavour (WOF) in cured meat. *Food Chem.* **18**, 1–18.

Ito, T., Cassens, R.G., Greaser, M.L., Lee, M. and Izumi, K. 1983. Lability and reactivity of nonheme protein-bound nitrite. *J. Food Sci.* **48**, 1204–1207.

Johns, A.M., Birkinshaw, L.H. and Ledward, D.A. 1989. Catalysts of lipid oxidation in meat products. *Meat Sci.* **25**, 209–220.

Johnson, P.B. and Civille, G.V. 1986. A standardized lexicon of meat WOF descriptors. *J. Sensory Studies* **1**, 99–104.

Kanner, J. 1979. S-Nitrosocysteine (RSNO), an effective antioxidant in cured meat. *J. Am. Oil Chem. Soc.* **56**, 74–76.

Kanner, J. 1994. Oxidative processes in meat and meat products: Quality implications. *Meat Sci.* **36**, 169–189.

Kanner, J., Ben-Gera, I. and Berman, S. 1980. Nitric-oxide myoglobin as an inhibitor of lipid oxidation. *Lipids* **15**, 944–948.

Kanner, J. and Doll, L. 1991. Ferritin in turkey muscle tissue: A source of catalytic iron ions for lipid peroxidation. *J. Agric. Food Chem.* **39**, 247–249.

Kanner, J., Harel, S. and Granit, R. 1991. Nitric oxide as an antioxidant. *Arch. Biochem. Biophys.* **289**, 130–136.

Kanner, J., Harel, S. and Granit, R. 1992. Nitric oxide, an inhibitor of lipid oxidation by lipoxygenase, cyclooxygenase and hemoglobin. *Lipids* **27**, 46–49.

Kanner, J., Harel, S., Shagalovich, J. and Berman, S. 1984. Antioxidative effect of nitrite in cured meat products: Nitric oxide-iron complexes of low molecular weight. *J. Agric. Food Chem.* **32**, 512–515.

Kanner, J. and Juven, B.J. 1980. S-Nitrosocysteine as an antioxidant, color-developing, and anticlostridial agent in comminuted turkey meat. *J. Food Sci.* **45**, 1105–1108, 1112.

Ke, P.J., Cervantes, E. and Robles-Martinez, C. 1984. Determination of thiobarbituric acid reactive substances (TBARS) in fish tissue by an improved distillation-spectrophotometric method. *J. Sci. Food Agric.* **35**, 1248–1254.

Ke, P.J., Nash, D.M. and Ackman, R.G. 1977. Mackerel skin lipids as an unsaturated fat model system for the determination of antioxidative potency of TBHQ and other antioxidant compounds. *J. Am. Oil Chem. Soc.* **54**, 417–420.

Ke, P.J. and Woyewoda, A.D. 1979. Microdetermination of thiobarbituric acid values in marine lipids by a direct spectrophotometric method with a monophasic reaction system. *Anal. Chim. Acta.* **106**, 279–284.

Kohn, H.I. and Liversedge, M. 1944. On a new aerobic metabolite whose production by brain is inhibited by apomorphine, emetine, ergotamine, epinephrine, and menadione. *J. Pharmacol Exp. Ther.* **82**, 292–300.

Kolodziejska, I., Skonieczny, S. and Rubin, L.J. 1990. Malondialdehyde-nitrite interactions in meat and model systems. *J. Food Sci.* **55**, 925–928, 946.

Kosugi, H., Kato, T. and Kikugawa, K. 1987. Formation of yellow, orange, and red pigments in the reaction of alk-2-enals with 2-thiobarbituric acid. *Anal. Biochem.* **165**, 456–464.

Kosugi, H., Kato, T. and Kikugawa, K. 1988. Formation of red pigment by a two-step 2-thiobarbituric acid reaction of alka-2,4-dienals. Potential products of lipid oxidation. *Lipids* **23**, 1024–1031.

Kosugi, H. and Kikugawa, K. 1986. Reaction of thiobarbituric acid with saturated aldehydes. *Lipids* **21**, 537–542.

Kosugi, H., Kojima, T. and Kikugawa, K. 1989. Thiobarbituric acid-reactive substances from peroxidized lipids. *Lipids* **24**, 873–881.

Lazarus, C.R., Deng, J.C. and Watson, C.M. 1977. Changes in the concentration of fatty acids from the nonpolar phospho- and glycolipids during storage of intact lamb muscles. *J. Food Sci.* **42**, 102–107.

Liu, H.-P. and Watts, B.M. 1970. Catalysts of lipid peroxidation in meats. 3. Catalysts of oxidative rancidity in meats. *J. Food Sci.* **35**, 596–598.

Liu, R-H., Conboy, J.J. and Hotchkiss, J.H. 1988. Nitrosation by nitro-nitroso derivatives of olefins: A potential mechanism for *N*-nitrosamine formation in fried bacon. *J. Agric. Food Chem.* **36**, 984–987.

Love, J. 1988. Sensory analysis of warmed-over flavor in meat. *Food Technol.* **42**(6), 140–143.

Love, J.D. 1972. A Comparison of Myoglobin and Non-heme Iron as Prooxidants in Cooked Meat and Dispersions of Phospholipid. Ph.D. Thesis, Michigan State University, East Lansing, MI.

Love, J.D. and Pearson, A.M. 1974. Metmyoglobin and nonheme iron as prooxidants in cooked meat. *J. Agric. Food Chem.* **22**, 1032–1034.

Love, J.D. and Pearson, A.M. 1976. Metmyoglobin and nonheme iron as prooxidants in egg-yolk phospholipid dispersions and cooked meat. *J. Agric. Food Chem.* **24**, 494–498.

MacDonald, B., Gray, J.I. and Gibbins, L.N. 1980*a*. Role of nitrite in cured meat flavor: Antioxidant role of nitrite. *J. Food Sci.* **45**, 893–897.

MacDonald, B., Gray, J.I., Kakuda, Y. and Lee, M.L. 1980*b*. Role of nitrite in cured meat flavor: Chemical analysis. *J. Food Sci.* **45**, 889–892.

MacDonald, B., Gray, J.I., Stanley, D.W. and Usborne, W.R. 1980*c*. Role of nitrite in cured meat flavor: Sensory analysis. *J. Food Sci.* **45**, 885–888, 904.

MacDougall, D.B. and Hetherington, M.J. 1992. The minimum quantity of nitrite required to stain sliced and homogenised cooked pork. *Meat Sci.* **31**, 201–210.

MacLeod, G. and Ames, J. 1988. Soy flavor and its improvement. *CRC Crit. Rev. Food Sci. Nutr.* **27**, 219–400.

Mai, J. and Kinsella, J.E. 1979. Lipid composition of dark and white muscle from white sucker (*Castostomus commersoni*). *J. Food Sci.* **44**, 1101–1105, 1109.

Marcuse, R. and Johansson, L. 1973. Studies on the TBA test for rancidity grading: II. TBA reactivity of different aldehyde classes. *J. Am. Oil Chem. Soc.* **50**, 387–391.

Matthews, R.F., Scanlan, R.A. and Libbey, L.M. 1971. Autoxidation products of 2,4-decadienal. *J. Am. Oil Chem. Soc.* **48**, 745–747.

Moerck, K.E. and Ball, H.R. Jr. 1974. Lipid autoxidation in mechanically deboned chicken meat. *J. Food Sci.* **39**, 876–879.

Monahan, F.J., Crackel, R.L., Gray, J.I., Buckley, D.J. and Morrissey, P.A. 1993. Catalysis of lipid oxidation in muscle model systems by haem and inorganic iron. *Meat Sci.* **34**, 95–106.

Morrissey, P.A. and Apte, S. 1988. Influence of species, haem and non-haem iron fractions and nitrite on hexanal production in cooked muscle systems. *Sci. Aliments* **8**, 3–14.

Morrissey, P.A. and Tichivangana, J.Z. 1985. The antioxidant activities of nitrite and nitrosylmyoglobin in cooked meats. *Meat Sci.* **14**, 175–190.

Nair, V. and Turner, G.A. 1984. The thiobarbituric acid test for lipid peroxidation: Structure of the adduct with malondialdehyde. *Lipids* **19**, 804–805.

Ohkawa, H., Ohishi, N. and Yagi, K. 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.* **95**, 351–358.

Onodenalore, A.C. 1993. Aqueous washing of mechanically deboned chicken meat: Effect on chemical and functional characteristics, M.Sc. thesis, Memorial University of Newfoundland, St. John's, NF, Canada.

Pearson, A.M., Love, J.D. and Shorland, F.B. 1977. "Warmed-over" flavor in meat, poultry, and fish. *Adv. Food Res.* **23**, 1–74.

Pegg, R.B. 1993. Development of Nitrite-free Meat Curing Systems. Ph.D. Thesis, Memorial University of Newfoundland, St. John's, NF, Canada.

Pegg, R.B. and Shahidi, F. 1991. Letters: Malondialdehyde-nitrite interactions in meat and model systems. *J. Food Sci.* **56**(1), v–vii.

Pegg, R.B., Shahidi, F. and Jablonski, C.R. 1992. Interactions of sulfanilamide and 2-thiobarbituric acid with malonaldehyde: Structure of adducts and implications in determination of oxidative state of nitrite-cured meats. *J. Agric. Food Chem.* **40**, 1826–1832.

Pikul, J., Leszczynski, D.E. and Kummerow, F.A. 1983. Elimination of sample autoxidation by butylated hydroxytoluene additions before thiobarbituric acid assay for malonaldehyde in fat from chicken meat. *J. Agric. Food Chem.* **31**, 1338–1342.

Pikul, J., Leszczynski, D.E. and Kummerow, F.A. 1989. Evaluation of three modified TBA methods for measuring lipid oxidation in chicken meat. *J. Agric. Food Chem.* **37**, 1309–1313.

Placer, Z.A., Cushman, L.L. and Johnson, B.C. 1966. Estimation of product of lipid peroxidation (malonyl dialdehyde) in biochemical systems. *Anal. Biochem.* **16**, 359–364.

Pokorný, J. and Dieffenbacher, A. 1989. Determination of 2-thiobarbituric acid value: Direct method. Results of a collaborative study and the standardised method. *Pure Appl. Chem.* **61**, 1165–1170.

Pokorný, J., Valentová, H. and Davídek, J. 1985. Modified determination of 2-thiobarbituric acid value in fats and oils. *Nahrung* **29**, 31–38.

Raharjo, S. and Sofos, J.N. 1993. Methodology for measuring malonaldehyde as a product of lipid peroxidation in muscle tissues: A review. *Meat Sci.* **35**, 145–169.

Ramarathnam, N., Rubin, L.J. and Diosady, L.L. 1991*a*. Studies on meat flavor. 1. Qualitative and quantitative differences in uncured and cured pork. *J. Agric. Food Chem.* **39**, 344–350.

Ramarathnam, N., Rubin, L.J. and Diosday, L.L. 1991*b*. Studies on meat flavor. 2. A quantitative investigation of the volatile carbonyls and hydrocarbons in uncured and cured beef and chicken. *J. Agric. Food Chem.* **39**, 1839–1847.

Rhee, K.S. 1978. Minimization of further lipid peroxidation in the distillation 2-thiobarbituric acid test of fish and meat. *J. Food Sci.* **43**, 1776–1778, 1781.

Robinson, M.E. 1924. Haemoglobin and methaemoglobin as oxidative catalysts. *Biochem. J.* **18**, 255–264.

Robles-Martinez, C., Cervantes, E. and Ke, P.J. 1982. Recommended method for testing the objective rancidity development in fish based on TBARS formation. *Can. Tech. Rep. Fish Aquatic Sci.* No. 1089.

St. Angelo, A.J., Crippen, K.L., Dupuy, H.P. and James, C. Jr. 1990. Chemical and sensory studies of antioxidant-treated beef. *J. Food Sci.* **55**, 1501-1505, 1539.

St. Angelo, A.J., Vercellotti, J.R., Dupuy, H.P. and Spanier, A.M. 1988. Assessment of beef flavor quality: A multidisciplinary approach. *Food Technol.* **42**(6), 133-138.

St. Angelo, A.J., Vercellotti, J.R., Legendre, M.G., Vinnett, C.H., Kuan, J.W., James, C. Jr. and Dupuy, H.P. 1987. Chemical and instrumental analyses of warmed-over flavor in beef. *J. Food Sci.* **52**, 1163-1168.

Salih, A.M., Smith, D.M., Price, J.F. and Dawson, L.E. 1987. Modified extraction 2-thiobarbituric acid method for measuring lipid oxidation in poultry. *Poultry Sci.* **66**, 1483-1488.

Sato, K. and Hegarty, G.R. 1971. Warmed-over flavor in cooked meats. *J. Food Sci.* **36**, 1098-1102.

Schieberle, P. and Grosch, W. 1981. Model experiments about the formation of volatile carbonyl compounds. *J. Am. Oil Chem. Soc.* **58**, 602-607.

Schmedes, A. and Hølmer, G. 1989. A new thiobarbituric acid (TBA) method for determining free malondialdehyde (MDA) and hydroperoxides selectively as a measure of lipid peroxidation. *J. Am. Oil. Chem. Soc.* **66**, 813-817.

Schricker, B.R. and Miller, D.D. 1983. Effects of cooking and chemical treatment on heme and nonheme iron in meat. *J. Food Sci.* **48**, 1340-1343, 1349.

Schricker, B.R., Miller, D.D. and Stouffer, J.R. 1982. Measurement and content of nonheme and total iron in muscle. *J. Food Sci.* **47**, 740-743.

Seman, D.L., Decker, E.A. and Crum, A.D. 1991. Factors affecting catalysis of lipid oxidation by a ferritin-containing extract of beef muscle. *J. Food Sci.* **56**, 356-358.

Shahidi, F. 1989*b*. Flavor of cooked meats. In *Flavor Chemistry. Trends and Developments*, ed. R. Teranishi, R.G. Buttery and F. Shahidi. ACS Symposium Series 388. Am. Chem. Soc., Washington, DC, pp. 188-201.

Shahidi, F. 1992. Prevention of lipid oxidation in muscle foods by nitrite and nitrite-free compositions. In *Lipid Oxidation in Food*, ed. A.J. St. Angelo. ACS Symposium Series 500. Am. Chem. Soc., Washington, DC, pp. 161-182.

Shahidi, F. and Hong, C. 1991*a*. Evaluation of malonaldehyde as a marker of oxidative rancidity in meat products. *J. Food Biochem.* **15**, 97-105.

Shahidi, F. and Hong, C. 1991*b*. Role of metal ions and heme pigments in autoxidation of heat-processed meat products. *Food Chem.* **42**, 339-346.

Shahidi, F. and Pegg, R.B. 1990. Reaction of malonaldehyde with 2-thiobarbituric acid and sulphanilamide: Spectroscopic studies. Presented at the 33rd Annual Conference of the Canadian Inst. of Food Sci. and Tech., June 3–6, Saskatoon, SK, Canada. Abstract 31.

Shahidi, F., Pegg, R.B. and Harris, R. 1991. Effects of nitrite and sulfanilamide on the 2-thiobarbituric acid (TBA) values in aqueous model and cured meat systems. *J. Muscle Foods* **2**, 1–9.

Shahidi, F., Rubin, L.J., Diosady, L.L. and Wood, D.F. 1985. Effect of sulfanilamide on the TBA values of cured meats. *J. Food Sci.* **50**, 274–275.

Shahidi, F., Rubin, L.J. and D'Souza, L.A. 1986. Meat flavor volatiles: A review of the composition, techniques of analysis, and sensory evaluation. *CRC Crit. Rev. Food Sci. Nutr.* **24**, 141–243.

Shahidi, F., Rubin, L.J. and Wood, D.F. 1987*a*. Control of lipid oxidation in cooked ground pork with antioxidants and dinitrosyl ferrohemochrome. *J. Food Sci.* **52**, 564–567.

Shahidi, F., Rubin, L.J. and Wood D.F. 1988. Stabilization of meat lipids with nitrite-free curing mixtures. *Meat Sci.* **22**, 73–80.

Shahidi, F. and Synowiecki, J. 1996. Alkali-assisted extraction of proteins from meat and bone residues of harp seal (Phoca groenlandica). *Food Chem.* **57**, 317–321.

Shahidi, F., Yun, J., Rubin, L.J. and Wood, D.F. 1987*b*. The hexanal content as an indicator of oxidative stability and flavour acceptability in cooked ground pork. *Can. Inst. Food Sci. Technol. J.* **20**, 104–106.

Sinnhuber, R.O. and Yu, T.C. 1958. 2-Thiobarbituric acid method for the measurement of rancidity in fishery products. II. The quantitative determination of malonaldehyde. *Food Technol.* **12**(1), 9–12.

Sinnhuber, R.O., Yu, T.C. and Yu, T.C. 1958. Characterization of the red pigment formed in the 2-thiobarbituric acid determination of oxidative rancidity. *Food Res.* **23**, 626–634.

Siu, G.M. and Draper, H.H. 1978. A survey of the malonaldehyde content of retail meats and fish. *J. Food Sci.* **43**, 1147–1149.

Skibsted, L.H. 1992. Cured meat products and their oxidative stability. In *The Chemistry of Muscle-based Foods*, ed. D.E. Johnston, M.K. Knight and D.A. Ledward. Special Publication No. 106, The Royal Society of Chemistry, Cambridge, UK, pp. 266–286.

Spanier, A.M. 1992. Current approaches to the study of meat flavor quality. In *Food Science and Human Nutrition, Vol. 29*, ed. G. Charalambous. Elsevier Science Publishers Ltd., Amsterdam, The Netherlands, pp. 695–709.

Spanier, A.M., Edwards, J.V. and Dupuy, H.P. 1988. The warmed-over flavor process in beef: A study of meat proteins and peptides. *Food Technol.* **42**(6), 110, 112–118.

Spanier, A.M., Miller, J.A. and Bland, J.M. 1992*a*. Lipid oxidation: Effect on meat proteins. In *Lipid Oxidation in Food*, ed. A.J. St. Angelo. ACS Symposium Series 500. Am. Chem. Soc., Washington, DC, pp. 104–119.

Spanier, A.M., Vercellotti, J.R. and James, C. Jr. 1992*b*. Correlation of sensory, instrumental and chemical attributes of beef as influenced by meat structure and oxygen exclusion. *J. Food Sci.* **57**, 10–15.

Stoick, S.M., Gray, J.I., Booren, A.M. and Buckley, D.J. 1991. Oxidative stability of restructured beef steaks processed with oleoresin rosemary, tertiary butylhydroquinone, and sodium tripolyphosphate. *J. Food Sci.* **56**, 597–600.

Su, Y., Ang, C.Y.W. and Lillard, D.A. 1991. Precooking method affects warmed-over flavor of broiler breast patties. *J. Food Sci.* **56**, 881–883, 898.

Tappel, A.L. 1952. Linoleate oxidation catalyzed by hog muscle and adipose tissue extracts. *Food Res.* **17**, 550–559.

Tarladgis, B.G., Pearson, A.M. and Dugan, L.R. Jr. 1964. Chemistry of the 2-thiobarbituric acid test for determination of oxidative rancidity in foods. II.-Formation of the TBA-malonaldehyde complex without acid-heat treatment. *J. Sci. Food Agric.* **15**, 602–607.

Tarladgis, B.G., Watts, B.M., Younathan, M.T. and Dugan, L.R. Jr. 1960. A distillation method for the quantitative determination of malonaldehyde in rancid foods. *J. Am. Oil Chem. Soc.* **37**, 44–48.

Tichivangana, J.Z. and Morrissey, P.A. 1984. Factors influencing lipid oxidation in heated fish muscle systems. *Ir. J. Fd Sci. Technol.* **8**, 47–57.

Tims, M.J. and Watts, B.M. 1958. Protection of cooked meats with phosphates. *Food Technol.* **12**, 240–243.

Tomás, M.C. and Funes, J. 1987. Application of 2-thiobarbituric acid reaction to exudates of frozen and refrigerated meats. *J. Food Sci.* **52**, 575–579.

Turner, E.W., Paynter, W.D., Montie, E.J., Bessert, M.W., Struck, G.M. and Olson, F.C. 1954. Use of the 2-thiobarbituric acid reagent to measure rancidity in frozen pork. *Food Technol.* **8**, 326–330.

Uchiyama, M. and Mihara, M. 1978. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. *Anal. Biochem.* **86**, 271–278.

Vercellotti, J.R., Kuan, J.W., Liu, R.H., Legendre, M.G., St. Angelo, A.J. and Dupuy, H.P. 1987*a*. Analysis of volatile heteroatomic meat flavor principles by purge-and-trap/gas chromatography-mass spectrometry. *J. Agric. Food Chem.* **35**, 1030–1035.

Vercellotti, J.R., Kuan, J.W., Spanier, A.M. and St. Angelo, A.J. 1989. Thermal generation of sulfur-containing flavor compounds in beef. In *Thermal Generation of Aromas*, ed. T.H. Parliment, R.J. McGorrin and C-T. Ho. ACS Symposium Series 409. Am. Chem. Soc., Washington, DC, pp. 452–459.

Verma, M.M., Paranjape, V. and Ledward, D.A. 1985. Lipid and haemoprotein oxidation in meat emulsions. *Meat Sci.* **14**, 91–104.

Walters, C.L., Hart, R.J. and Perse, S. 1979. The possible role of lipid pseudonitrosites in nitrosamine formation in fried bacon. *Z. Lebensm. Unters. Forsch.* **168**, 177–180.

Ward, D.D. 1986. The TBA assay and lipid oxidation: an overview of the relevant literature. *Milchwissenschaft* **40**, 583–588.

Warner, K., Evans, C.D., List, G.R., Dupuy, H.P, Wadsworth, J.I. and Goheen, G.E. 1978. Flavor score correlation with pentanal and hexanal contents of vegetable oil. *J. Am. Oil Chem. Soc.* **55**, 252–256.

Watts, B.M. 1954. Oxidative rancidity and discoloration in meat. *Adv. Food Res.* **5**, 1–52.

Wettasinghe, M. and Shahidi, F. 1997. Antioxidant activity of preformed cooked cured-meat pigment in a β-carotene/linoleate model system. *Food Chem.* **58**, 203–207.

Wills, E.D. 1966. Mechanisms of lipid peroxide formation in animal tissues. *Biochem. J.* **99**, 667–676.

Witte, V.C., Krause, G.F. and Bailey, M.E. 1970. A new extraction method for determining 2-thiobarbituric acid values of pork and beef during storage. *J. Food Sci.* **35**, 582–585.

Witz, G., Lawrie, N.J., Zaccaria, A., Ferran, H.E. Jr. and Goldstein, B.D. 1986. The reaction of 2-thiobarbituric acid with biologically active alpha, beta-unsaturated aldehydes. *J. Free Radic. Biol. Med.* **2**, 33–39.

Wong, D.W.S. 1989. Lipids. In *Mechanism and Theory in Food Chemistry*. Van Nostrand Reinhold, New York, NY, pp. 1–47.

Yamauchi, K., Nagai, Y. and Ohashi, T. 1980. Quantitative relationship between alpha-tocopherol and polyunsaturated fatty acids and its connection to development of oxidative rancidity in porcine skeletal muscle. *Agric. Biol. Chem.* **44**, 1061–1067.

Yamauchi, K., Nagai, Y. and Ohashi, T. 1982. Quantitative relationship between alpha-tocopherol and polyunsaturated fatty acids and its connection to development of oxidative rancidity in chicken skeletal muscle. *Agric. Biol. Chem.* **46**, 2719–2724.

Younathan, M.T. and Watts, B.M. 1959. Relationship of meat pigments to lipid oxidation. *Food Res.* **24**, 728–734.

Younathan, M.T. and Watts, B.M. 1960. Oxidation of tissue lipids in cooked pork. *Food Res.* **25**, 538–543.

Zipser, M.W., Kwon, T-W. and Watts, B.M. 1964. Oxidative changes in cured and uncured frozen cooked pork. *J. Agric. Food Chem.* **12**, 105–109.

Zipser, M.W. and Watts, B.M. 1962. A modified 2-thiobarbituric acid (TBA) method for the determination of malonaldehyde in cured meats. *Food Technol.* **16**(7), 102–104.

Zubillaga, M.P., Maerker, G. and Foglia, T.A. 1984. Antioxidant activity of sodium nitrite in meat. *J. Am. Oil Chem. Soc.* **61**, 772–776.

CHAPTER 5

FLAVOR OF MEAT

Flavor is an important sensory characteristic that contributes to the overall acceptability of muscle foods. The flavors associated with cooked meats have proven to be particularly difficult to characterize, both for the sensory analyst and the flavor chemist. Meat flavor is influenced not only by compounds contributing to the sense of taste, but also by those stimulating the olfactory organ. Meat flavor is a complex stimulus involving not just taste and aroma, but other sensory attributes such as mouthfeel, juiciness and temperature (Gray *et al.* 1981). Nonetheless, the volatile compounds of cooked meat determine the aroma attributes and contribute most to the characteristic flavors of meat (Mottram 1998). After all, aroma often affects our judgement of a food even before consumption; it is a significant factor influencing the public's food-buying decisions and their perception of food quality. Major advances in the identification of compounds which give cooked meat its characteristic meaty flavor have been made this past decade, but many questions pertaining to meat flavor and its precursors remain unanswered.

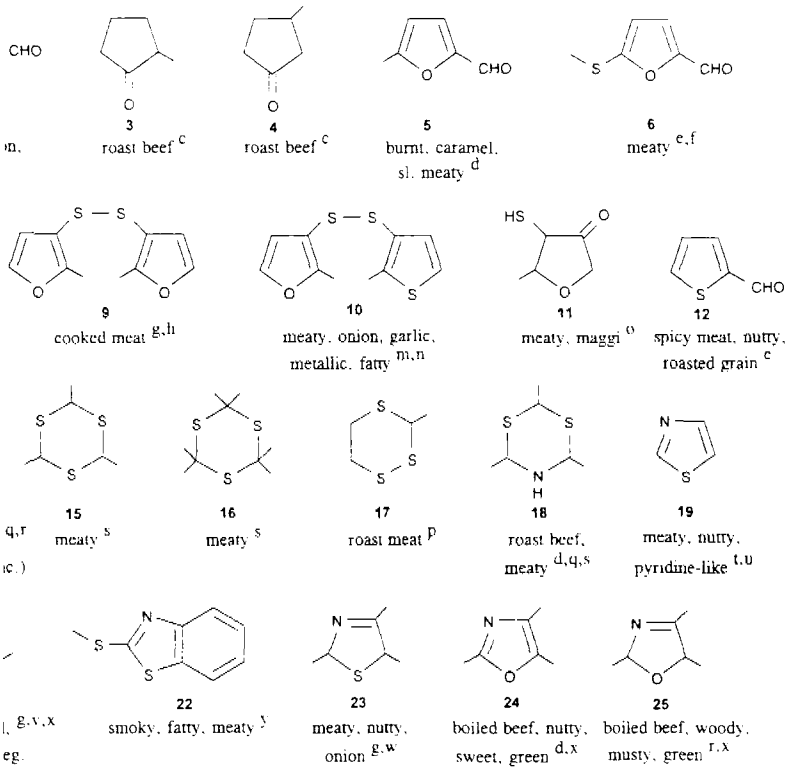
Flavor of Uncured Meat

Raw meat has a slight serum-like odor and a blood-like taste but serves as a rich reservoir of compounds with taste tactile properties as well as aroma precursors and flavor enhancers (Crocker 1948; Bender and Ballance 1961; Shahidi 1998). Upon thermal processing, numerous chemical reactions occur between the many non-volatile compounds that are present in meat, and yield a heterogeneous system comprising: (1) a myriad of volatile compounds with odor properties; (2) non-volatile compounds with taste properties; and (3) potentiators and synergists. The method of cooking employed, such as boiling, frying, broiling and roasting, as well as the final internal temperature contributes significantly to the formation and stability of both volatile and non-volatile compounds, and thereby relates to differences in the overall meat flavor sensation (MacLeod and Seyyedain-Ardebili 1981; Spanier *et al.* 1990; Mottram 1991; Spanier and Boylston 1994).

Volatile compounds produced during thermal processing of meat are believed to be derived from non-volatile precursors, most of which are water-soluble. Flavor appears to be a combination of thermal degradation products of low-molecular-weight precursors which include reducing sugars, vitamins, amino

acids, peptides and nucleotides, as well as products of browning (Maillard) reaction and fat oxidation (Batzer *et al.* 1962; Wasserman and Gray 1965; Mottram *et al.* 1982; Mottram and Edwards 1983). Upon heat processing, free amino acids in meat, such as cysteine, cleaved from protein by the action of proteolytic enzymes during the postmortem period, react with reducing sugars, products of glycolysis, and vitamins such as thiamine (Shahidi 1989). Often products of one reaction become precursors for others. Interaction of these volatiles with lipid-derived products can produce desirable flavors, but the progress of oxidation can mask the natural flavor of heat-processed meats and eventually leads to MFD.

Analyzing the volatile organic compounds that impact meat flavor can be a daunting task. To date, more than 1000 compounds have been identified in the volatile constituents of cooked red meats and poultry by gas chromatographic-mass spectrometric techniques (Shahidi *et al.* 1986; Shahidi 1989; Imafidon and Spanier 1994). These volatiles are representative of most classes of organic compounds such as hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids, esters, lactones, ethers, furans, pyridines, pyrazines, pyrroles, oxazoles and oxazolines, thiazoles and thiazolines, thiophenes and other sulfur- and halogen-containing compounds. Yet, obtaining useful information from the analysis of meat volatiles can be even more challenging than the isolation/identification steps themselves. The critical question becomes, "What is the relative sensory significance of these volatiles?" The answer is not totally clear, but many volatiles are relatively unimportant. Chang and Peterson (1977) reported that aliphatic and aromatic hydrocarbons, saturated alcohols, carboxylic acids, esters, ethers, and carbonyl compounds (*i.e.*, aldehydes and ketones) were probably not the main contributors to meat flavor. Rather, lactones, acyclic sulfur-containing compounds (*i.e.*, mercaptan and sulfides), non-aromatic heterocyclic compounds containing either sulfur, nitrogen or oxygen (*e.g.*, hydrofuranoids) and aromatic heterocyclic compounds containing either sulfur, nitrogen or oxygen (*e.g.*, pyrazines and thiophenes) possessed characteristic meaty flavor notes. Figure 5.1 depicts some of the compounds identified from cooked beef aromas which possess a meaty flavor. Whether a compound is a key odor impact substance depends on both its concentration and odor threshold (*i.e.*, how sensitive the nose is to that particular compound). For example, many heterocyclic thiols and disulfides contribute to a truly specific meaty odor and are therefore considered aroma impact compounds. The meaty character of these compounds depends on the position of the thiol group and the degree of unsaturation; furans and thiophenes with a thiol group in the 3-position tend to possess a meaty aroma while those with the thiol in the 2-position tend to be burnt and sulfurous (Evers *et al.* 1976; Farmer and Patterson 1991). According to van den Ouweland *et al.* (1989), the best meat-like aroma is given when there is a methyl group adjacent to the thiol group and the ring contains at least one



OM COOKED BEEF AROMAS (Maarse and Visscher 1989; Werkhoff *et al.* 1989, 1990) AND REPORTED TO POSSESS MEATY ODOR

1972; [h]Evers *et al.* 1976; [u]Furia and Bellanca 1975; [i]IFF Inc. 1979; [g]Katz 1981; [q]Kubota *et al.* 1980; man *et al.* 1976; [c]Nishimura *et al.* 1980; [d]Ohloff and Flament 1978; [e]Pittet and Hruza 1974; [f]Rödel and Tressl and Silwar 1981; [r]Tressl *et al.* 1983; [o]van den Ouweland and Peer 1975; [t]van der Linde *et al.* 2; [n]Werkoff *et al.* 1989, [m]1990; [s]Wilson *et al.* 1974). (From MacLeod 1998).

double bond. Yet, the odors from these sulfur volatiles, which are pleasant at the concentrations found in meat, become objectionable at higher levels. Therefore, when assessing the flavor quality of muscle foods, both qualitative and quantitative aspects of volatiles must be considered.

Heat-Induced Meat Flavor

The Breakdown of Individual Substances. In order to degrade mono- and oligosaccharides as well as amino acids to yield volatile compounds, higher temperatures than those generally associated with the cooking of meat are required. Although some breakdown of simple sugars to furanones and furfurals can occur during the thermal processing of meat, amino acids are more stable and unlikely to undergo pyrolysis. However, some pyrolysis of amino acids resulting in decarboxylation and deamination might occur at the surface of grilled or roasted meat, where localized dehydration allows the temperature to rise significantly above the boiling point of water (Mottram 1991). These compounds are taste-active but contribute very little, if any, to the aroma of cooked meat.

Thiamine (*i.e.*, vitamin B_1) is a sulfur-containing compound with a thiazole and pyrimidine ring system. When meat is thermally processed, the vitamin degrades and potent aromas are generated, of which some have been described as meaty (Fig. 5.2). Thiamine's breakdown products include thiophenes, thiazoles, furans, furanthiols, and bicyclic heterocyclic compounds (MacLeod and Seyyedain-Ardebili 1981; Hartman *et al.* 1984; Werkhoff *et al.* 1990; Mottram 1991). An important thiamine degradation product is 5-hydroxy-3-mercapto-2-pentanone. This very reactive compound is the intermediate for a number of thiols including 2-methyl-4,5-dihydro-3-furanthiol and 2-methyl-3-furanthiol as well as the mercaptoketones (*i.e.*, 3-mercapto-2-butanone, 3-mercapto-2-pentanone and 2-mercapto-3-pentanone) (Güntert *et al.* 1993*a*,*b*; Mottram *et al.* 1995).

Maillard Reaction. Free amino groups of amino acids or peptides can react with reducing sugars in meat and undergo a series of complex non-enzymatic browning reactions known simply as the Maillard reaction. The Maillard reaction, which does not require the very high temperatures associated with sugar caramelization and protein pyrolysis, is one of the most important routes for the generation of flavors in cooked foods. Its products include high molecular weight brown-colored compounds, known as melanoidins, and volatile aroma compounds (Salter *et al.* 1988; Farmer *et al.* 1989). The first step in the Maillard reaction involves the formation of a N-substituted glycosylamine via the addition of the carbonyl group of the open-chain form of a reducing sugar to a primary amino group of an amino acid, peptide or protein. The glycosylamine

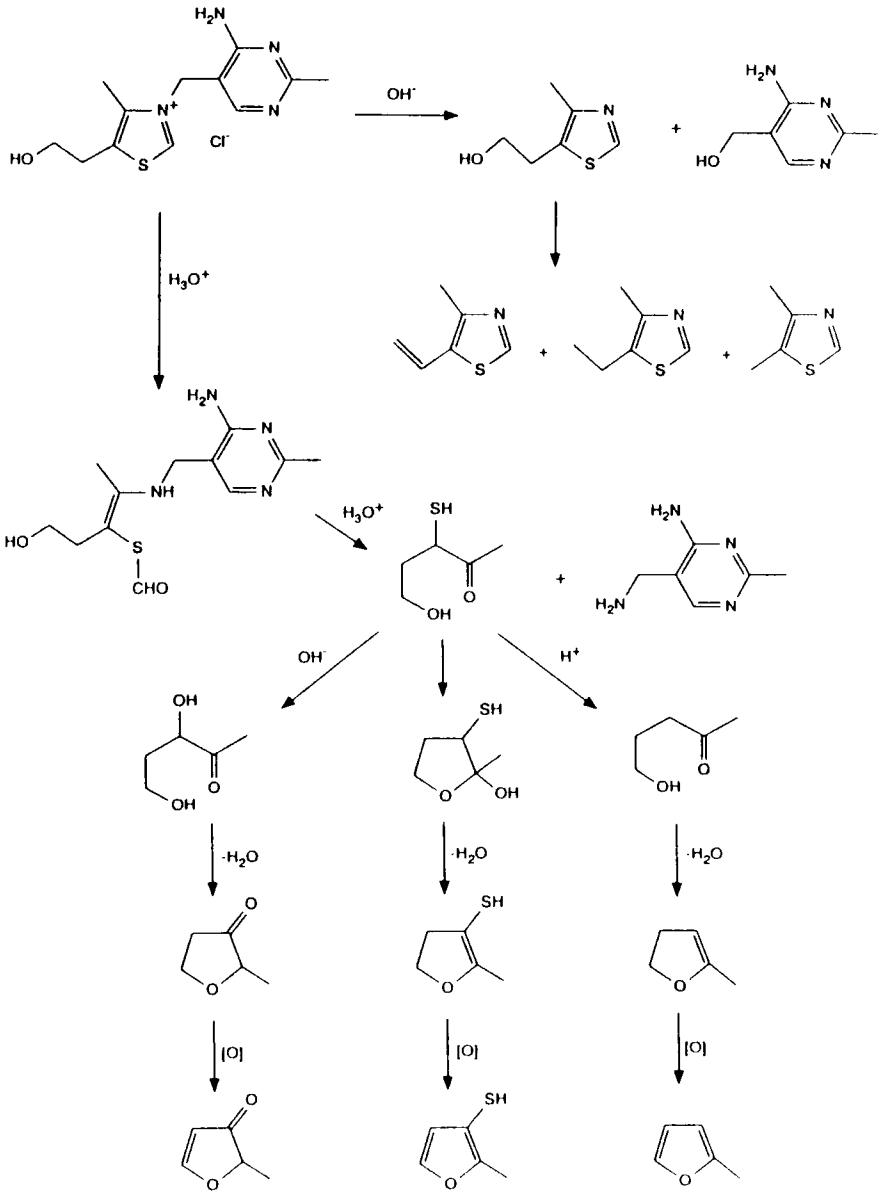


FIG. 5.2. DEGRADATION PRODUCTS FROM THE BREAKDOWN OF THIAMINE.
(From Mottram 1991).

rearranges itself forming an Amadori compound which can degrade further, thus generating compounds such as furfurals, furanones and dicarbonyls. These compounds may themselves make some contribution to meat flavor, but they are more important as reactants for other aroma volatiles (Mottram 1998). They can interact with reactive compounds such as amines, amino acids, hydrogen sulfide, thiols, ammonia, acetaldehyde, and other aldehydes. These additional reactions lead to many important classes of flavor compounds such as pyrazines, oxazoles, thiophenes, thiazoles and other heterocyclic sulfur compounds. Although the Maillard reaction can take place in aqueous solution, it occurs much more readily at low moisture levels; hence, in meat, flavor compounds produced by the Maillard reaction tend to form on the surface of the meat where some dehydration has occurred. A simplistic view of the Maillard reaction, as it results in the generation of flavor volatiles in meat, is presented in Fig. 5.3.

Lipid Oxidation. Hornstein and Crowe (1960) suggested that meat aroma, derived from water-soluble precursors, was similar in all meat and that the characteristic species differences were due to the contribution of volatiles derived from the lipid fraction. They postulated that lipids provide volatile compounds that give the characteristic flavors of different species, and that elimination of the lipid-derived flavors should reveal the true-to-nature flavor of meat itself. Fat influences flavor by formation of organoleptically significant amounts of carbonyl compounds (*i.e.*, aldehydes and ketones) resulting from the oxidation of unsaturated fatty acids, and by acting as a depot of fat-soluble compounds that volatilize upon heating. The spectrum of secondary products of lipid oxidation will depend, of course, on the fatty acid composition of the lipids, which also varies from one species to another. One of the main functions of thermal processing is to produce flavor and flavor precursors from lipids, many of which possess intense odors, as well as to allow intimate mixing of fat-soluble and water-soluble compounds (Herz and Chang 1970). Yet, Mottram *et al.* (1982) emphasized that although species-characteristic aromas have been found on heating fatty tissues, lipids alone are not responsible. These authors reported a comparison of the flavor volatiles from cooked beef and pork systems. The addition of minced subcutaneous adipose tissue to the meat cakes increased the lipid content nine-fold. The addition of pork fat to either beef or pork lean resulted in a substantial increase in hexanal, but only small changes in most other volatiles. The general lack of relationship between volatiles and adipose fat level suggested that the triacylglycerols of adipose tissues may not be the major source of volatiles and that the intramuscular triacylglycerols and phospholipids may be more important.

Interaction Between Lipid Oxidation and Maillard Reaction Products. For years, sensory panels and consumer studies had failed to show any

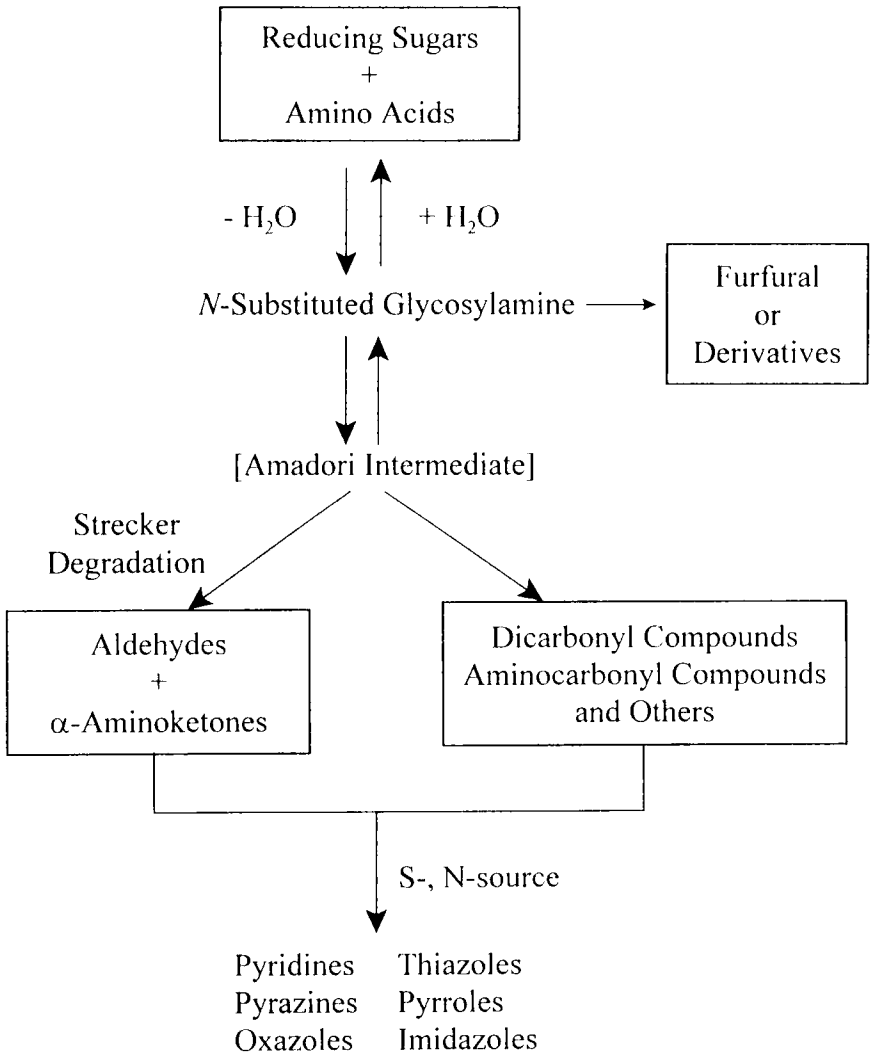


FIG. 5.3. A SIMPLISTIC VIEW OF THE MAILLARD REACTION AS IT RELATES TO FLAVOR GENERATION IN MEAT.

relationship between the flavor of lean meat and the amount of fat on the carcass; thereby, apparently confirming early meat flavor research that associated meatiness with the water-soluble flavor precursors while species characteristics were associated with the lipids (Hornstein and Crowe 1964). However, the volatiles of cooked meat are often dominated by lipid-derived volatiles, and such volatiles are expected to have some effect on meaty flavor (Mottram 1998). Farmer (1990) examined the role of triacylglycerols and structural phospholipids in the formation of meat aroma. Triacylglycerols alone or the total lipids, including phospholipids, were extracted from meat and the aroma profiles of the thermally processed meat were then studied. This researcher reported that removal of triacylglycerols had little effect on the aroma of the cooked meat whereas this was not so when phospholipids were removed. Elimination of phospholipids resulted in a marked loss in the meat character of the odor; the aroma was changed from meaty to roast or biscuit-like. Removal of phospholipids also caused a significant increase in certain volatile heterocyclic compounds, notably alkylpyrazines (Mottram 1991). As the primary source of these compounds in cooked meats comes from the Maillard reaction, it appeared that interaction of phospholipids in the Maillard reaction could be important for the characteristic aroma of cooked meat (Whitfield *et al.* 1988; Farmer 1990; Mottram 1991). In other words, the implications from this study were that in normal meat, lipids or their degradation products inhibit the formation of heterocyclic compounds by participating in the Maillard reaction (Mottram 1998).

Species Effect. As previously mentioned, the characteristic flavor of the different meat species is generally believed to be derived from the lipid sources. Yet, it has been suggested that interaction of lipids with other meat components may also be involved (Wasserman and Spinelli 1972). The chemical nature of many flavor volatiles of meat from different species is similar qualitatively, but different quantitatively. However, lamb, mutton and goat meat are exceptions. Wong *et al.* (1975*a*) identified a number of volatile medium-chain fatty acids (C_5 - C_{12}) in sheep and goat meats, which included some methyl-branched chain-homologues. These saturated fatty acids had not been reported in other meats and were associated with the characteristic flavor of cooked sheep meat that results in its low consumer acceptance in many countries. In particular, 4-methyloctanoic and 4-methylnonanoic acids were considered to be primarily responsible for this flavor (Wong *et al.* 1975*b*). Kim *et al.* (1993) confirmed this finding and reported that 4-ethyloctanoic as well as 4-methyloctanoic acids were key flavor impact compounds in goat meat. It has also been reported that mutton aromas contain a higher concentration of 3,5-dimethyl-1,2,4-trithiolane and 2,4,6-trimethylperhydro-1,3,5-dithiazine (thialdine) as compared to those of other species. Additional sulfur-containing compounds were present in high

concentration, and this was attributed to the high content of sulfurous amino acids in mutton as compared with those of beef and pork. Similarly, a greater concentration of alkyl-substituted heterocycles and alkylphenols was noted in mutton volatiles (Buttery *et al.* 1977; Ha and Lindsay 1991).

Others. Besides the effect of breed and sex, the age of the animal, the dietary regime and pH of the meat are among factors which have been ascribed to affect the flavor of cooked meat. Finally, in the evaluation of flavor quality of meat, the contribution to taste by amino acids, peptides and nucleotides must be considered. These compounds not only interact with other components to produce flavor volatiles, but they also contribute to the sweet, salty, bitter, sour and umami sensation of muscle foods. In the production of soups and gravies, proteins are partially hydrolyzed to enhance the products' taste sensation. Therefore, studies in this area would permit us to optimize conditions to yield products with a maximum level of acceptability.

Flavor of Nitrite-Cured Meat

Nitrite is responsible for the production of the characteristic flavor of cured meat (*i.e.*, a flavor that distinguishes cooked ham from pork). The role of nitrite in cured meat flavor is complex and the chemical changes that are responsible for this unique flavor in meat are not entirely understood (Shahidi 1989). Cured meat flavor is probably a composite sensation derived from contributions of many odoriferous compounds (National Academy of Sciences 1982). Research into cured meat flavor has been divided into two main areas, namely the sensory evaluation of flavor imparted to meat by nitrite, and the qualitative and quantitative identification of volatile and non-volatile components responsible for it, but caution must be exercised. A compound-by-compound search of meat flavor volatiles may misidentify the true nature of cured meat flavor, since a mixture of two or more odors can produce an aroma that is perceived as qualitatively distinct from the odors of each component.

The relationship of nitrite to cured meat flavor was first described by Brooks *et al.* (1940) who concluded that the characteristic flavor of bacon was primarily due to the action of nitrite. They further suggested that a satisfactory bacon product could be produced using only sodium chloride and sodium nitrite and that an adequate cured flavor could be obtained with a nitrite concentration as low as 10 ppm. Mottram and Rhodes (1974) studied the effect of varying nitrite concentrations in bacon by sensory analyses. Brines containing 20% (w/v) sodium chloride and sodium nitrite at concentrations ranging between 0 and 2000 mg/l were used to cure pork middles. Sensory data showed a significant difference ($P<0.01$) between the flavor of uncured and that of cured pork. Sensory studies with similar findings have been reported (Cho and Bratzler

1970; Herring 1973; Simon *et al.* 1973). MacDougall *et al.* (1975) showed that taste panel scores of bacon flavor were linearly related to the logarithm of the nitrite concentration in the brines, but the level of nitrite required for a satisfactory flavor varied between products depending on the nature of the meat. MacDonald *et al.* (1980) demonstrated through sensory evaluation studies that 50 ppm of nitrite were required to develop a significant ($P<0.05$) cured-meat flavor as opposed to 10 ppm proposed by Brooks *et al.* (1940). In addition to nitrite, the effect and concentration of other curing ingredients, namely salt, sugar, polyphosphate and smoke, play an overriding role in the appreciation of cured meat flavor (MacDougall *et al.* 1975) as does the holding time, temperature, and storage conditions employed (Kemp *et al.* 1975).

Nitrite's role in the development of cured meat flavor involves its antioxidative activity which, as described in Chapter 4, retards the breakdown of unsaturated fatty acids and the formation of secondary oxidation products. Numerous researchers have attempted to identify the volatile compounds produced during the thermal processing of cured meat (Ockerman *et al.* 1964; Cross and Ziegler 1965; Lillard and Ayres 1969; Mottram and Rhodes 1974; Ho *et al.* 1983). Ockerman *et al.* (1964) extracted volatile compounds from dry-cured hams by vacuum distillation and cold trap collection. The main components identified by GC retention times and verified by IR spectroscopy included 6 aldehydes, 3 ketones, 5 acids, ammonia and methylamine, and hydrogen sulfide; however, all of the compounds identified were contributors to the aroma of uncured cooked meat. Using vacuum distillation, Lillard and Ayres (1969) identified a total of 42 compounds in country-cured ham, of which 24 were aldehydes. Ho *et al.* (1983) isolated volatile aroma compounds from fried bacon and subjected them to extensive gas-chromatographic fractionation, thus, enabling the pure fractions obtained to be identified by IR spectroscopy and mass spectrometry. In all, 135 compounds were identified, which included hydrocarbons, alcohols, ketones, furans, pyrazines, and sulfur- and nitrogen-containing heterocycles. These authors postulated that some compounds identified in their study, which had not been detected in other investigations of cooked cured-meat volatiles, were possibly due to the smoking and frying of bacon. In contrast with the large number of papers published on the chemistry of uncured meat flavor, the literature available on the flavor attributes of cured meat has only been reported by a handful of researchers. Shahidi *et al.* (1986) provided an extensive listing of volatile compounds in uncured and cured pork. The volatile constituents identified in nitrite-cured ham are presented in Table 5.1 (Ramarathnam *et al.* 1991*a*; Ramarathnam 1998).

Cross and Ziegler (1965) examined the volatile constituents isolated from uncured and cured hams by a GC methodology. Qualitatively, the volatile compounds of cured ham were similar to uncured samples, but were quantitatively different. They reported that hexanal and pentanal were present

TABLE 5.1
VOLATILE CONSTITUENTS IDENTIFIED IN NITRITE-CURED HAM

Aldehydes
formaldehyde[2,3]
ethanal[1-4]
propanal[1-3]
β-methylthio-propanal[4]
2-methylpropanal[2,3]
2-methylbutanal[1]
3-methylbutanal[1,2,4]
pentanal[1-4]
hexanal[1-3]
2-hexenal[3,4]
heptanal[3,4]
2-heptenal[3]
2,4-heptadienal[3]
octanal[3,4]
2-octenal[3]
nonanal[3,4]
2-nonenal[3,4]
2,4-nonadienal[3,4]
decanal[3,4]
2-decenal[3]
2,4-decadienal[3,4]
undecanal[3,4]
2-undecenal[3]
2,4-undecadienal[3]
dodecanal[3,4]
2-dodecenal[3,4]
2,4-dodecadienal[3]

Carboxylic Acids
methanoic (formic)[2]
ethanoic (acetic)[2,4]
propanoic[2]
butanoic[2,4]
4-methylpentanoic[2]
hexanoic[4]
octanoic[3,4]
decanoic[3]
dodecanoic[3]
tetradecanoic[3]
hexadecanoic[3]
hexadecenoic[3]
octadecanoic[3]
octadecenoic[3]
octadecadienoic[3]
octadecatrienoic[3]
eicosanoic[3]
eicosenoic[3]
eicosadienoic[3]
eicosatrienoic[3]

Alcohols
methanol[3]
ethanol[2]
propanol[3]
3-methylbutanol[4]
hexanol[3,4]
4-hexanol[4]
heptanol[3]
octanol[3,4]
2-octanol[3]

Esters
ethylmethanoate[3]
methylethanoate[3]
methylpropanoate[3]
ethylbutanoate[3]
methylhexanoate[3]
ethylhexanoate[3]
methyloctanoate[3]
methyldecanoate[3]
methyldodecanoate[3]

Furans
methylfuran[4]
pentylfuran[4]
heptylfuran[4]
2-acetylfuran[4]
furyl alcohol[4]

Hydrocarbons
heptane[4]
pentadecane and its isomers[4]
1-pentadecene[4]
hexadecane and its isomers[4]

Ketones
2-propanone[1,2,4] (acetone)
2-butanone[2,3]
2,3-butanedione[2-4]
2-pentanone[3]
2,3-pentanedione[4]
2,6-hexanedione[3]
2-octanone[4]
2-nonanone[4]
2-undecanone[4]
2-dodecanone[4]
2-tridecanone[4]
pentadecanone[4]

Phenols
dimethylphenol[4]

Pyridines
2-methylpyridine[4]

Pyrazines
2-methylpyrazine[4]

Other Nitrogen Compounds
ammonia[2]
methylamine[2]
N-ethylpyrrolidone[4]

Sulfur Compounds
hydrogen sulfide[2]
methylmercaptan[4,5]
ethylmercaptan[5]
n-propylmercaptan[5]
n-butylmercaptan[5]
3-methylbutyl-mercaptan[5]
4-methyl-4-mercaptan-pentane-2-one[5]
methyl ethylsulfide[5]
ethylenesulfide[5]
propylenesulfide[5]
ethyl isobutylsulfide[5]
2,2′-bis(ethylthio)-propane[5]
thiacyclohexane[4]
ethylthioacetate[5]
n-butylthiopropionate[5]
iso-butylthio-butanoate[5]
methylthioacetate[5]
dimethyldisulfide[4,5]
ethyl methyl-disulfide[4,5]
diethyldisulfide[5]
1,3-thioxalane[5]
dimethyltrisulfide[4,5]
methyl ethyltrisulfide[5]
diethyltrisulfide[5]
3,5-dimethyl-1,2,4-trithiolane[4]
3,6-dimethyl-1,2,4-trithiolane[4]
thiophene[5]
2-methylthiophene[5]
2-formylthiophene[5]
2,4,6-triethyl perhydro-1,3,5-dithiazine (thialdine)[4]

Halogenated Compounds
dichlorobenzene[4]

Miscellaneous Compounds
benzonitrile[6]
phenylacetonitrile[6]
nonanenitrile[6]
decanenitrile[6]
undecanenitrile[6]
dodecanenitrile[6]
tridecanenitrile[6]
pentylnitrate[6]
hexylnitrate[6]
heptylnitrate[6]
octylnitrate[6]

[1]Cross and Ziegler (1965); [2]Ockerman *et al.* (1964); [3]Lillard and Ayres (1969); [4]Bailey and Swain (1973); [5]Golovnya *et al.* (1982); and [6]Mottram (1984). (From Ramarathnam 1998).

in appreciable amounts in the volatiles of uncured, but were barely detectable in the volatiles of cured ham; it was suggested that the absence of these aldehydes was responsible for the flavor differences between cured and uncured ham. Swain (1972) concurred with this finding and reported that nitrite appeared to retard the formation of higher molecular weight aldehydes (*i.e.*, $> C_5$). Cross and Ziegler (1965) also noted that the volatiles, after passage through a solution of 2,4-dinitrophenylhydrazine (DNPH), had the characteristic cured-ham aroma, regardless of whether cured or uncured hams were used. Cured and uncured chicken and beef volatiles, after stripping their carbonyl compounds by passage through DNPH solutions, also possessed an aroma similar to that of cured ham. Cross and Ziegler (1965) concluded that treating meat with nitrite does not seem to contribute any new volatile compounds to the flavor of cooked meats, with the exception of nitrogen oxides that are not present in cooked uncured meat. Therefore, they postulated that cured-ham aroma represents the basic flavor of meat derived from precursors other than triacylglycerols, and that the aromas of various types of cooked meat depend on the spectrum of carbonyl compounds derived by lipid oxidation.

Shahidi (1989) reported that the elimination of lipid oxidation, either by curing or by stripping of carbonyl compounds from volatiles of untreated cooked meats, caused a major effect on the flavor perception of meats, but this author noted that qualitative differences due to the possible presence of less active flavor components can not be ruled out. Nonetheless, GC analyses of the volatiles of cured meat revealed a much simpler spectrum than their uncured counterparts, with drastic suppression in the content of major aldehydes, such as hexanal and pentanal, which are known to be responsible for MFD. Ramarathnam (1991*a*,*b*), who examined and provided quantitative information on the carbonyls and hydrocarbons present in the aroma concentrates of uncured and nitrite-cured pork, beef and chicken, concurred with this finding. Shahidi (1989) proposed that any agent, or combination of agents that prevents lipid oxidation, with the exception of nitrite precursors, would in principal, duplicate the antioxidant role of nitrite in the curing process, thereby preventing hexanal generation and MFD. According to Shahidi (1992), this is in line with findings of other researchers and its validity was confirmed by preliminary sensory evaluations, but mutton was not included in these studies.

A simplistic view, attempting to present a unifying theory of the origin of the basic flavor of meat, species differentiation, and MFD is provided in Fig. 5.4. It postulates that meat, when cooked, acquires its characteristic species flavor which is caused by volatile carbonyl compounds, such as hexanal and pentanal, formed by oxidation of its lipid components (*i.e.*, primarily phospholipids). Further oxidation during storage of cooked meat results in the deterioration of its flavor. Curing with nitrite suppresses the formation of oxidation products. It may be assumed that the flavor of nitrite-cured meats is

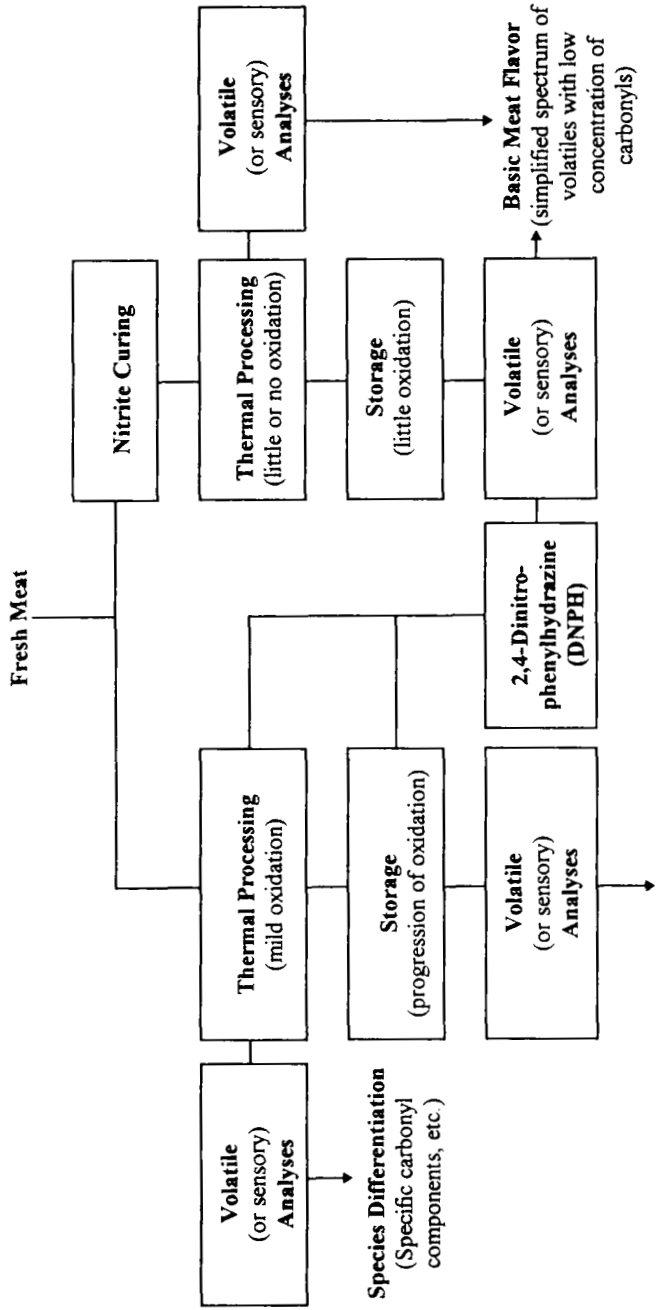


FIG. 5.4. CONSEQUENCE OF THERMAL PROCESSING, CURING AND STORAGE ON THE FLAVOR OF COOKED MEATS AND DEVELOPMENT OF MEAT FLAVOR DETERIORATION
(From Shahidi 1992).

actually the basic natural flavor of meat from different species without being influenced by overtone carbonyls derived from oxidation of their lipid components. Further support for this view has been provided by Ramarathnam *et al.* (1991*a*,*b*; 1993*a*,*b*), but the postule does not easily explain the fact that the intensity of cured meat flavor is proportional to the logarithm of nitrite concentrations as reported by MacDougall *et al.* (1975), or the apparent persistence of the characteristic "mutton" flavor after nitrite curing of sheep meat (Reid *et al.* 1993; Young and Braggins 1998). These authors reported that curing had no effect on panelists' abilities to distinguish between the flavor of mutton and other red meats. They suggested that lipid oxidation products from adipose tissue contribute very little to mutton aroma and possibly also to species flavor difference in lean meats.

The presence or absence of bacteria in cures/pickles may also contribute to the type and intensity of meat flavor volatiles formed. Hinrichsen and Andersen (1994) and Andersen and Hinrichsen (1995) reported that changes in process technology in bacon production have given rise to a product with an inferior flavor profile. Data showed that a greater number of volatile compounds were generated in bacon produced according to traditional process technology compared to a bacon product prepared by new technology in which the tank curing process was left out. These authors suggested that factors other than sodium chloride and sodium nitrite levels underlie cured flavor development in such products. Further studies revealed that halotolerant bacteria, such as *Vibrio* spp. isolated from cover brines, have an impact on the flavor volatiles formed. Two volatile compounds, namely 2-methylbutanal and 3-methylbutanal, appeared. These branched aldehydes can react with hydrogen sulfide, ammonia and ammonium sulfide in meat leading to the formation of 3,5-diisobutyl-1,2,4-trithiolane and 5,6-dihydro-2,4,6-triisobutyl-4*H*-1,3,5-dithiazine, both of which are claimed to display cured aroma (Shu and Mookherjee 1985; Shu *et al.* 1985). It is therefore likely that the presence of *Vibrio* spp. is a factor in connection with the formation of an overall flavor in tank-cured bacon products (Hinrichsen and Andersen 1994).

Flavor of Dry-Cured Hams

Dry-curing of hams is a traditional process in the Mediterranean region that leads to products with unique flavor profiles (Flores and Toldrá 1993). The distinctive flavors are due to interactions among various flavor precursors originating from meat proteins, lipids and carbohydrates and the added salt and nitrate. The nonvolatile components, which mainly comprise peptides and amino acids, constitute taste-active compounds and have a large impact on the final flavor of the product (Flores *et al.* 1997; 1998*a*). On the other hand, the typical aroma of dry-cured ham is mainly associated with the generation of volatile

compounds (*i.e.*, lipid oxidation products) during the later stages of processing. Not only does the ripening/aging step markedly affect the volatile composition in dry-cured hams, but so does the analytical methodology employed to investigate the resulting flavor (*e.g.*, solvent extraction, vacuum distillation, aroma extraction dilution analysis, dynamic headspace analysis). Berdagué *et al.* (1991) isolated the volatile constituents of dry-cured ham by vacuum distillation. The structures of seventy-six compounds were identified by mass spectrometry, cochromatography or measured Kováts indices. Included amongst the carbonyls, hydrocarbons and alcohols were two new heterocyclic nitrogen compounds and two lactones. Olfactory examination of the separated components revealed that some of the unidentified compounds had a strong flavor note associated with dry-cured ham. Flores *et al.* (1998*b*) reported that more than 250 compounds have been identified to date from various studies on French, Italian and Spanish dry-cured hams (Table 5.2). These authors noted that more carboxylic acids, lactones and aliphatic hydrocarbons were detected in dry-cured hams that had been subjected to vacuum distillation as compared to dynamic headspace analysis. The major organic classes of compounds identified in Serrano dry-cured ham were: (1) hydrocarbons (*i.e.*, alkanes, methyl-branched alkanes and aromatic hydrocarbons) arising from autoxidation of lipids; (2) aldehydes with 6 or more carbon atoms resulting from oxidation of free fatty acids (hexanal was the most abundant); (3) alcohols arising from oxidative decomposition of lipids and methyl-derived ones from Strecker degradation of amino acids; (4) ketones resulting as products of either β-keto acid decarboxylation or saturated fatty acids via β-oxidation; (5) free fatty acids resulting from the hydrolysis of triacylglycerols and phospholipids; (6) γ-lactones arising from dehydration and cyclization of the γ-hydroxyacids; (7) esters resulting from the interaction of free fatty acids and alcohols generated by lipid oxidation in the intramuscular tissue; (8) carboxylic acids; and (9) other miscellaneous compounds, including benzene derivatives, amines and amides. Studies on the aroma constituents of Serrano dry-cured hams that had been subjected to long (12 months) and short (7 months) curing periods revealed that there were some quantitative differences in the volatile compounds detected. However, none of the volatile compounds identified had a characteristic cured aroma. A few of the key impact meaty aroma notes were 2-pentylfuran (ham-like), 1*H*-pyrrole (meaty) and 2-butoxyethanol (dark toast-meaty) (Flores *et al.* 1998*b*). Many desirable meaty flavor compounds have very low flavor threshold values, thus making their identification difficult.

These volatile components come from the muscle enzyme system which acts on the proteins and lipids of meat. For example, during the early stages of postmortem storage, calpains (*i.e.*, cysteine endopeptidases) and cathepsins (*i.e.*, lysosomal proteinases) break down myofibrillar proteins and increase the tenderness of the product. Afterward, these and additional muscle proteases

TABLE 5.2
VOLATILE COMPOUNDS PREVIOUSLY REPORTED IN DRY-CURED HAM

Aldehydes
(E)-2-heptenal[4]
(E)-2-nonenal[2]
(E)-2-octenal[4]
(E)-2-pentenal[4]
(E,E)-2,4-decadienal[2]
(E,E)-2,4-pentadienal[4]
(E,Z)-2,4-decadienal[2]
(Z)-2-decenal[4]
(Z)-2-nonenal[4]
2,4-octadienal[3]
2,4-nonadienal[3]
2-decenal[1]
2-dodecenal[1]
2-furaldehyde[1]
2-heptenal[3,7]
2-hexenal[1,3,4,6]
2-methyl-2-butenal[3]
2-methyl-2-pentenal[1]
2-methyl-2-propenal[3]
2-methylbutanal[2,3,4,7]
2-methylpropanal[3,6]
2-nonenal[5]
2-octenal[3,7]
3-methyl-2-butenal[1]
3-methylhexanal[6]
4-methyl-2-pentenal[1]
9-octadecanal[5]
acetaldehyde[3]
benzaldehyde[1,3,4]
benzenacetaldehyde[3,5]
butanal[2,4]
decanal[1,6]
dodecanal[1,5]
heptanal[1,4,7]
hexadecanal[2,5]
hexanal[1,7]
hexenal[3]
nonanal[1,3,5,6]
octanal[1,3,7]
octadecanal[5]
octadecenal[5]
pentanal[1,4,6,7]
pentadecanal[5]
phenylacetaldehyde[1,2]
propanal[3]
tetradecanal[5]
undecanal[5]

Alcohols
(E)-2-octen-1-ol[4]
(Z)-2-octen-1-ol[4]
1.4-butanediol[1]
1,2-propanediol[4]
1-butanol[4,6,7]
1-butoxyethoxyethanol[5]
1-butoxy-2-propanol[4,7]
1-decanol[1]
1-dodecanol[1]
1-ethylcyclopropanol[3]
1-heptanol[2,4,7]
1-hexanol[1,5,7]
1-octanol[1,4,5]
1-octen-3-ol[2,7]
1-pentanol[1,7]
1-penten-3-ol[3,4,6,7]
1-propanol[3,4,7]
1-tetradecanol[2]
2-butanol[3,4,7]
2-butoxyethanol[2,4,6,7]
2-ethyl-1-hexanol[4]
2-ethoxyethanol[2]
2-hepten-1-ol[4]
2-hexanol[4]
2-methyl-1-propanol[4,6,7]
2-methyl-2-buten-1-ol[4,7]
2-methyl-3-buten-2-ol[2,4,7]
2-methylbutan-1-ol[1-3,5,6]
2-pentanol[3,4,7]
2-propanol[3]
3,7-dimethyl-1-octen-3-ol[4]
3-methyl-3-buten-1-ol[4,6,7]
3-methyl-butan-1-ol[1-3,5,6]
2-pentanol[3,4,7]
2-propanol[3]
3,7-dimethyl-1-octen-3-ol[4]
3-methyl-buten-1-ol[4,6,7]
3-methyl-butan-1-ol[1,7]
3-penten-1-ol[3]
5-methylheptan-2-ol[1]
cis-3-hexen-1-ol[1]
epoxydihydrolinalool[4]
ethanol[3,6]
furfurylalcohol[1]
methanol[3]
phenylethanol[2,5]

Aliphatic Hydrocarbons
alkyl cyclopentane[6]
1-heptene[4,7]
1-octene[4,6,7]
2,2,3-trimethylpentane[4,5]
2,2,4,6,6-pentamethylheptane[6]
3-methylnonane[6]
4-methylheptane[5]
4,6,8-trimethylnonene[4]
branched alkane[1]
decane[2,5,6]
decene[3]
dimethylundecane[3]
docosane[2]
dodecane[2,5]
heneicosane[2,5]
heptadecane[2,5]
heptane[3,4,6,7]
hexane[6]
hexadecane[2]
myrcene[1]
methyldecane[5]
nonadecane[2]
nonane[1,3,5,6]
nonene[3]
octadecane[2,5]
octane[3,4,6,7]
pentane[4,6]
pentadecane[2,5]
tetradecane[2]
tridecane[2,5]
undecane[2,5]

Aromatic Hydrocarbons
1,2,3-trimethylbenzene[1,4,6]
1,2-dimethylbenzene[1,3-6]
1,2-dimethyl-3-ethylbenzene[1]

Table 5.2 *continued*

1,2,4-trimethylbenzene[5]
1,3-dimethylbenzene[4,7]
1,4-dimethylbenzene[1,4,6,7]
1-ethyl-2-methylbenzene[4,6]
1-methoxyhexane[4]
1-methyl-3-methylethylbenzene[1]
benzene[3,4,7]
benzonitrile[4]
ethenylbenzene[1,4,6]
ethylbenzene[1,4,5,7]
isopropylbenzene[4]
methylvinylbenzene[4]
tert-butylbenzene[4]
toluene[3,7]

Ketones
1-hydroxy-2-propanone[4]
2-hexanone[6]
2-hydroxy-3-pentanone[6]
2-methyloctan-3-one[2]
2-propanone[3,4,6,7]
2-undecanone[4]
3-hexanone[6]
3-hydroxybutan-2-one[2,4-7]
3-methyl-2-butanone[2,4-7]
3-methyl-2-pentanone[7]
3-octen-2-one[1]
4-octen-3-one[3,4,7]
4-methyl-2-pentanone[7]
6-methyl-5-hepten-2-one[4,6,7]
branched ketone[1]
butan-2-one[2,7]
butan-2,3-dione[2,3,6,7]
cyclohexanone[4]
heptan-2-one[2,3,5,6]
nonan-2-one[3]
octan-2-one[5,6]
pentan-2,3-dione[2,4,6]
pentan-2-one[1,3,4,6,7]
trans-geranylacetone[5]

Carboxylic Acids
2-methylpropanoic acid[3]
3-methylbutanoic acid[3]
9-hexadecenoic acid[5]
9,12-octadecadienoic acid[5]
9-octadecenoic acid[5]
acetic acid[1,3,4]
butanoic acid[1,4]
hexanoic acid[1,4,5]
hexadecanoic acid[5]
heptanoic acid[5]
isohexanoic acid[1]
isooctanoic acid[1]
octanoic acid[1,5]
pentadecanoic acid[2,5]
pentanoic acid[1,3,4]
propanoic acid[3,4]
nonanoic acid[5]
undecanoic acid[5]

Esters
alkyl phathalate[2]
butyl acetate[6]
ethyl acetate[3,4,6,7]
ethyl butanoate[3,4,7]
ethyl decanoate[2,4]
ethyl 2-methylbutanoate[2,4,6,7]
ethyl 3-methylbutanoate[3,4,7]
ethyl pentanoate[3]
ethyl propanoate[3,4]
ethyl hexanoate[1,4,7]
ethyl hexadecanoate[2]
ethyl heptanoate[3]
ethyl octanoate[2,4,7]
hexyl butyrate[1]
linalyl acetate[1]
methyl acetate[4]
methyl benzoate[4]
methyl butanoate[4,7]
methyl carbamate[1]
methyl decanoate[4]
methyl hexanoate[4,7]
methyl hexadecanoate[5]
methyl heptanoate[4,7]
methyl nonanoate[4]
methyl octanoate[4,7]
methylpentanoate[4,7]
methyl propanoate[4,7]
methyl 2-methylbutanoate[4,6,7]
methyl 2-methylpropanoate[4,7]
methyl 3-hexenoate[4]
methyl 3-methylbutanoate[4,7]
methyl 3-octenoate[4]
methyl 4-methylhexanoate[4,7]
methyl 4-methylpentanoate[4]
methyl 4-decenoate[4]
methyl 5-hexenoate[4,7]
methyl 6-methylheptanoate[4]
propyl acetate[3]

Lactones
δ-butyrolactone[1,3]
γ-butyrolactone[2]
γ-hexalactone[2,5,6]
γ-nonalactone[2,5]
γ-octalactone[2]
γ-pentalactone[6]
γ-valerolactone[2]

Sulfur Compounds
3-methylthiopropanol[2]
dimethyl disulfide[3,4,6,7]
dimethyl trisulfide[3,4,7]
dimethyl tetrasulfide[4]
methyl 3-(methylthio)-propanoate[4]
methyl ethyl disulfide[3]
methyl *n*-pentyl disulfide[2]

Chloride Compounds
2-chloronaphthalene[2]
2,2-dichloroethanol[6]
dichloromethane[6]
dichlorobenzene[6]
tetrachloroethene[6]
trichloromethane[2,5,6]

Furans
2-ethylfuran[4,6]
2-pentylfuran[4,7]
2-methyl-4,5-dihydrofurane[1]

Table 5.2. *continued*

2,2,4-trimethyl-2,5-dihydro-furane[1]	trimethylpyrazine[4]	BHT[2,5]
2,5-dimethyltetrahydro-furane[1]	**Miscellaneous**	dimethylphenol[6]
	1-methyl-2-pyrrolidinone[5]	farnesol[5]
Pyrazines	1,2-benzenedicarboxylic acid[5]	isovaleramide[1]
pyrazine[4]	2,4,6-trimethyl-1,3,5-trioxane[5]	limonene[4,6]
2-methylpyrazine[4]	3-vinylpyridine[4]	pentyloxyrane[1]
2,6-dimethylpyrazine[4,7]		phenylethylamine[1]
		propanodiamide[1]
		pyrrole lactone[2,4]

[1]López *et al.* (1992); [2]Garcia *et al.* (1991); [3]Hinrichsen and Pedersen (1995); [4]Barbieri *et al.* (1992); [5]Berdagué *et al.* (1991); [6]Buscailhon *et al.* (1993); and [7]Bolzoni *et al.* (1996).
(From Flores *et al.* 1998*b*)

(*e.g.*, aminopeptidases) take over and degrade protein constituents further. For example, cathepsin D has been found to be especially active against myosin heavy chains, titin and also on M and C proteins, topomyosin and troponins T and I (Zeece and Katoh 1989). Low-molecular-weight polypeptide fragments as well as peptides and free amino acids are generated by these proteolytic enzymes. Peptide mapping from high performance liquid chromatography and capillary electrophoresis studies has shown that these protein degradation products contribute to the typical flavor of dry-cured ham by exaggerating bitter, metallic and savory flavor notes (Aristoy and Toldrá, 1995; Rodríguez-Nuñez *et al.* 1995). Furthermore, amino acids play an important role in the generation of volatile flavor compounds through Strecker and Maillard reactions. Protease activity during the dry curing of hams can be controlled in various ways, the easiest of which is by regulating the relative humidity and temperature. Addition of salt can also control an excess of proteolysis by its inhibitory effect on cathepsins and aminopeptidases. Table 5.3 illustrates the changes in the free amino acids' content in pork muscle during the processing (0, 7 and 15 months) of dry-cured ham.

Lipolysis greatly influences the flavor of dry-cured hams. Lipases and phospholipases are most active during the initial five months of processing and hydrolyze fatty acid residues of adipose and intramuscular tissue (Motilva *et al.* 1993*a*,*b*). From adipose tissue, the chief lipolytic products are myristic, heptadecanoic, linolenic and arachidonic acids (Toldrá *et al.* 1997). From muscle tissue, the majority of free fatty acids are generated by phospholipases. Free fatty acids tend to accumulate as the process progresses for up to 10 months and then start to decrease as a consequence of lipid oxidation. The resulting oxidation products act as flavor precursors to a great number of volatile compounds. In fact, Flores *et al.* (1985) reported that the generation of the characteristic aroma of dry-cured ham was in agreement with the onset of lipid oxidation.

TABLE 5.3
CHANGES IN THE FREE AMINO ACIDS' CONTENT IN THE MUSCLE *BICEPS FEMORIS* DURING THE PROCESSING (0, 7 AND 15 MONTHS) OF DRY-CURED HAM.[a]

Amino acids	Raw	7 months	15 months	Δ[b]
Aspartic acid	0.70	197.0	301.1	300.4
Glutamic acid	5.40	354.0	498.6	493.2
Serine	2.38	183.6	250.3	248.0
Asparagine	1.03	46.9	27.1	26.1
Glycine	7.30	155.9	216.1	208.8
Glutamine	25.43	46.9	24.6	-0.8
Taurine	34.15	59.5	81.2	47.1
Tyrosine	2.20	127.2	171.9	169.7
Proline	3.18	195.9	288.6	285.5
Alanine	14.50	270.7	389.3	374.8
Ornithine	1.21	29.7	56.2	55.0
Arginine	3.88	236.8	230.8	227.0
Threonine	3.30	193.9	279.9	276.6
Valine	3.40	221.4	315.5	312.1
Methionine	1.37	90.8	133.7	132.3
Isoleucine	1.78	155.7	218.3	216.5
Leucine	2.82	246.9	342.7	339.9
Phenylalanine	2.03	149.3	209.2	207.1
Tryptophan	0.75	25.7	31.4	30.7
Histidine	3.20	70.0	98.0	94.8
Lysine	3.12	509.0	734.6	731.5

[a]Mean free amino acid content (mg amino acid/100 g muscle) from 6 muscles.
[b]Net increments in relation to raw meat. (From Toldrá *et al.* 1997).

REFERENCES

Andersen, H.J. and Hinrichsen, L.L. 1995. Changes in curing agents, microbial counts and volatile components during processing of green bacon using two different production technologies. *J. Sci. Food Agric.* **68**, 477–487.

Arctander, S. 1969. Perfume and Flavor Chemicals, published by the author, New Jersey.

Aristoy, M-C. and Toldrá, F. 1995. Isolation of flavor peptides from raw pork meat and dry-cured ham. In *Food Flavors: Generation, Analysis and Process Influence*, ed. G. Charalambous. Elsevier Science Publishers B.V., Amsterdam, The Netherlands, pp. 1323–1344.

Bailey, M.E. and Swain, J.W. 1973. Influence of nitrite on meat flavor. In *Proceedings of the Meat Industry Research Conference*. American Meat Institute Foundation, Arlington, VA, pp. 29–45.

Baltes, W. 1979. Röstaromen. *Deutsch. Lebensm. Rundsch.*, **75**, 2-7.

Barbieri, G., Bolzoni, L., Parolari, G., Virgili, R., Buttini, R., Careri, M. and Mangia, A. 1992. Flavour compounds of dry-cured ham. *J. Agric. Food Chem.* **40**, 2389-2394.

Batzer, O.F., Santoro, A.T. and Landmann, W.A. 1962. Identification of some beef flavor precursors. *J. Agric. Food Chem.* **10**, 94-96.

Bender, A.E. and Ballance, P.E. 1961. A preliminary examination of the flavour of meat extract. *J. Sci. Food Agric.* **12**, 683-687.

Berdagué, J.-L., Denoyer, C., Le Quéré, J.-L. and Semon, E. 1991. Volatile components of dry-cured ham. *J. Agric. Food Chem.* **39**, 1257-1261.

Bolzoni, L., Barbieri, G. and Virgili, R. 1996. Changes in volatile compounds of Parma ham during maturation. *Meat Sci.* **43**, 301-310.

Brinkman, H.W., Copier, H., de Leuw, J.J.M. and Tjan, S.B. 1972. Components contributing to beef flavor. Analysis of the headspace volatiles of beef broth. *J. Agric. Food Chem.* **20**, 177-181.

Brooks, J., Haines, R.B., Moran, T. and Pace, J. 1940. The function of nitrate, nitrite and bacteria in the curing of bacon and hams. Dept. of Sci. and Indust. Res., Food Investigation. Special Report 49, His Majesty's Stationery Office, London, UK, 32 pp.

Buscailhon, S., Berdagué, J.L. and Monin, G. 1993. Time-related changes in volatile compounds of lean tissue during processing of French dry-cured ham. *J. Sci. Food Agric.* **63**, 69-75.

Buttery, R.G., Ling, L.C., Teranishi, R. and Mon, T.R. 1977. Roasted lamb fat: Basic volatile components. *J. Agric. Food Chem.* **25**, 1227-1229.

Chang, S.S. and Peterson, R.J. 1977. Symposium: The basis of quality in muscle foods. Recent developments in the flavor of meat. *J. Food Sci.* **42**, 298-305.

Cho, I.C. and Bratzler, L.J. 1970. Effect of sodium nitrite on flavor of cured pork. *J. Food Sci.* **35**, 668-670.

Crocker, E.C. 1948. Flavor of meat. *Food Res.* **13**, 179-183.

Cross, C.K. and Ziegler, P. 1965. A comparison of the volatile fractions from cured and uncured meat. *J. Food Sci.* **30**, 610-614.

Evers, W.J., Heinsohn, H.H. Jr., Mayers, B.J. and Sanderson, A. 1976. Furans substituted at the three position with sulfur. In *Phenolic, Sulfur, and Nitrogen Compounds in Food Flavors,* ed. G. Charalambous and I. Katz. Am. Chem. Soc., Washington, DC, pp. 184-193.

Farmer, L.J. 1990. Effect of lipid on the formation of volatile aroma compounds by the Maillard reaction. Ph.D. Thesis, University of Bristol, Bristol, UK.

Farmer, L.J., Mottram, D.S. and Whitfield, F.B. 1989. Volatile compounds produced in Maillard reactions involving cysteine, ribose and phospholipid. *J. Sci. Food Agric.* **49**, 347-368.

Farmer, L.J. and Patterson, R.L.S. 1991. Compounds contributing to meat flavour. *Food Chem.* **40**, 201–205.

Flores, J., Nieto, P., Bermell, S. and Miralles, M.C. 1985. Cambios en los lípidos del jamón durante el proceso de curado, lento y rápido, y su relación con la calidad. *Rev. Agroquim. Tecnol. Aliment.* **25**, 117–124.

Flores, J. and Toldrá, F. 1993. Curing. In *Encyclopedia of Food Science, Food Technology and Nutrition*, ed. R. Macrae, R.K. Robinson and M.J. Sadler. Academic Press Inc., London, UK, pp. 1277–1282.

Flores, M., Grimm, C.C., Toldrá, F. and Spanier, A.M. 1997. Correlations of sensory and volatile compounds of Spanish "Serrano" dry-cured ham as a function of two processing times. *J. Agric. Food Chem.* **45**, 2178–2186.

Flores, M., Sanz, Y., Spanier, A.M., Aristoy, M-C. and Toldrá, F. 1998*a*. Contribution of muscle and microbial aminopeptidases to flavor development in dry-cured meat products. In *Food Flavors: Formation, Analysis and Packaging Influences*, ed. E.T. Contis, C.-T. Ho, C.J. Mussinan, T.H. Parliment, F. Shahidi and A.M. Spanier. Developments in Food Science 40. Proceedings of the 9th International Flavor Conference, The George Charalambous Memorial Symposium, Limnos, Greece, 1-4 July 1997. Elsevier Science B.V., Amsterdam, The Netherlands, pp. 547–557.

Flores, M., Spanier, A.M. and Toldrá, F. 1998b. Flavour analysis of dry-cured ham. In *Flavor of Meat, Meat Products and Seafoods, Second Edition*, ed. F. Shahidi. Blackie Academic & Professional, London, UK, pp. 320–341.

Furia, T.E. and Bellanca, N. 1975. Fenaroli's Handbook of Flavor Ingredients. 2nd ed., Vol. 1, CRC Press, Boca Raton, Fla., 551 pp.

Garcia, C., Berdagué, J.J., Antequera, T., López-Bote, C., Córdoba, J.J. and Ventanas, J. 1991. Volatile components of dry cured Iberian ham. *Food Chem.* **41**, 23–32.

Golovnya, R.V., Garbuzov, V.G., Grigoryeva, I.Y., Zharich, S.L. and Bolshakov, A.S. 1982. Gas chromatographie analysis of volatile sulfur-containing compounds of salted and salted-boiled pork. *Nahrung* **26**, 89–96.

Gray, J.I., MacDonald, B., Pearson, A.M. and Morton, I.D. 1981. Role of nitrite in cured meat flavor: A review. *J. Food Prot.* **44**, 302–312, 319.

Güntert, M., Bertram, H.-J., Emberger, R., Hopp, R., Sommer, H. and Werkhoff, P. 1993b. New aspects of the thermal generation of flavour compounds from thiamin. In *Progress in Flavour Precursor Studies. Analysis-Generation-Biotechnology*, ed. P. Schreier and P. Winterhalter. Allured Pub. Corp., Carol Stream, IL, pp. 361–378.

Güntert, M., Bertram, H.-J., Hopp, R., Silberzahn, W., Sommer, H. and Werkhoff, P. 1993*a*. Thermal generation of flavor compounds from thiamin and various amino acids. In *Recent Developments in Flavor and Fragrance Chemistry*, ed. R. Hopp and K. Mori. VCH, Weinheim, Germany, pp. 215–240.

Ha, J.K. and Lindsay, R.C. 1991. Volatile alkylphenols and thiophenol in species-related characterizing flavors of red meat. *J. Food Sci.* **56**, 1197–1202.

Hartman, G.J., Carlin, J.T., Scheide, J.D. and Ho, C-T. 1984. Volatile products formed from the thermal degradation of thiamin at high and low moisture levels. *J. Agric. Food Chem.* **32**, 1015–1018.

Herring, H.K. 1973. Effect of nitrite and other factors on the physico-chemical characteristics and nitrosamine formation in bacon. In *Proceedings of the Meat Industry Research Conference*. American Meat Institute Foundation, Arlington, VA, pp. 47–60.

Herz, K.O. and Chang, S.S. 1970. Meat flavor. *Adv. Food Res.* **18**, 1–83.

Hinrichsen, L.L. and Andersen, H.J. 1994. Volatile compounds and chemical changes in cured pork: Role of three halotolerant bacteria. *J. Agric. Food Chem.* **42**, 1537–1542.

Hinrichsen, L.L. and Pedersen, S.B. 1995. Relationship among flavor, volatile compounds, chemical changes, and microflora in Italian-type dry-cured ham during processing. *J. Agric. Food Chem.* **43**, 2932–2940.

Ho, C-T., Lee, K.N. and Jin, Q.Z. 1983. Isolation and identification of volatile flavor compounds in fried bacon. *J. Agric. Food Chem.* **31**, 336–342.

Hornstein, I. and Crowe, P.F. 1960. Flavor studies on beef and pork. *J. Agric. Food Chem.* **8**, 494–498.

Hornstein, I. and Crowe, P.F. 1964. Meat flavor — A review. *J. Gas Chromatogr.* **2**, 128–131.

International Flavors and Fragrances, Inc. 1979. 3-Furylaklyl sulfides useful as flavorings. British Patent 1,543,653.

Imafidon, G.I. and Spanier, A.M. 1994. Unraveling the secret of meat flavor. *Trends Food Sci. Technol.* **5**, 315–321.

Katz, I. 1981. Recent progress in some aspects of meat flavor chemistry. In *Flavor Research. Recent Advances*, ed. R. Teranishi, R.A. Flath and H. Sugisawa. Marcel Dekker, New York, NY, pp. 217–229.

Kemp, J.D., Langlois, B.E., Fox, J.D. and Varney, W.Y. 1975. Effects of curing ingredients and holding times and temperatures on organoleptic and microbiological properties of dry-cured sliced ham. *J. Food Sci.* **40**, 634–636.

Kim, J-O., Ha, Y.L. and Lindsay, R.C. 1993. Role of volatile branched-chain and other fatty acids in species-related red meat flavors. *J. Korean Soc. Food Nutr.* **22**, 300–306.

Kubota, K., Kobayashi, A. and Yamanishi, T. 1980. Some sulfur containing compounds in cooked odor concentrate from boiled Antarctic krills (*Euphausia superba* Dana). *Agric. Biol. Chem.* **44**, 2677–2682.

Lillard, D.A. and Ayres, J.C. 1969. Flavor compounds in country cured hams. *Food Technol.* **23**(2), 117–120.

López, M.O., de la Hoz, L., Cambero, M.I., Gallardo, E., Reglero, G. and Ordóñez, J.A. 1992. Volatile compounds of dry hams from Iberian pigs. *Meat Sci.* **31**, 267–277.

Maarse, H. and Visscher, C.A. 1989. Volatile Compounds in Food — Qualitative and Quantitative Data, 6th Edition. TNO-CIVO Food Analysis Institute, Zeist, The Netherlands.

MacDonald, B., Gray, J.I., Stanley, D.W. and Usborne, W.R. 1980. Role of nitrite in cured meat flavor: Sensory analysis. *J. Food Sci.* **45**, 885–888, 904.

MacDougall, D.B., Mottram, D.S. and Rhodes, D.N. 1975. Contribution of nitrite and nitrate to the colour and flavour of cured meats. *J. Sci. Food Agric.* **26**, 1743–1754.

MacLeod, G. 1986. The scientific and technological basis of meat flavours. In *Developments in Food Flavours,* ed. G.G. Birch and M.G. Lindley. Elsevier Applied Science, London, UK, pp. 191–223.

MacLeod, G. 1998. The flavour of beef. In *Flavor of Meat, Meat Products and Seafoods, Second Edition*, ed. F. Shahidi. Blackie Academic & Professional, London, UK, pp. 27–60.

MacLeod, G. and Ames, J. 1986. 2-Methyl-3-(methylthio)furan: a meaty character impact aroma compound identified from cooked beef. *Chem. Ind. (London)* 175–177.

MacLeod, G. and Seyyedain-Ardebili, M. 1981. Natural and simulated meat flavors (with particular reference to beef). *CRC Crit. Rev. Food Sci. Nutr.* **14**, 309–437.

Motilva, M-J., Toldrá, F., Aristoy, M-C. and Flores, J. 1993*a*. Subcutaneous adipose tissue lipolysis in the processing of dry-cured ham. *J. Food Biochem.* **16**, 323–335.

Motilva, M-J., Toldrá, F., Nieto, P. and Flores, J. 1993*b*. Muscle lipolysis phenomena in the processing of dry-cured ham. *Food Chem.* **48**, 121–125.

Mottram, D.S. 1984. Organic nitrates and nitriles in the volatiles of cooked cured pork. *J. Agric. Food Chem.* **32**, 343–345.

Mottram, D.S. 1991. Meat. In *Volatile Compounds in Foods and Beverages,* ed. H. Maarse. Marcel Dekker, New York, NY, pp. 107–177.

Mottram, D.S. 1998. The chemistry of meat flavour. In *Flavor of Meat, Meat Products and Seafoods, Second Edition*, ed. F. Shahidi. Blackie Academic & Professional, London, UK, pp. 5–26.

Mottram, D.S. and Edwards, R.A. 1983. The role of triglycerides and phospholipids in the aroma of cooked beef. *J. Sci. Food Agric.* **34**, 517–522.

Mottram, D.S., Edwards, R.A. and MacFie, H.J.H. 1982. A comparison of the flavour volatiles from cooked beef and pork meat systems. *J. Sci. Food Agric.* **33**, 934–944.

Mottram, D.S., Madruga, M.S. and Whitfield, F.B. 1995. Some novel meatlike aroma compounds from the reactions of alkanediones with hydrogen sulfide and furanthiols. *J. Agric. Food Chem.* **43**, 189–193.

Mottram, D.S. and Rhodes, D.N. 1974. Nitrite and the flavour of cured meat I. In *Proceedings of the International Symposium on Nitrite in Meat Products*, Zeist, The Netherlands, September 10–14, 1973, ed. B. Krol and B.J. Tinbergen. Wageningen Centre for Agricultural Publishing and Documentation, Wageningen, The Netherlands, pp. 161–171.

Mussinan, C.J., Wilson, R.A., Katz, I., Hruza, A. and Vock, M.H. 1976. Identification and flavor properties of some 3-oxazolines and 3-thiazolines isolated from cooked beef. In *Phenolic, Sulfur, and Nitrogen Compounds in Food Flavors*, ed. G. Charalambous and I. Katz. Am. Chem. Soc., Washington, DC, pp. 133–145.

National Academy of Sciences. 1982. Alternatives to the Current Use of Nitrite in Foods. National Academy Press, Washington, DC.

Nishimura, O., Mihara, S. and Shibamoto, T. 1980. Compounds produced by the reaction of 2-hydroxy-3-methyl-2-cyclopenten-1-one with ammonia and hydrogen sulfide. *J. Agric. Food Chem.* **28**, 39–43.

Ockerman, H.W., Blumer, T.N. and Craig, H.B. 1964. Volatile chemical compounds in dry-cured hams. *J. Food Sci.* **29**, 123–129.

Ohloff, G. and Flament, I. 1978. Heterocyclic constituents of meat aroma. *Heterocycles* **11**, 663–695.

Pittet, A.O. and Hruza, D.E. 1974. Comparative study of flavor properties of thiazole derivatives. *J. Agric. Food Chem.* **22**, 264–269.

Ramarathnam, N. 1998. Flavour of cured meat. In *Flavor of Meat, Meat Products and Seafoods, Second Edition*, ed. F. Shahidi. Blackie Academic & Professional, London, UK, pp. 290–319.

Ramarathnam, N., Rubin, L.J. and Diosday, L.L. 1991*a*. Studies on meat flavor. 1. Qualitative and quantitative differences in uncured and cured pork. *J. Agric. Food Chem.* **39**, 344–350.

Ramarathnam, N., Rubin, L.J. and Diosday, L.L. 1991*b*. Studies on meat flavor. 2. A quantitative investigation of the volatile carbonyls and hydrocarbons in uncured and cured beef and chicken. *J. Agric. Food Chem.* **39**, 1839–1847.

Ramarathnam, N., Rubin, L.J. and Diosday, L.L. 1993*a*. Studies on meat flavor. 3. A novel method for trapping volatile components from uncured and cured pork. *J. Agric. Food Chem.* **41**, 933–938.

Ramarathnam, N., Rubin, L.J. and Diosday, L.L. 1993*b*. Studies on meat flavor. 4. Fractionation, characterization, and quantitation of volatiles from uncured and cured beef and chicken. *J. Agric. Food Chem.* **41**, 939–945.

Reid, D.H., Young, O.A. and Braggins, T.J. 1993. The effects of antioxidative treatments on mutton flavour/odour intensity and species flavour differentiation. *Meat Sci.* **35**, 171–182.

Rodríguez-Nuñez, E., Aristoy, M-C. and Toldrá, F. 1995. Peptide generation in the processing of dry-cured ham. *Food Chem.* **53**, 187–190.

Rödel, W. and Kruse, H.-P. 1980. Present problems of meat aroma research. *Nahrung* **24**, 129–139.

Salter, L.J., Mottram, D.S. and Whitfield, F.B. 1988. Volatile compounds produced in Maillard reactions involving glycine, ribose and phospholipid. *J. Sci. Food Agric.* **46**, 227–242.

Self, R., Casey, J.C. and Swain, T. 1963. The low-boiling volatiles of cooked foods. *Chem. Ind. (London)*, 863–864.

Shahidi, F. 1989. Flavor of cooked meats. In *Flavor Chemistry. Trends and Developments*, ed. R. Teranishi, R.G. Buttery and F. Shahidi. ACS Symposium Series 388. Am. Chem. Soc., Washington, DC, pp. 188–201.

Shahidi, F. 1992. Prevention of lipid oxidation in muscle foods by nitrite and nitrite-free compositions. In *Lipid Oxidation in Food*, ed. A.J. St. Angelo. ACS Symposium Series 500. Am. Chem. Soc., Washington, DC, pp. 161–182.

Shahidi, F. 1998. Flavour of muscle foods — an overview. In *Flavor of Meat, Meat Products and Seafoods, Second Edition*, ed. F. Shahidi. Blackie Academic & Professional, London, UK, pp. 1–4.

Shahidi, F., Rubin, L.J. and D'Souza, L.A. 1986. Meat flavor volatiles: A review of the composition, techniques of analysis, and sensory evaluation. *CRC Crit. Rev. Food Sci. Nutr.* **24**, 141–243.

Shibamoto, T. 1980. Heterocyclic compounds found in cooked meats. *J. Agric. Food Chem.* **28**, 237–243.

Shu, C-K. and Mookherjee, B.D. 1985. Volatile components of the phenolic fraction of cooked bacon. *J. Agric. Food Chem.* **33**, 1107–1109.

Shu, C-K., Mookherjee, B.D., Bondarovich, H.A. and Hagedorn, M.L. 1985. Characterization of bacon odor and other flavor components from the reaction of isovaleraldehyde and ammonium sulfide. *J. Agric. Food Chem.* **33**, 130–132.

Simon, S., Ellis, D.E., MacDonald, B.D., Miller, D.G., Waldman, R.C. and Westerberg, D.O. 1973. Influence of nitrite and nitrate curing ingredients on quality of packaged frankfurters. *J. Food Sci.* **38**, 919–923.

Spanier, A.M. and Boylston, T.D. 1994. Effect of temperature on the analysis of beef flavor volatiles: Focus on carbonyl and sulfur-containing compounds. *Food Chem.* **50**, 251–259.

Spanier, A.M., McMillin, K.W. and Miller, J.A. 1990. Enzyme activity levels in beef: Effect of postmortem aging and end-point cooking temperature. *J. Food Sci.* **55**, 318–322, 326.

Swain, J.W. 1972. Volatile flavor constituents of pork cured with and without nitrite. Ph.D. Thesis, University of Missouri, Columbia, MO.

Toldrá, F., Flores, M. and Sanz, Y. 1997. Dry-cured ham flavour: enzymatic generation and process influence. *Food Chem.* **59**, 523–530.

Tressl, R., Helak, B., Grunewald, K.-G. and Silwar, R. 1983. Formation of flavor components from proline, hydroxyproline and sulphur containing amino acids. In *Colloque International sur les Arômes Alimentaires*, ed. J. Adda and H. Richard. Technique et documentation-Lavoisier, Paris, pp. 207–230.

Tressl, R. and Silwar, R. 1981. Investigation of sulfur-containing components in roasted coffee. *J. Agric. Food Chem.* **29**, 1078–1082.

van den Ouweland, G.A.M., Demole, E.P. and Enggist, P. 1989. Process meat flavor development and the Maillard reaction. In *Thermal Generation of Aromas*, ed. T.H. Parliment, R.J. McGorrin and C-T. Ho. ACS Symposium Series 409. Am. Chem. Soc., Washington, DC, pp. 433–441.

van den Ouweland, G.A.M. and Peer, H.G. 1975. Components contributing to beef flavor. Volatile compounds produced by the reaction of 4-hydroxy-5-methyl-3(2*H*)-furanone and its thio analog with hydrogen sulfide. *J. Agric. Food Chem.* **23**, 501–505.

van der Linde, L.M., van Dort, J.M., de Valois, P., Boelens, H. and de Rijke, D. 1979. Volatile components from thermally degraded thiamine. In *Progress in Flavour Research*, ed. D.G. Land and H.E. Nursten. Applied Science, London, UK, pp. 219–224.

Vernin, G. 1980. Recent progress in food flavours: the role of heterocyclic compounds. *Ind. Aliment. Agric. (Paris)* **97**, 433–449.

Vernin, G. 1982. *Chemistry of Heterocyclic Compounds in Flavours and Aromas*. Ellis Horwood Limited, Chichester, UK.

Wasserman, A.E. and Gray, N. 1965. Meat flavor. I. Fractionation of water-soluble flavor precursors of beef. *J. Food Sci.* **30**, 801–807.

Wasserman, A.E. and Spinelli, A.M. 1972. Effect of some water-soluble components on aroma of heated adipose tissue. *J. Agric. Food Chem.* **20**, 171–174.

Werkhoff, P., Brüning, J., Emberger, R., Güntert, M., Köpsel, M., Kuhn, W. and Surburg, H. 1990. Isolation and characterization of volatile sulfur-containing meat flavor components in model systems. *J. Agric. Food Chem.* **38**, 777–791.

Werkhoff, P., Emberger, R., Güntert, M. and Köpsel, M. 1989. Isolation and characterization of volatile sulfur-containing meat flavor components in model systems. In *Thermal Generation of Aromas*, ed. T.H. Parliment, R.J. McGorrin and C-T. Ho. ACS Symposium Series 409. Am. Chem. Soc., Washington, DC, pp. 460–478.

Whitfield, F.B., Mottram, D.S., Brock, S., Puckey, D.J. and Salter, L.J. 1988. Effect of phospholipid on the formation of volatile heterocyclic compounds in heated aqueous solutions of amino acids and ribose. *J. Sci. Food Agric.* **42**, 261–272.

Wilson, R.A., Katz, I., Vock, M.H. and Shuster, E.J. 1974. Aroma Mixtures. British Patent 1,364,747.

Wong, E., Nixon, L.N. and Johnson, C.B. 1975*a*. Volatile medium chain fatty acids and mutton flavor. *J. Agric. Food Chem.* **23**, 495–498.

Wong, E., Johnson, C.B. and Nixon, L.N. 1975*b*. The contribution of 4-methyloctanoic (hircinoic) acid to mutton and goat meat flavour. *N. Z. J. Agric. Res.* **18**, 261–266.

Young, O.A. and Braggins, T.J. 1998. Sheepmeat odour and flavour. In *Flavor of Meat, Meat Products and Seafoods, Second Edition*, ed. F. Shahidi. Blackie Academic & Professional, London, UK, pp. 101–130.

Zeece, M.G. and Katoh, K. 1989. Cathepsin D and its effects on myofibrillar proteins: A review. *J. Food Biochem.* **13**, 157–178.

CHAPTER 6

MEAT MICROBIOLOGY

Meat is an ideal medium for many organisms to grow because it is high in moisture, rich in nitrogenous compounds (*e.g.*, amino acids, peptides, proteins) and plentifully supplied with minerals and accessory growth factors. Furthermore, it has some fermentable carbohydrates, usually glycogen, and is at a favorable pH for most microorganisms. Consequently, meat and meat products are extremely perishable unless modified or stored in an environment designed to retard microbial activity and reproduction (Frazier and Westhoff 1988; van Laack 1994).

Certain precautionary measures are employed throughout the industry to retard deteriorative changes and to extend the period of acceptability of meat and meat products. Improper sanitation of facilities usually increases the rate and degree of these deteriorative changes which can lead to spoilage and ultimately putrefaction. With the exception of external surfaces (*i.e.*, hide, hair and hooves) and the gastrointestinal and respiratory tracts, tissues of living animals are essentially free of microorganisms (Patterson and Gibbs 1978). Once the animal is slaughtered, however, the internal defense mechanisms that combat infectious agents in the living body are lost. Thus, from the point of exsanguination and at every stage thereafter, measures must be taken to ensure the minimization of microbial contamination and proliferation (Hedrick *et al.* 1994).

On dressed red meat carcasses, the majority of bacteria originate from the hide (Newton *et al.* 1978; Grau 1986). Yet many different types of microorganisms from soil, water, air, feed and manure, as well as natural surface flora and intestinal organisms, can contaminate the carcass during processing operations. Other sources of contaminants can come from floors, walls, contact surfaces, knives, hands and the clothing of workers. Unlike cattle and sheep, the skin of hogs is not usually removed from the carcass; rather, it is scalded. Although scalding reduces the number of microorganisms on the skin, contamination of the carcass can occur during dehairing and evisceration processes (Grau 1986). In general, the numbers and types of microorganisms present in meat and meat products will be influenced by the following: (1) the environmental conditions of the meat processing facility; (2) the microbiological quality of the meat in its raw and unprocessed state; (3) the sanitary conditions under which the meat is handled and processed; and (4) the adequacy of subsequent packaging, handling and storage conditions in maintaining flora at a low level. Rapid chilling of carcasses at low temperatures and relative humidities and at high air velocities

may result in a reduction of bacterial populations, whereas milder chilling conditions may allow the growth of psychrotrophs, increasing the population compared to that of mesophiles (Jackson *et al.* 1997). Of course, proper sanitation minimizes microbial contamination, but if microorganisms are present, their activity seldom can be completely curtailed, no matter what control measures are applied. Thus, the initial level of microbial contamination is an important factor in determining the shelf-life and acceptability of all meat products (Hedrick *et al.* 1994).

Spoilage of Meat and Meat Products

Meat is described as spoiled when organoleptic changes make it unacceptable to the consumer. Factors associated with spoilage include color defects, growth of mold, development of off-flavors, changes in texture, formation of slime or any other characteristics making the meat undesirable for consumption (Jackson *et al.* 1997). It is generally accepted that spoilage occurs when bacterial numbers reach 10^7 CFU/cm^2 on the meat surface, but there remains some disagreement on these numbers relative to quality acceptance (Rahman 1999). During aerobic spoilage, off-odors are first detected when populations reach 10^7 CFU/cm^2, but when numbers reach 10^8 CFU/cm^2, the surface of muscle tissue begins to feel tacky, representing the first stage in slime formation (Ingram and Dainty 1971). The onset of spoilage is subjective and influenced by culture, economic factors, sensory acuity and the intensity of the defect. Yet, despite variation in expectations, most consumers would agree that gross discoloration, strong off-odors, and the development of slime constitute spoilage (Gill 1983). While enzymatic activity changes muscle tissue during storage, organoleptically detectable spoilage is generally a result of decomposition and the formation of metabolites caused by the growth of microorganisms (Jackson *et al.* 1997).

Microorganisms found on or in meat consist of molds, yeasts and bacteria. Under similar conditions, bacteria generally grow faster than yeasts, and yeasts outgrow molds. Type and number of organisms present are important factors contributing to the rate of meat spoilage. Even though meat always contains some level of microorganisms, several properties inherent to specific products and surrounding environments affect markedly the rate, type and degree of spoilage. The initial microflora of muscle foods is highly variable, but the organisms that eventually grow to a level to cause spoilage are those that find existing conditions most favorable. The microflora is altered so that it is dominated by a few, or often a single microbial species, usually of the genus *Pseudomonas, Lactobacillus, Moraxella, Acinetobacter*, or *Brochothrix thermosphacta* (Gill 1986). A more detailed listing of the bacteria genera most often found on fresh and spoiled meats, poultry and seafood is provided in Table 6.1.

TABLE 6.1
GENERA OF BACTERIA MOST FREQUENTLY FOUND ON MEATS, POULTRY AND SEAFOOD

Genus	Gram reaction	Fresh meats	Fresh livers	Processed meats	Vacuum packed meats	Bacon	Poultry	Fish and Seafood
Acinetobacter	–	XX	X	X	X	X	XX	X
Aeromonas	–	XX			X	X	X	X
Alcaligenes	–	X	X			X	X	X
Bacillus	+	X		X		X	X	X
Brochothrix	+	X	X	X	XX			
Campylobacter	–						XX	
Carnobacterium	+	X			XX			
Citrobacter	–	X					X	
Clostridium	+	X					X	
Corynebacterium	+	X	X	X	X	X	XX	X
Enterobacter	–	X		X	X		X	X
Enterococcus	+	XX	X	X	XX	X	X	X
Escherichia	–	X					X	X
Flavobacterium	–	X	X				XX	X
Hafnia	–	X			X			
Kurthia	+	X			X			
Lactococcus	+	X		X				
Lactobacillus	+	X		XX	XX			X
Leuconostoc	+	X	X	X	X			
Listeria	+	X		X			XX	X
Microbacterium	+	X		X	X	X	X	X
Micrococcus	+	X	XX	X	X	X	X	
Moraxella	–	XX	X			X	X	X
Pantoea	–	X					X	
Pediococcus	+	X		X	X			
Proteus	–	X					X	
Pseudomonas	–	XX			X		XX	XX
Psychrobacter	–	XX					X	X
Salmonella	–	X					X	
Serratia	–	X		X	X		X	
Shewanella	–	X						XX
Staphylococcus	+	X	X	X	X	X	X	
Vagococcus	+						XX	
Vibrio	–					X		X
Yersinia	–	X			X			

X = known to occur, XX = most frequently reported. (From Jay 1996).

Parameters that affect microbial growth in meat include intrinsic factors such as moisture content, pH, oxidation-reduction potential, available nutrients and the presence or absence of inhibitory substances. Furthermore, extrinsic factors affect microbial growth and include temperature, relative humidity, presence or absence of oxygen and the physical form of the meat (*i.e.*, whether carcass, wholesale or retail cuts, or comminuted forms). Temperature in particular greatly influences the rate, type and extent of microbial growth. Although molds, yeast and bacteria have some genera which are psychrophiles, few microorganisms can grow at temperatures around 0°C. Meat processors know that temperatures below 5°C greatly retard the proliferation of more prevalent spoilage microorganisms and also prevent growth of nearly all pathogens. Thus, 5°C is the critical temperature during meat handling and storage, and it cannot be exceeded for significant periods without substantial reduction in the quality and appearance of meat. Even with reliable refrigeration, psychrophilic bacteria from the genera *Pseudomonas* spp., *Achromobacter* spp., *Micrococcus* spp., *Lactobacillus* spp., *Streptococcus* spp., *Leuconostoc* spp., *Pediococcus* spp., *Flavobacterium* spp. and *Proteus* spp. can be found on meat. Of these bacteria, *Pseudomonas* spp. are able to compete successfully on aerobically stored refrigerated meat (Kraft 1992). This is because they show a competitive growth rate even at refrigeration temperatures, are able to use low-molecular-weight nitrogen compounds as sources of energy and are able to grow within the usual pH range of muscle foods (5.5 to 7.0). Bacteria from *Moraxella* and *Acinetobacter* spp. are less capable of competing at refrigeration temperatures and at the lower pH range of meat (Jackson *et al.* 1997). Therefore, to keep microorganisms in check (*i.e.*, to retard microbial proliferation), the main objective of the meat processor is to prolong the lag phase as long as possible.

Based on the available oxygen, meat spoilage is classified as either aerobic or anaerobic. All molds that grow on meat are aerobic and yeasts also grow best under such conditions. On the other hand, bacteria found in meat may be aerobic, anaerobic or facultative (*i.e.*, microorganisms that grow either with or without oxygen) organisms. Aerobic conditions exist in meat stored in air, but only on or near the surface because oxygen diffusion through the tissue is limited. Therefore, microbial growth occurring on meat surfaces is largely that of aerobes with some facultative organisms, while the interior portions of meat contain primarily anaerobic and facultative bacteria. The atmosphere surrounding meat affects markedly the composition of microbial populations and their metabolic activity. Use of casings, vacuum packaging, modified atmosphere packaging (*i.e.*, MAP) and sealed containers suppresses or entirely prevents the activity of aerobic microorganisms. Under these conditions, lactic acid bacteria and *Brochothrix thermosphacta* predominate (Jackson *et al.* 1997). Lactic acid

bacteria are favored by their growth rate, their fermentative metabolism and also their ability to grow in the pH range of meat (Egan 1983).

Aerobic spoilage of meat by bacteria and yeasts usually results in slime formation, undesirable flavors and color changes, whereas that by molds generally leads to the formation of "whiskers." Long-aged meat almost always exhibits some mold growth. Furthermore, all types of microorganisms result in lipolysis which enhances oxidative rancidity. Aerobic spoilage is essentially limited to the surface of meat where oxygen is readily available; therefore, the infected areas can be trimmed off. Hence, meat underneath the infected surface generally is acceptable for human consumption. It has been reported that increased tenderness and unique flavors develop in aged meat (e.g., European dry-cured hams). This may be a consequence of microbial enzyme action in addition to that of endogenous enzymes present in the tissue.

Anaerobic spoilage occurs in the interior of meat, vacuum packaged products, or sealed containers where oxygen is either absent or present in limited quantities. This type of spoilage is caused by facultative and anaerobic bacteria and is usually described by the terms taint, souring and putrefaction. Taint is a nonspecific term used to describe any off-taste or off-odor, while souring results mainly from the build-up of short-chain fatty acids during bacterial enzymatic degradation of complex molecules (Sutherland *et al.* 1976). Souring of cured meat products such as frankfurters, bologna, sausages and luncheon meats generally results from growth of lactobacilli, enterococci and related organisms. The souring comes from utilization of lactose (*i.e.*, in milk solids) and other sugars by the organisms with the production of acids (Jay 1996). Proteolysis without putrefaction may also contribute to souring and is sometimes referred to as "stinking sour fermentation." Cured hams undergo a type of spoilage different from that of fresh or smoked hams. This is due primarily to the fact that curing solutions pumped into the hams contain sugars that are fermentable by the natural flora of the ham and also by those pumped into the product in the curing solution, such as lactic acid bacteria. This premature spoilage is generally evident from a milkiness in the purge of vacuum-packaged sliced ham. Although cured, most hams are at more risk of spoilage than other types of processed meat products because they contain considerably higher concentrations of carbohydrates, *ca.* 2-7%, usually in the form of dextrose and corn syrup solids (Bautista *et al.* 2000).

The meat industry offers various forms of meat preservation, but is restricted with respect to the choice of preservatives and bactericidal agents. A number of ingredients commonly added to meat products during processing impart preservative effects, but to varying degrees. For example, salt provides a limited preservative action against microorganisms by lowering water activity; nitrite has bacteriostatic properties and when added at reduced levels, it functions synergistically with salt to give certain cured meat products effective

preservation; sugar added to fermented sausages indirectly serves as a preservative due to the lactic acid formed by starter cultures which results in lower pHs; and various constituents of wood smoke impart bacteriostatic and bactericidal effects (Hedrick *et al.* 1994).

Clostridium botulinum in Meat

No meat product is completely sterile and a potential always exists for deteriorative changes and spoilage, as well as food infections and poisoning. Food intoxication is defined as an illness caused by ingestion of toxins, whereas food infection is defined as the consumption of pathogenic organisms that proliferate and cause illness in the host (Hedrick *et al.* 1994). In compromised meat products, illnesses which result from ingestion are usually of bacterial origin. The bacterial toxins themselves are relatively odorless and tasteless and thus are readily consumed by unsuspecting victims. Of particular concern in vacuum-packaged meat products is the toxin produced from the bacterium *Clostridium botulinum*. Spores from *C. botulinum* are widely distributed throughout nature and they may find their way into processed meat through raw food materials or by contamination of the meat after processing. Given the right conditions, the spores can develop and grow. Unless processors and consumers take preventive measures to eliminate *C. botulinum* or to inhibit growth and toxin production by this organism, botulism outbreaks will occur.

The causative organism was first isolated in 1896 by van Ermengen from a salted ham that was involved in several deaths in humans (Pierson and Reddy 1988). The isolated organism was a Gram-positive, spore-forming, gas-forming, anaerobic bacillus which produced a heat-labile toxin that was lethal to a variety of animals. van Ermengen named the organism *Bacillus botulinus,* but in 1923 it was renamed *Clostridium botulinum*. To date, there are seven recognized serotypes of *C. botulinum* (A, B, C, D, E, F and G), but only serotypes A, B, E and F are involved in human botulism (Pierson and Reddy 1988). There are marked differences among the strains in their tolerance to sodium chloride and water activity, minimum growth temperature, proteolytic activity, and in the heat resistance of their spores. Types A and B are of most concern to the food processor since both form extremely heat-resistant spores.

Foodborne botulism results from consumption of food in which *C. botulinum* has grown and produced toxin. The botulinal neurotoxins are proteins that are produced intracellularly as protoxins. They are liberated when the botulinum vegetative cell lyses, and are activated to the maximum toxic state by proteolytic enzymes. Serotype A toxin is more lethal than strains B and E. The toxin is absorbed and bound irreversibly to peripheral nerve endings. Signs and symptoms of botulism poisoning include nausea, vomiting, fatigue, dizziness, headache, dryness of skin, mouth and throat, constipation, paralysis of muscles,

double vision, and difficulty in breathing; these may develop within 12-72 hours after consumption of the toxin-containing food. Treatment of the poisoning includes administration of botulinal antitoxin and appropriate supportive care, particularly respiratory assistance. Recovery may take several weeks to a month if death does not occur, but today the mortality rate is less than 10% (Pierson and Reddy 1988). Some characteristics of botulism, as well as those of other common food poisonings and infections are presented in Table 6.2.

Conditions that favor growth and toxin production by *C. botulinum* include a relatively high-moisture, low-salt, low-acid (*i.e.*, pH > 4.6) food that is stored anaerobically and at temperatures in excess of 3.3°C. Unfortunately, meat provides an adequate medium with nutrients for the growth of *C. botulinum* and toxin production. The low incidence of *C. botulinum* in cured meats has been largely attributed to the use of nitrite as a curing ingredient. Many studies have been published on the efficacy of sodium nitrite in inhibiting *C. botulinum* growth and toxin production in perishable cured meats such as wieners, bacon, canned ham, luncheon meat, and canned comminuted meat. Safety cannot be totally attributed to nitrite alone, but rather to a variety of factors, such as heat treatment, acidity (pH), salt and bacterial spore levels. Other curing adjuncts, such as ascorbic acid and sodium erythorbate, have been reported to influence the efficacy of nitrite.

Bacteriostatic Properties of Nitrite

Cured and smoked meat as well as most types of processed sausage either contain antimicrobial ingredients or are treated in such a manner that restricts microbial growth and activity. In the case of cured meat specifically, nitrite's role as an antimicrobial agent against botulinal toxin production has received considerable attention. Tompkin (1993) has published an elegant review of the discovery of nitrite as an antibotulinal agent in meat and meat products from a chronological perspective. In this manner, insight is provided into why the research was performed, the selection of the experimental procedure and the information that was available to influence the researchers' conclusions. Tompkin (1993) noted that many unanswered questions still remain as to what the significant factors are that influence the efficacy of nitrite, or for that matter, the value of nitrate. Aspects of his review have been incorporated into this work.

It was during the 1920s that investigation of the antibacterial properties of nitrite and nitrate commenced. It was first observed by MacNeal and Kerr (1929) that nitrate exerted no special restrictive effect on bacterial activity in neutral or alkaline solutions, but in acid solutions, a marked inhibition was evident. This effect was incomparably greater than that of salt and these authors attributed it to the production of small amounts of nitric and nitrous acids in

TABLE 6.2
CHARACTERISTICS OF SOME COMMON FOOD POISONINGS AND INFECTIONS

Illness	Causative agent	Symptoms	Time	Foods usually involved	Preventive measures
Botulism (food poisoning)	Toxins produced by *Clostridium botulinum*.	Impaired swallowing, speaking, respiration, coordination. Dizziness and double vision.	12-48 h	Canned low-acid foods including canned meat and seafood, smoked and processed fish.	Proper canning, smoking and processing procedures. Cooking to destroy toxins, proper refrigeration and sanitation.
Staphyloccoccal (food poisoning)	Enterotoxin produced by *Staphylococcus aureus*.	Nausea, vomiting, abdominal cramps due to gastroenteritis (*i.e.*, inflammation of the lining of the stomach and intestines).	3-6 h	Custard and cream-filled pastries, potato salad, dairy products, cooked ham, tongue and poultry.	Pasteurization of susceptible foods, proper refrigeration and sanitation.
Clostridium perfringens (food poisoning)	Toxin produced by *Clostridium perfringens* (live cell intoxication).	Nausea, occasional vomiting, diarrhea and abdominal pain and flatulence.	8-24 h	Cooked meat, poultry and fish held at non-refrigerated temperatures for long periods of time.	Prompt refrigeration of unconsumed cooked meat, poultry or fish; maintain proper refrigeration and sanitation.
Salmonellosis (food infection)	Infection produced by ingestion of any of more than 1200 species of *Salmonella* that can grow in the human gastrointestinal tract.	Nausea, vomiting, diarrhea, fever, abdominal pain; may be preceded by chills and headache.	6-24 h	Insufficiently cooked or warmed-over meat, poultry, eggs and dairy products; products are quite susceptible when not refrigerated for a long time.	Cleanliness and sanitation of handlers and equipment; pasteurization, proper refrigeration and packaging.
Trichinosis (infection)	*Trichinella spiralis* (nematode worm) found in pork.	Nausea, vomiting, diarrhea, profuse sweating, fever and muscle soreness.	2-28 d	Insufficiently cooked pork and products containing pork.	Thorough cooking of pork (to an internal temperature of 59°C or higher); freezing and storage of uncooked pork at -15°C or lower for a minimum 20 days; avoid feeding pigs raw garbage.
Campylobacter (food infection)	*Campylobacter jejuni* and *Campylobacter coli*.	Headache, fever, abdominal pains and diarrhea.	1-7 d	Insufficiently cooked poultry and meat products, unpasteurized milk and dairy products.	Cleanliness and sanitation of handlers and equipment, pasteurization and proper cooking.

(From Hedrick *et al.* 1994).

mixtures containing reducing substances. Yet, the fundamental importance of pH as it relates to nitrite and nitrate chemistry was not greatly pursued over the next two decades. Subsequent research by investigators such as Tanner and Evans (1933; 1934) and Jensen *et al.* (1934) gave varied results concerning the importance of, or lack of, nitrite and nitrate mixtures in meat products as preservatives. Tarr (1941; 1942) reported the bacteriostatic action of sodium nitrite at a concentration of 200 mg/kg in fish muscle against *Achromobacter*, *Aerobacter*, *Escherichia*, *Micrococcus* and *Pseudomonas*. Subsequent tests (Tarr 1941) demonstrated the importance of pH to the efficacy of nitrite. For example, at pH 7.0 little or no inhibition was observed, unlike that at pH 5.7 and 6.0 where complete or strong microbial inhibition occurred. Tarr (1942) further documented the effect of pH in a series of tests in nutrient broth inoculated with a variety of aerobes as well as *C. botulinum* and *C. sporogenes*. At the same time, researchers such as Jensen and Hess (1941) were promoting the virtues of nitrate and perpetuated the belief that the sole function of nitrite was for development of cured meat color. By the beginning of the 1950s, (1) the relative roles of nitrate and nitrite as preservatives in cured meats were unclear. Strong opinions were expressed that nitrite alone was ineffective at the levels used in commercial practice; (2) nitrate was promoted as an inhibitor of anaerobic spoilage and to enhance the swelling of perishable canned cured meat by *Bacillus* spp. when temperature abused; (3) nitrite was clearly shown to be an effective antimicrobial agent, especially if the product pH was below 7.0; (4) the disappearance of nitrite during processing and storage was assumed to make it an unreliable preservative; (5) salt was considered the cornerstone for the preservation of cured meats based on the levels used during this period; (6) the concept of a combined effect of heating in the presence of curing salts was proposed. Although thermal destruction was not shown to be enhanced by the presence of curing salts, there was increased inhibition of outgrowth of surviving spores by salt and, to some degree, by nitrite.

During the 1950s, the relative significance of salt, nitrite and nitrate as preservatives was somewhat clarified. Steinke and Foster (1951) demonstrated the antibotulinal effects of nitrite in a processed meat product that had been packaged with an oxygen-impermeable film. They reported the development of botulinal toxin after 9-23 days in liver sausages that had been incubated at 30°C and which contained 2.5% salt, 0.1% sodium nitrate and an inoculum of 5000 spores/g. The inhibitory period was extended markedly by a slight increase in salt concentration, a decrease in the incubation temperature, or a decrease in the spore inoculum level. Adding 200 mg/kg of sodium nitrite to the sausage was considerably more inhibitory to toxin formation than the addition of 1000 mg/kg sodium nitrate. Of course, the initial contamination level or incidence of *C. botulinum* spores in fresh meat to be cured can greatly affect the control of *C. botulinum* growth and toxin formation in the finished product. In general, it is

considered that the incidence of *C. botulinum* spores is low in fresh meat, but some concern has been shown with respect to spore incidence in bacon products following studies such as those by Roberts and Smart (1976).

Bulman and Ayres (1952) found that levels of salt or sodium nitrate in excess of 4.4% and 4.0%, respectively, were required to prevent spoilage from anaerobic spore formers in pork. In the absence of salt, an increased inhibitory effect against PA 3679 was noted as sodium nitrite levels went up from 400 to 800 mg/kg. Most interesting was that a cure containing salt and sodium nitrite at 3.5% and 150-170 mg/kg, respectively, was much more effective than that containing either compound alone. The same was not true when nitrate was used at 1250 mg/kg in the presence of either salt or nitrite. However, a mixed cure of the three yielded the maximum degree of inhibition. These investigators pondered whether the growth of spoilage organisms was influenced by the depletion of nitrite to noninhibitory levels.

A key study by Silliker *et al.* (1958) on shelf-stable canned cured luncheon meat and a subsequent review (Silliker 1959) helped to establish the future direction for nitrite research. Although shelf-stable canned cured luncheon meats had been produced for years, the reasons for their stability were still not clearly defined. By using this class of product, Silliker *et al.* (1958) demonstrated that nitrate played absolutely no role in retarding putrid spoilage. In fact, it actively stimulated spoilage by aerobic spore formers. Addition of salt alone was not responsible for the stability of the product. It was suggested that the key to stability was the addition of sodium nitrite at a 78 mg/kg level and heat injury to the small number of indigenous spores. Silliker *et al.* (1958) concluded that the stability of shelf-stable cured meat given less than a botulinal cooking was due to the combined effect of nitrite, salt, thermal injury to the spores and a low indigenous spore level. As viable spores could be recovered from stable commercial products, the primary effect of nitrite was to prevent germination and/or outgrowth of heat-injured spores. By the end of the decade, investigations were suggesting that (1) nitrate had no antimicrobial effect, other than its possible influence on water activity; (2) nitrate could serve as an electron acceptor, permitting the growth of aerobes, such as micrococci and bacilli. Several reports of unstable commercially canned cured meats containing nitrate appeared (*e.g.*, Eddy and Ingram 1956); (3) although nitrite was recognized as an effective antimicrobial agent, its value as a preservative in perishable cured meats was still in doubt; (4) nitrite was shown to play a significant role in the stability of shelf-stable canned meat. The system providing stability consisted of the combined effect of nitrite, salt and thermal injury to the low indigenous level of anaerobic spores. The suspected role of nitrite was to prevent germination or outgrowth of surviving heat-injured spores; and (5) brine content was shown to influence botulinal outgrowth and toxin formation. At brine levels approaching the inhibitory level, toxin was produced without obvious organoleptic spoilage.

During the 1960s, an increased emphasis was placed on understanding the role of nitrite in the total inhibitory system in cured meats and its effect on thermally injured spores. This was especially important as the increased use of vacuum packaging raised new questions regarding the microbiological safety of cured meats. Research in this area was fueled by outbreaks of botulism arising from temperature-abused vacuum-packed smoked fish products. Pivnick and Bird (1965) found that the oxygen permeability of packaging films influences spoilage, but not toxinogenesis of sliced cured meats inoculated with *C. botulinum*. Particularly significant were their findings that the type of cured meat influenced toxin production. For example, bologna was relatively resistant to type A toxin production, unlike that of ham. Cooked sliced ham from five producers inoculated with type E spores yielded variable results at 30°C, which was thought to be due to product differences in brine and residual nitrite levels. However, on sliced bologna, type E toxin was not produced at 30°C. The combination of brine (3.75%), residual nitrite, a fermentable carbohydrate and decreasing pH during temperature abuse was attributed as inhibitory factors. Similar results were obtained for other cured products. In a later study, Pivnick *et al.* (1967) reported that at any given temperature, delay in spoilage and toxin production was directly related to the concentration of nitrite in the product and that heat injury of spores was not necessary for sodium nitrite to inhibit toxinogenesis.

Shank *et al.* (1962) examined whether nitrite or nitrogen oxides were involved in the inhibition of Gram-negative bacteria in cured meats. These investigators concluded that nitrite and nitrate have either neutral or stimulatory effects on bacteria and that nitric oxide has virtually no effect. Nitrous acid was considered to be primarily responsible for both the qualitative and quantitative changes that occurred in the bacterial flora when meat was cured. It was postulated that nitrous acid reacts either with the cell itself or with various constituents of the medium, thereby making them unavailable for subsequent metabolism.

Perigo *et al.* (1967) offered a new possibility for the stability of shelf-stable canned cured meat. These researchers found that nitrite heated in bacteriological media was more inhibitory toward vegetative cells of PA 3679 than nitrite added aseptically after the medium had been autoclaved. This effect was found to occur in the temperature range of 95 to 125°C at pH values greater than 6.0. At pH 6.0, heating in the range of 100 to 110°C enhanced this inhibitory effect tenfold or greater. Perigo *et al.* (1967) postulated that an unknown substance might have formed from nitrite that disappeared during thermal processing. Thus, in the case of shelf-stable canned cured meat, the level of residual nitrite remaining after processing may be an inappropriate measurement of the inhibitory capacity of the product. Since the formation of the inhibitor required substantial heating, the researchers reasoned that the inhibitor might play a complementary role in

the stability of shelf-stable, but not perishable, canned cured meats. This inhibitory substance has since become known as the Perigo factor or Perigo-type factor and considerable effort was applied toward isolating and characterizing it (Pivnick and Chang 1974). Perigo and Roberts (1968) confirmed these findings and showed the enhanced inhibitory effect of nitrite against vegetative cells of 30 clostridia strains including 14 strains of *C. botulinum* serotypes A, B, E and F in laboratory media after heat treatment. Studies by Roberts (1975) also indicated that a reducing agent and protein source were necessary components of the laboratory media in order to observe the Perigo effect. Roussin's salt, nitrosocysteine and cysteine-NO-Fe complexes have been suggested as being responsible for the Perigo factor inhibition. However, the Perigo factor is neutralized by meat particles (Johnston *et al.* 1969) and meat does not reach 105°C during thermal processing, which is the minimum temperature reported for Perigo inhibitor formation. Evidence strongly suggests that while there is an inhibitor formed in meat following the addition of nitrite, this inhibitor, which is not nitrite itself, is significantly different from the Perigo inhibitor formed in laboratory media (Lee *et al.* 1978; Holley 1981).

Although the inhibition of *C. botulinum* spores and those of other clostridia species by nitrite has been extensively studied, the exact mechanism by which nitrite exerts this action remains unanswered. Johnston *et al.* (1969) have suggested that nitrite's mode of action in cured meat might be an enhanced destruction of spores by heat, an increased germination of spores during the heat treatment followed by thermal destruction of the germinated spores, an inhibited germination and outgrowth of spores surviving the heat process, or a reaction with some components in the meat system to produce a more inhibitory compound. Progress made during the 1960s toward defining the role of nitrite in cured meats showed that (1) the antimicrobial effect of nitrite is related to the formation of nitrous acid; (2) a number of investigators reached the same general conclusions about the shelf stability of canned cured meats given less than a botulinal cooking as that set forth by Silliker *et al.* (1958); (3) the concept of thermal injury to spores that survive the processing of shelf-stable canned cured meats was valid; (4) the process of outgrowth was more sensitive than spore germination to nitrite; (5) an inhibitory substance was formed from nitrite which disappeared during thermal process; (6) an early test on the effect of vacuum packaging on the safety of perishable cured meat led to the recommendation that they should be frozen to prevent the development of microbial health hazards; (7) certain cured meats are more prone to supporting botulinal growth. Factors influencing growth included brine level, pH, residual nitrite, storage temperature and inoculum level; (8) the significance of decreasing pH on botulinal inhibition in cooked cured meats with the presence of sufficient fermentable carbohydrate; and (9) the growth of enterococci in perishable canned hams can inhibit several clostridial species.

During the 1970s and 80s, the discovery of *N*-nitrosamines in cured meats became an issue of great concern. Throughout this period, pressure mounted to reduce or eliminate nitrate and/or nitrite addition to meat products. Research continued to examine the Perigo factor, nitrite's role in perishable canned cured meats and to unravel the mechanism of nitrite's inhibitory efficacy against botulinal toxin formation. It was recognized that the safety of cured meats such as wieners, bacon, canned ham and luncheon meat cannot be totally attributed to nitrite alone. A variety of factors such as heat treatment, acidity, reductants, salt concentration, storage temperature, bacterial spore level and their interactions with nitrite provide the safety from botulism afforded cured meat. Thermal processes used on cured meat are sufficient to inactivate vegetative bacterial cells, with the occasional exception of the relatively heat-resistant enterococci (Roberts 1975). Processors are most concerned, however, with residual *C. botulinum* spores in cured meat after thermal processing, and the effect of nitrite on outgrowth of bacteria when the meat is subjected to temperature abuse. If refrigerated storage could be assured, then there would be no potential botulinal hazard in bacon, sausage, wieners and luncheon meat products.

By the 1970s, it was generally accepted that the inhibitory efficacy of nitrite on *C. botulinum* growth and toxin production increases with the concentration of nitrite. Numerous studies utilizing inoculum levels of *C. botulinum* spores in a variety of cured meat products have demonstrated that as the spore concentration increases, the inhibitory effects of nitrite and other curing adjuncts can eventually be overcome, thus allowing *C. botulinum* growth and toxin production. Ham, bacon, dry sausage, frankfurters and other cured meats differ from one another in many respects (*e.g.*, formulation, processing techniques, packaging, and manner of marketing). For this reason, the determination of nitrite and nitrate levels needed to control botulinal hazard must be made individually for each class of product (Christiansen *et al.* 1973).

Christiansen *et al.* (1973) studied the relationship between nitrite concentration and spore levels added to perishable canned hams (containing 2.2% NaCl). Nitrite concentrations from 0 to 500 mg/kg were added to the cured pork while a mixture of spores of five type A and five type B strains of *C. botulinum* were used to inoculate the meat at either a 100 or 10,000 spores/g of meat level. In temperature-abused samples (27°C), Christiansen *et al.* (1973) found that both the rate of toxin production and number of toxic cans were dependent upon the level of nitrite initially added to the meat. Furthermore, the level of nitrite necessary to inhibit toxin production was dependent upon the spore inoculum level. Nontoxic spoilage occurred at 7°C in uninoculated product formulated without nitrite. At the low spore inoculum level, toxin was confirmed in the product with up to 150 mg/kg of nitrite while at the higher level, toxin was confirmed up to 400 mg/kg of nitrite. However, only 8 of 280 samples with

nitrite levels of 200 mg/kg or greater were botulinogenic at the high spore inoculum concentration. Using multiple linear regression analysis, Christiansen *et al.* (1973) prepared two probability curves which confirmed the observations that as the level of nitrite increased, the probability of botulinal toxin production decreased and at a given nitrite concentration, the probability of toxin production was greater at the high spore inoculum level than at the low one. Statistical analysis of residual nitrite levels of each sample during storage revealed that botulinal toxicity could more accurately be predicted from the nitrite level added at the time of formulation rather than the residual nitrite level. However, Christiansen (1980) postulated that the nitrite level present at the time of temperature abuse is an important factor in determining outgrowth of *C. botulinum*. Because nitrite levels decrease in meat during storage, bacterial growth occurs only when there is an insufficient concentration of nitrite to check outgrowth. In other words, the extent of nitrite inhibition of *C. botulinum* can be explained as a race between nitrite depletion and death of germinated clostridial spores (Christiansen *et al.* 1978). This agreed with a study by Tompkin *et al.* (1978*a*) involving a product held at 4.4 or 10°C for up to half a year before placing it at 27°C. The longer the product was held at refrigeration temperature, the more the residual nitrite level declined and the less inhibitory the product became when subjected to temperature abuse at 27°C. Factors such as pH and ascorbate concentration affect inhibition because they influence the rate of nitrite depletion (Tompkin *et al.* 1978*b*). Chelating agents such as ascorbate, ethylenediaminetetraacetic acid and cysteine can also enhance the efficacy of nitrite by sequestering ferrous and ferric ions (Tompkin *et al.* 1978*c*; 1979*a,b*). This made it possible to explain the differences in the relative efficacy of nitrite in various meats on the basis of their iron content; however, beef liver, despite its relatively high iron content (49 μg/g), did not cause a substantial loss in the antibotulinal efficacy of nitrite.

Tompkin *et al.* (1978*b*) showed that erythorbate, ascorbate and cysteine enhanced the anticlostridial efficacy of nitrite in cured meat by sequestering metal ions in the meat rather than by an antioxidative or reductive mechanism. It was proposed that an essential metabolic step involving a cation is blocked by the reaction of nitric oxide within the germinated botulinal cell. These authors suggested that ferredoxin, an iron-sulfur protein cofactor for electron transport and energy production in clostridia, might be one such compound, and that uptake of undissociated nitrous acid or a nitrogen oxide (*e.g.*, NO) interferes with energy metabolism in the vegetative cell to prevent outgrowth. Subsequent outgrowth could occur when (1) residual nitrite depletes to noninhibitory levels, allowing the dissociation of the nitrogen oxide from the iron, as can occur with nondenatured (*i.e.*, nonheated) heme compounds; (2) repair through the formation of new unreacted ferredoxin by the vegetative cell occurs; and/or (3)

residual nitrite depletes to noninhibitory levels, allowing the germination of dormant spores.

Benedict (1980), in his review of the biochemical basis for nitrite's inhibition of *C. botulinum*, reported that in cured meat it was most likely due to several interacting factors, namely (1) reaction and oxidation of cellular biochemicals within the spores and vegetative cells; (2) restriction of the use of iron, or other essential metal ions, through inhibition of solubilization, transport or assimilation, thereby interfering with metabolism and repair mechanisms; and (3) cell surface membrane activity limiting substrate transport by the outgrowing cell. Yarbrough *et al.* (1980) proposed that nitrite has several sites of attack in the bacterial cell. First, it interferes with energy conservation by inhibiting oxygen uptake, oxidative phosphorylation and proton-dependent active transport. Second, nitrite acts as an uncoupler, causing a collapse of the proton gradient. Third, certain metabolic enzymes such as aldolase are inhibited. In the end, evidence suggests that nitrite delays, but does not entirely prevent, clostridial outgrowth. Although it does not inhibit spore germination, nitrite's inhibitory effect can be seen upon emergence of the vegetative cell from the spore during cell division (Tompkin 1978; Genigeorgis and Riemann 1979; Sofos *et al.* 1979). Inhibition of energy-dependent transport systems within the cell results from the presence of the anion, but growth inhibition is believed to be caused by undissociated nitrous acid (Freese *et al.* 1973).

Reddy *et al.* (1983) employed electron paramagnetic resonance (EPR) spectroscopy to examine the spectra of botulinal cells treated with nitrite and a reducing agent. EPR spectroscopy demonstrated the presence of iron-sulfur proteins within botulinal cells. Signals were observed at $g = 2.02$ in washed, sonicated cells and at $g = 1.94$ in cell preparations reduced with sodium dithionite. As a single iron-sulfur protein is not capable of producing signals at both g values, these results were indicative of at least two different iron-sulfur proteins. Treatment of the cells with 200 mg/kg sodium nitrite at 35°C for 45 min resulted in the appearance of a signal at $g = 2.035$, characteristic of iron-nitrosyl complexes, and disappearance of the signal at $g = 1.94$. Reddy *et al.* (1983) concluded that nitric oxide inactivation of iron-sulfur proteins was the probable mechanism of botulinal inhibition in nitrite-cured meats. Their results support the theory that nitrite is inhibitory to *C. botulinum* by inactivating iron-sulfur enzymes, such as ferredoxin, that are essential for growth.

REFERENCES

Bautista, D.A., Pegg, R.B. and Shand, P.J. 2000. Effect of L-glucose and D-tagatose on bacterial growth in media and a cooked cured ham product. *J. Food Prot.* **63**, 71-77.

Benedict, R.C. 1980. Biochemical basis for nitrite-inhibition of *Clostridium botulinum* in cured meat. *J. Food Prot.* **43**, 877-891.

Bulman, C. and Ayres, J.C. 1952. Preservative effect of various concentrations of curing salts in comminuted pork. *Food Technol.* **6**, 255-259.

Christiansen, L.N. 1980. Factors influencing botulinal inhibition by nitrite. *Food Technol.* **34**(5), 237-239.

Christiansen, L.N., Johnston, R.W., Kautter, D.A., Howard, J.W. and Aunan, W.J. 1973. Effect of nitrite and nitrate on toxin production by *Clostridium botulinum* and on nitrosamine formation in perishable canned comminuted cured meat. *Appl. Microbiol.* **25**, 357-362.

Christiansen, L.N., Tompkin, R.B. and Shaparis, A.B. 1978. Fate of *Clostridium botulinum* in perishable canned cured meat at abuse temperature. *J. Food Prot.* **41**, 354-355.

Eddy, B.P. and Ingram, M. 1956. A salt-tolerant denitrifying *Bacillus* strain which 'blows' canned bacon. *J. Appl. Bacteriol.* **19**, 62-70.

Egan, A.F. 1983. Lactic acid bacteria of meat and meat products. *Antonie Leeuwenhoek J. Microbiol.* **49**, 327-336.

Frazier, W.C. and Westhoff, D.C. 1988. Contamination, preservation, and spoilage of meats and meat products. In *Food Microbiology, Fourth Edition*. McGraw-Hill, New York, pp. 218-242.

Freese, E., Sheu, C.W. and Galliers, E. 1973. Function of lipophilic acids as antimicrobial food additives. *Nature* **241**, 321-325.

Genigeorgis, C. and Riemann, H. 1979. Food processing and hygiene. In *Food-Borne Infections and Intoxications, Second Edition*, ed. H. Riemann and F.L. Bryan. Academic Press, San Diego, pp. 613-713.

Gill, C.O. 1983. Meat spoilage and evaluation of the potential storage life of fresh meat. *J. Food Prot.* **46**, 444-452.

Gill, C.O. 1986. The control of microbial spoilage in fresh meats. In *Advances in Meat Research, Volume 2. Meat and Poultry Microbiology*, ed. A.M. Pearson and T.R. Dutson. AVI Publishing Co., Westport, Conn., pp. 49-88.

Grau, F.H. 1986. Microbial ecology of meat and poultry. In *Advances in Meat Research, Volume 2. Meat and Poultry Microbiology*, ed. A.M Pearson and T.R. Dutson. AVI Publishing Co., Westport, Conn., pp. 1-47.

Hedrick, H.B., Aberle, E.D., Forrest, J.C., Judge, M.D. and Merkel, R.A. 1994. Principles of Meat Science, Third Ed. Kendall/Hunt Publishing Company, Dubuque, IA.

Holley, R.A. 1981. Review of the potential hazard from botulism in cured meats. *Can. Inst. Food Sci. Technol. J.* **14**, 183-195.

Ingram, M. and Dainty, R.H. 1971. Changes caused by microbes in spoilage of meats. *J. Appl. Bacteriol.* **34**, 21-39.

Jackson, T.C., Acuff, G.R. and Dickson, J.S. 1997. Meat, poultry, and seafood. In *Food Microbiology. Fundamentals and Frontiers*, ed. M.P. Doyle, L.R. Beuchat and T.J Montville. ASM Press, Washington, DC, pp. 83–100.

Jay, J.M. 1996. Fresh meats and poultry and processed meats and poultry. In *Modern Food Microbiology, Fifth Edition*. Chapman & Hall, New York, pp. 69–117.

Jensen, L.B. and Hess, W.R. 1941. A study of the effects of sodium nitrate on bacteria in meat. *Food Manuf.* **16**, 157–160.

Jensen, L.B., Wood, I.H. and Jansen, C.E. 1934. Swelling in canned chopped hams. *Ind. Eng. Chem.* **26**, 1118–1120.

Johnston, M.A., Pivnick, H. and Samson, J.M. 1969. Inhibition of *Clostridium botulinum* by sodium nitrite in a bacteriological medium and in meat. *Can. Inst. Food Sci. Technol. J.* **2**, 52–55.

Kraft, A.A. 1992. *Psychrotrophic Bacteria in Foods: Disease and Spoilage*. CRC Press, Boca Raton, FL.

Lee, S.H., Cassens, R.G. and Sugiyama, H. 1978. Factors affecting inhibition of *Clostridium botulinum* in cured meats. *J. Food Sci.* **43**, 1371–1374.

MacNeal, W.J. and Kerr, J.E. 1929. The influence of potassium nitrate on the action of bacteria and enzymes. In *Studies in Nutrition. An Investigation of the Influence of Saltpeter on the Nutrition and Health of Man with Reference to its Occurrence in Cured Meats. Volume II. Discussion and Interpretation of the Data Relating to the Health and Physical Condition of the Men*, ed. W. J. MacNeal with the assistance of J.E. Kerr and W.S. Chapin. Univ. of Illinois Press, Urbana, Ill., pp. 358–389.

Newton, K.G., Harrison, J.C.L. and Wauters, A.M. 1978. Sources of psychrotrophic bacteria on meat at the abattoir. *J. Appl. Bacteriol.* **45**, 75–82.

Patterson, J.T. and Gibbs, P.A. 1978. Sources and properties of some organisms isolated in two abattoirs. *Meat Sci.* **2**, 263–273.

Perigo, J.A. and Roberts, T.A. 1968. Inhibition of clostridia by nitrite. *J. Food Technol.* **3**, 91–94.

Perigo, J.A., Whiting, E. and Bashford, T.E. 1967. Observations on the inhibition of vegetative cells of *Clostridium sporogenes* by nitrite which has been autoclaved in a laboratory medium, discussed in the context of sub-lethally processed cured meats. *J. Food Technol.* **2**, 377–397.

Pierson, M.D. and Reddy, N.R. 1988. *Clostridium botulinum*. *Food Technol.* **42**(4), 196–198.

Pivnick, H. and Bird, H. 1965. Toxinogenesis by *Clostridium botulinum* types A and E in perishable cooked meats vacuum-packed in plastic pouches. *Food Technol.* **19**, 1156–1164.

Pivnick, H. and Chang, P-C. 1974. Perigo effect in pork. In *Proceedings of the International Symposium on Nitrite in Meat Products*, Zeist, The Netherlands, September 10-14, 1973, ed. B. Krol and B.J. Tinbergen. Wageningen Centre for Agricultural Publishing and Documentation, Wageningen, The Netherlands, pp. 111-116.

Pivnick, H., Rubin, L.J., Barnett, H.W., Nordin, H.R., Ferguson, P.A. and Perrin, C.H. 1967. Effect of sodium nitrite and temperature on toxinogenesis by *Clostridium botulinum* in perishable cooked meats vacuum-packed in air-impermeable plastic pouches. *Food Technol.* **21**(2), 100-102.

Reddy, D., Lancaster, J.R. Jr. and Cornforth, D.P. 1983. Nitrite inhibition of *Clostridium botulinum*: Electron spin resonance detection of iron-nitric oxide complexes. *Science* **221**, 769-770.

Rahman, M.S. 1999. Postharvest handling of foods of animal origin. In *Handbook of Food Preservation*, ed. M.S. Rahman. Marcel Dekker, New York, pp. 47-73.

Roberts, T.A. 1975. The microbiological role of nitrite and nitrate. *J. Sci. Food Agric.* **26**, 1755-1760.

Roberts, T.A. and Smart, J.L. 1976. The occurrence and growth of *Clostridium* spp. in vacuum-packed bacon with particular reference to *Cl. perfringens* (*welchii*) and *Cl. botulinum*. *J. Food Technol.* **11**, 229-244.

Shank, J.L., Silliker, J.H. and Harper, R.H. 1962. The effect of nitric oxide on bacteria. *Appl. Microbiol.* **10**, 185-189.

Silliker, J.H. 1959. The effect of curing salts on bacterial spores. In *Proceedings of the Meat Industry Research Conference*. American Meat Institute Foundation, Arlington, VA, pp. 51-60.

Silliker, J.H., Greenberg, R.A. and Schack, W.R. 1958. Effect of individual curing ingredients on the shelf stability of canned comminuted meats. *Food Technol.* **12**, 551-554.

Sofos, J.N., Busta, F.F. and Allen, C.E. 1979. Sodium nitrite and sorbic acid effects on *Clostridium botulinum* spore germination and total microbial growth in chicken frankfurter emulsions during temperature abuse. *Appl. Environ. Microbiol.* **37**, 1103-1109.

Steinke, P.K.W. and Foster, E.M. 1951. Botulinum toxin formation in liver sausage. *Food Res.* **16**, 477-484.

Sutherland, J.P., Gibbs, P.A., Patterson, J.T. and Murray, J.G. 1976. Biochemical changes in vacuum packaged beef occurring during storage at 0-2°C. *J. Food Technol.* **11**, 171-180.

Tanner, F.W. and Evans, F.L. 1933. Effect of meat curing solutions on anaerobic bacteria. I. Sodium chloride. *Zentrabl. Bakteriol. Parasitenkd Infektionskr. Hyg. Abt 2.* **88**, 44-54.

Tanner, F.W. and Evans, F.L. 1934. Effect of meat curing solutions on anaerobic bacteria. III. Sodium nitrite. *Zentrabl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 2.* **91**, 1-14.

Tarr, H.L.A. 1941. The action of nitrites on bacteria. *J. Fish Res. Bd. Can.* **5**, 265-275.

Tarr, H.L.A. 1942. The action of nitrites on bacteria: Further experiments. *J. Fish Res. Bd. Can.* **6**, 74-89.

Tompkin, R.B. 1978. The role and mechanism of the inhibition of *C. botulinum* by nitrite — Is a replacement available? In *Proceedings of the 31st Annual Reciprocal Meat Conference*, June 18-22, Storrs, CT. American Meat Institute Foundation, Arlington, VA, pp. 135-147.

Tompkin, R.B. 1993. Nitrite. In *Antimicrobials in Foods. Second Edition, Revised and Expanded*, ed. P.M. Davidson and A.L. Branen. Marcel Dekker, New York, pp. 191-262.

Tompkin, R.B., Christiansen, L.N. and Shaparis, A.B. 1978*a*. Effect of prior refrigeration on botulinal outgrowth in perishable canned cured meat when temperature abused. *Appl. Environ. Microbiol.* **35**, 863-866.

Tompkin, R.B., Christiansen, L.N. and Shaparis, A.B. 1978*b*. Antibotulinal role of isoascorbate in cured meat. *J. Food Sci.* **43**, 1368-1370.

Tompkin, R.B., Christiansen, L.N. and Shaparis, A.B. 1978*c*. The effect of iron on botulinal inhibition in perishable canned cured meat. *J. Food Technol.* **13**, 521-527.

Tompkin, R.B., Christiansen, L.N. and Shaparis, A.B. 1979*a*. Iron and the antibotulinal efficacy of nitrite. *Appl. Environ. Microbiol.* **37**, 351-353.

Tompkin, R.B., Christiansen, L.N. and Shaparis, A.B. 1979*b*. Isoascorbate level and botulinal inhibition in perishable canned cured meat. *J. Food Sci.* **44**, 1147-1149.

van Laack, R.L.J.M. 1994. Spoilage and preservation of muscle foods. In *Muscle Foods: Meat, Poultry and Seafood Technology*, ed. D.M. Kinsman, A.W. Kotula and B.C. Breidenstein. Chapman & Hall, New York, pp. 378-405.

Yarbrough, J.M., Rake, J.B. and Eagon, R.G. 1980. Bacterial inhibitory effects of nitrite: Inhibition of active transport, but not of group translocation, and of intracellular enzymes. *Appl. Environ. Microbiol.* **39**, 831-834.

CHAPTER 7

THE FATE OF NITRITE

Nitrite is a very reactive entity that can act as an oxidizing, reducing and nitrosating agent. It can be converted to various forms such as nitrate, nitric oxide, dinitrogen trioxide and nitrous acid. When added to meat, evidence of its reactiveness comes not only from well-recognized changes in the color, flavor and shelf-life of meat products, as previously discussed, but also because a significant portion of that added disappears. Once processing of cured meat products is complete, less than 50% of the nitrite added can be analyzed chemically (Cassens *et al.* 1974). Nitrite depletes continuously in products and its depletion rate is dependent upon product formulation, pH, time and temperature relations during processing and storage (Nordin 1969; Kubberød *et al.* 1974). What is even more perplexing is the fact that it is possible to add widely different amounts of nitrite to two samples and, after processing, find the same amount of residual nitrite in both (Fiddler 1977). There is general agreement that most of the nitrite added to meat exists in a form other than the nitrite anion, and for this reason ought to be the subject of further scrutiny (Cassens *et al.* 1977). Knowledge of reaction products formed from nitrite and various meat constituents is at least as important, if not more so, as information pertaining to the amount of nitrite added during curing or remaining after processing.

When addressing the fate of nitrite, clarification in terminology is necessary. Research studies have shown that nitrite can react with proteins, lipids, pigments and other constituents of meat, but a portion of it remains unreacted in the free form (*i.e.*, NO_2^- and HNO_2). This unreacted nitrite is often referred to as free or residual nitrite, whereas nitrite that has reacted with constituents of the meat matrix is termed bound nitrite. Residual nitrite is the quantity of nitrite that can be detected by present analytical methodology, but the accuracy of the assay is questionable (Goutefongea *et al.* 1977). Conventionally, nitrosyl refers to NO which is liganded to a metal ion such as the ferrous ion of the heme moiety in myoglobin; thus, NOMb is termed as a nitrosylheme complex. Nitroso refers to NO which has reacted with a non-metal constituent (*e.g.*, *C* — NO, *S* — NO); a *N*-nitrosamine is a classic example of *N*-nitrosation. Nitroso-nitrite derivatives are referred to as nitrosites while nitroso-nitro derivatives are pseudonitrosites.

As aforementioned, the concentration of residual nitrite in meat depends on factors such as the type of muscle cured, pH and temperature of the system (Olsman and Krol 1972). It seems, however, that loss of nitrite is primarily a

function of time and temperature. During storage, a decrease in its concentration takes place and by the time meat products reach market, they contain only 5-30 mg/kg residual nitrite (Cassens *et al.* 1979). The question is, "what happens to nitrite/where does it go?" In an attempt to answer this, a great deal of research on the fate of nitrite in meat products has been carried out; the details are outlined below.

Reactions of Nitrite

Reaction with Heme Compounds. In fresh meat, nitrite reacts with the native muscle pigment myoglobin (Mb) to form nitrosylmyoglobin (NOMb) which is then converted upon thermal processing to nitrosyl myochromogen, the cooked cured-meat pigment (CCMP); details of the chemistry behind these reactions have been described in Chapter 3. Bonnett and co-workers reported that the reaction of $NaNO_2$ with hemoproteins under mildly acidic conditions can occur at the ferrous ion to give the nitrosylheme pigment (Bonnett *et al.* 1980*a*), in the porphyrin ring (Bonnett *et al.* 1978; 1980*b*) or in the protein (Bonnett and Nicolaidou 1979). Sebranek *et al.* (1973) had calculated that Mb binds *ca.* 15 mg/kg of the added nitrite. However, MacDougall and Hetherington (1992) reported that the theoretical minimum quantity of $NaNO_2$ required for 50% conversion of pork muscle Mb to CCMP was slightly greater than 3 mg/kg. They attempted to determine the minimum quantity of nitrite necessary to stain sliced homogenized cooked pork. Without addition of salt or reducing compounds, they found that less than 2 mg/kg was needed to produce a definite pink color in the sample. Of course, the amount of $NaNO_2$ required for color fixation is greatly dependent on the Mb concentration of the muscle tissue in question.

Nitrite can react with other porphyrin-containing pigments in muscle tissue such as cytochromes and hemoglobin, but the quantity of these compounds in a well bled animal is small compared to that of Mb. Walters *et al.* (1967) reported on the importance of nitrosylferricytochrome *c*, formed by anaerobic cytochrome oxidase action in the presence of nitrite during the curing reaction. These researchers incubated nitrosylferricytochrome c and metMb with muscle mitochondria and reduced nicotinamide adenine dinucleotide (NADH) under a nitrogen atmosphere. The nitrosylferricytochrome *c* was converted to ferrocytochrome *c*, which does not form a nitrosyl complex, and a stoichiometric equivalent of NO. The NO generated by this enzymatic pathway was transferred to metMb by NADH-cytochrome *c* reductase action and the NOmetMb intermediate formed was readily reduced to NOMb by mitochondria.

Formation of Nitrate. The fact that nitrate can be detected in meat products that were cured solely with nitrite is not surprising. Möhler (1971) found that *ca.* 20% of the nitrite added to a beef product was converted to nitrate within 2 h of processing. Nitrate formation was noted during incubation before thermal processing, whereas after cooking only slight nitrate formation was detected. Upon storage, the conversion of nitrite to nitrate continued. Herring (1973) found a conspicuous level of nitrate in bacon formulated only from nitrite. As greater concentrations of nitrite were added to the bellies, a higher content of nitrate was detected in the finished product. They reported that 30% of the nitrite added to bacon was converted to nitrate in less than one week and the level of nitrate continued to increase to *ca.* 40% of the added nitrite until about 10 weeks of storage. Möhler (1974) suggested that when nitrite is added to meat, a simultaneous oxidation of nitrite to nitrate and the ferrous ion of MbO_2 to the ferric ion of metMb occurs. He predicted the equation below and concluded that additional nitrate may be formed as a result of secondary oxidation, in which dismutation of nitrous acid could play a role, as could the oxidation of NO.

$$4\ MbO_2 + 4NO_2^- + 2\ H_2O \rightarrow 4\ metMbOH + 4\ NO_3^- + O_2 \qquad (1)$$

The addition of ascorbate, or its isomer erythorbate, to cured meat products seems to play a key role in the conversion of nitrite to nitrate. Newmark *et al.* (1974) suggested that a high concentration of nitrate formation in bacon occurs when elevated levels of sodium ascorbate are employed. In cured pork products, Lee *et al.* (1978) found that an appreciable quantity of nitrate was formed in the absence of ascorbate, but an even higher level was detected when ascorbate was present. For products devoid of ascorbate, endogenous enzymatic or nonenzymatic reducing agents in meat might play a role similar to that of ascorbate in forming nitrate before thermal processing (Walters *et al.* 1967; Cheah 1976; Lee *et al.* 1978). Based on model system studies using nitrite, metMb and ascorbate, Lee *et al.* (1978) found that when metMb was incubated with nitrite alone, no nitrate was detected during 96 h of storage. Similarly, nitrate was not detected when nitrite was incubated with ascorbate alone for a period of 48 h. However, when metMb, ascorbate and nitrite were added in combination, the formation of nitrate was promoted. These authors favored the following pathway for the formation of nitrate: Ascorbate reduces the added metMb to Mb. In the presence of oxygen, Mb and nitrite are then simultaneously oxidized to metMb and nitrate, respectively. Ascorbate is then regenerated from the reduction of dehydroascorbate.

$$2\ metMb + ascorbate \rightleftharpoons 2\ Mb + dehydroascorbate \qquad (2)$$

$$2\ Mb + O_2 + NO_2^- \rightleftharpoons 2\ metMb + 2\ NO_3^- + 2\ e^- \quad (3)$$

$$dehydroascorbate + 2\ e^- \rightarrow ascorbate \quad (4)$$

Fujimaki *et al.* (1975) investigated the fate of nitrite in model systems containing various ratios of Mb, nitrite and sodium ascorbate. To simulate curing conditions, these systems were stored at 4°C and then heated to *ca.* 70-80°C. All of the added nitrite was accounted for as residual nitrite, nitrate, CCMP and gaseous nitrogen compounds. Fujimaki *et al.* (1975) concluded that most of the added nitrite was converted to nitrate except for that which was fixed to CCMP or converted to gas. These investigators reasoned that more NO is generated from nitrite in the presence of ascorbate and that the NO then reacts with oxygen to form nitrate. Myoglobin was not suggested as being involved in the conversion of nitrite to nitrate; however, these authors did note that surplus ascorbate reacted with Mb and resulted in the decomposition of the porphyrin ring leading to greening.

Evolution of Gases. Nitrite can be lost through the formation of gaseous products during curing. In the van Slyke reaction, nitrous acid derived from nitrite reacts with free α-amino groups and nitrogen gas evolves (*n.b.*, R denotes an alkyl group).

$$RCHNH_2COOH + HNO_2 \rightarrow RCHOHCOOH + N_2 + H_2O \quad (5)$$

This reaction is a function of both temperature and pH; it occurs more rapidly at higher temperatures and under mild acid conditions where a larger proportion of the nitrite is in the undissociated form.

Fox and Nicholas (1974) examined the fate of nitrite in meat and found that the disappearance of nitrite was related to the production of NO. The formation of NO, or the anhydride N_2O_3, from NO_2^- and HNO_2 is fundamental to the chemistry of curing. Factors controlling NO production in meat, and the reactions it undergoes once formed, will impact the residual nitrite levels detected. For example, ascorbate, which is added to cured meats, acts as a strong nucleophile and is even more reactive toward nitrite on a mole basis in forming NO than cysteine. It has been suggested that the reaction involves the formation of nitrosoascorbate intermediates, but these are unstable and do not accumulate (Fox *et al.* 1981). Fox and Nicholas (1974) reported that nitrite loss and NO formation followed the same kinetics, that is, first order in nitrite and 0.5 order in reductant (RdH).

$$2HNO_2 \rightleftharpoons N_2O_3 + H_2O \quad (6)$$

$$N_2O_3 + RdH \rightleftharpoons RdNO + HNO_2 \quad (7)$$

$$2\ RdNO + H_2O \rightleftharpoons 2\ RdH + N_2O_3 \quad (8)$$

$$RdNO \rightleftharpoons Rd^{\bullet} + NO \quad (9)$$

Walters and Taylor (1964) reported that not all of nitrite's loss in meat could be accounted for as NO. Nitric oxide may be converted back to nitrite, react with heme pigments to form NOMb, oxidize to nitrogen dioxide, NO_2, and be given off as gas, or dismutate in water to form nitrate and nitrite. Walters and Taylor (1964) and Woolford *et al.* (1972) detected both nitrous oxide (N_2O) and NO in the headspace gases from anaerobic incubation of nitrite and minced porcine skeletal muscle. Nitric oxide production increased linearly with nitrite concentration up to 14 mM; the N_2O level increased but to a much lesser extent than that of NO. Olsman and Krol (1972) also detected NO, N_2O and N_2 in the headspace gas of a heated cured meat product.

Production and evolution of NO, N_2O and N_2 by reaction of nitrite with meat account for some of the loss of nitrite, but not all nitrite nitrogen is evolved from the finished product as a gas. A significant portion remains in the sample in combination with either a meat constituent (*i.e.*, protein or lipid) or an added reductant.

Role of Non-Heme Proteins and Sulfhydryl Groups. One of the major pathways for loss of nitrite in cured meats is through its reaction with non-heme protein. Proteins contain a number of potential reaction sites for nitrite, and muscle provides an enormous spectrum of proteins of widely varying properties and amino acid compositions (Cassens *et al.* 1979). Many studies have concentrated on nitrite's interaction with sulfhydryl/thiol residues of protein; both the amount and changes in sulfhydryl content of meats and the chemical properties and stabilities of generated *S*-nitrosothiols have been studied. Thiols such as reduced glutathione are readily converted by nitrite to *S*-nitroso derivatives, although most such compounds are labile and short-lived; the S — N bond is weak and NO, one of the products of homolysis, is a stable radical. In the presence of iron(II) salts, nitrite can react with thiols to yield iron-sulfur nitrosyl salts, such as the sodium salt of Roussin's black anion, $Na[Fe_4S_3(NO)_7]$ (Glidewell and Glidewell 1993). These authors identified two iron complexes ($Na[Fe_4S_3(NO)_7]$ and $[Fe_2(SCH_3)_2(NO)_4]$) that formed from the reaction of iron(II) sulfate, nitrite, cysteine, methionine and their derivatives under a range of experimental conditions relevant to food processing operations. The formation of these complexes can account for nitrite loss during processing and in some circumstances, complete conversion of added nitrite to $Na[Fe_4S_3(NO)_7]$ was observed.

Mirna and Hofmann (1969) made an important discovery with regard to nitrite loss when they observed that the depletion of added nitrite in meat was equaled by a reduction in the content of free sulfhydryl groups. They demonstrated that nitrite can react with sulfhydryl groups of meat proteins (*ca.* 20 mmol/kg meat) to form nitrosothiol compounds (RSNO), and speculated that these compounds might be involved in nitrite disappearance, possibly from the oxidation of sulfhydryl by nitrite. Olsman and Krol (1972) investigated the function of the sulfhydryl groups in terms of nitrite loss by blocking the thiol moieties with the alkylating reagent *N*-ethylmaleimide. This procedure suppressed the depletion of nitrite, but not to an extent that would suggest that thiol groups played a significant role. Moreover, the further addition of sulfhydryl groups to meat in the form of reduced glutathione did not greatly affect the depletion rate of nitrite. Kubberød *et al.* (1974) found that nitrite reacted with sulfhydryls to form RSNO, but noted that this reaction in a rabbit myosin model system was low at conditions similar to those found in meat (pH 5.5-6.0 and a $NaNO_2$/SH molar ratio of *ca.* 1/10). These authors assumed that the direct reaction between nitrite and sulfhydryls in myosin was responsible for only a small proportion of the total nitrite lost in the curing process. On the other hand, when cysteine (30 mM) was incubated with nitrite (4 mM) for 5 h at 60°C in a meat slurry, the nitrite loss was greater by 30-50% than that of its counterpart with no added cysteine (Fox and Nicholas 1974). Woolford (1974) reported that the amount of RSNO formed was linear with respect to increasing nitrite levels added to fresh meat. Thus, nitrosothiol derivatives are likely formed in proteins containing free cysteinyl residues, but at the same time they are easily destroyed. Olsman (1977) found that half of the free nitrite lost during the first few days of storage could be recovered as protein-bound nitrite. He suggested that the formation of RSNO is reversible and may act as a pool of nitrosating potential to be used through an intermediary nitrosating agent to form stable nitrosated compounds as the free nitrite in the system declines. Thus, RSNO formation contributes to the so-called bound nitrite which can be released upon decomposition by heterolytic and homolytic mechanisms. According to Bonnett and Nicolaidou (1979), the limited evidence suggests that the heterolytic mechanism could lead to *N*-nitrosamine formation, while the homolytic process is expected to generate NO, another possible route for NOMb formation in the curing process. Once formed, RSNO can undergo a redox reaction resulting in a disulfide R-S-S-R and the liberation of NO.

$$R_2NH + RS — N = O \rightarrow R_2NNO + RSH \qquad (10)$$

$$RSH + HONO \rightarrow RSNO + H_2O \rightarrow RS^{\bullet} + NO + H_2O \qquad (11)$$

Nitrosation model studies have shown that α-amino acids, other than cysteine, can be nitrosated. For example, nitosation of proline gives the *N*-nitrosoamine which, although apparently not itself a carcinogen, can be decarboxylated to *N*-nitrosopyrrolidine, a noted carcinogen from animal studies (Preussmann and Stewart 1984). Nitrosation of arginine derivatives such as *N*-acetylarginamide gives *N*-nitrosocyanamide, which has mutagenic properties. Philpot and Small (1938) found that tyrosine was nitrosated, but to a lesser extent than that of tryptophan; indoles react readily with nitrous acid and exhibit a wide variety of reactions. Malin *et al.* (1989) investigated the deamination of lysine ϵ-amino groups in different proteins in an effort to monitor the fate of nitrite and to detect reactions that proteins could undergo in nitrite-processed foods. They reported severe losses in lysine and the subsequent formation of hydroxynorleucine derivatives; 6-hydroxynorleucine is believed to arise by nitrous acid deaminaiton of lysine (Gilbert *et al.* 1975).

Woolford *et al.* (1976), using a nitrite-bovine serum albumin model system, tried to quantitatively account for all of the added nitrite, but failed to do so. As the nitrite level fell to 40% of its original value (*i.e.*, from 200 to 80 mg/kg) after 160 h of incubation, 30% of the missing nitrite nitrogen was determined to be bound to the protein (Fig. 7.1). Using porcine myosin, the most abundant non-heme protein in meat, Woolford *et al.* (1976) found 3-nitrotyrosine to be a major reaction product of nitrite with myosin; 3-nitrotyrosine accounted for 10-20% of the total protein bound nitrite. Mellet *et al.* (1992) speculated that tyrosine residues, when in contact with nitrite, can also form diazonium salt derivatives which then participate in the formation of intermolecular bonds.

Reaction with Adipose Tissue. Adipose tissue is not inert towards reaction with nitrite. Even though its amount, type and distribution in cured meats varies greatly, little attention has been paid towards interactions between lipids and nitrite. Bacon is the exception, however, on account of the frequent occurrence of small quantities of *N*-nitrosopyrrolidine detected after its frying (Mottram *et al.* 1977).

Using $^{15}NO_2^-$, Cassens and coworkers have demonstrated that only a small portion (1-5%) of the added nitrite becomes associated with lipids (Sebranek *et al.* 1973; Cassens *et al.* 1978; 1979). For the most part, however, the nature of this association remains unknown. Goutefongea *et al.* (1977) studied the reaction of sodium nitrite in brine with adipose tissue, both intact and comminuted, and found that a small portion of the added nitrite was bound to lipid. Between 80 to 90% of the added nitrite was analyzable as free nitrite in cured whole adipose tissue. These researchers also found that fatty acids and triacylglycerols showed a binding towards nitrite that was related to the degree of lipid unsaturation; nitrosated adipose derivatives were extracted along with lipids according to the

Folch method (1957). Gray *et al.* (1983) demonstrated a correlation between increasing levels of unsaturated fatty acids in uncured pork with increasing amounts of *N*-nitrosamines formed during bacon frying. Hotchkiss *et al.* (1985) reported the existence of a lipid-bound nitrite derivative and showed that it was responsible for nitrosating amines in fried-out bacon fat and extracted raw bacon fat.

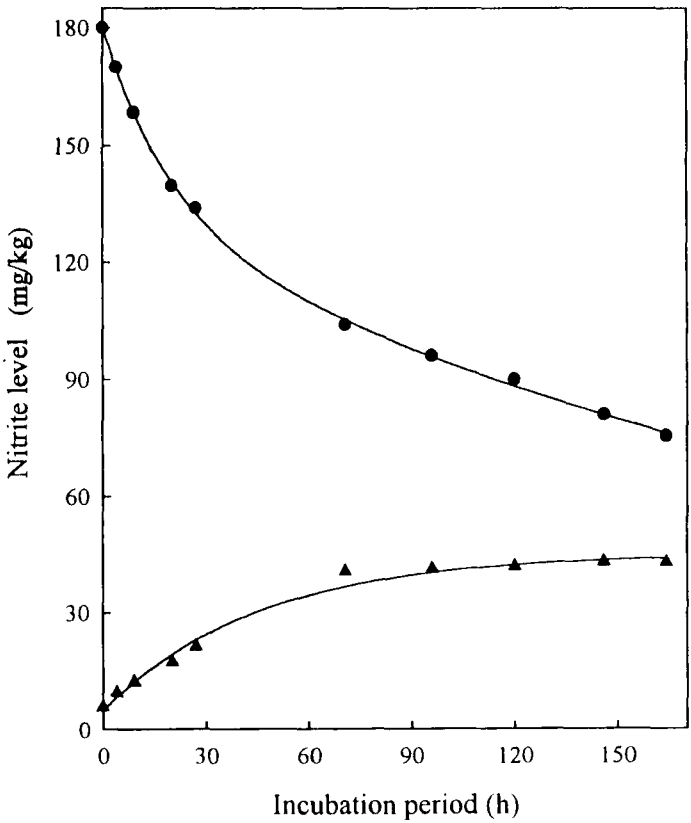


FIG. 7.1. RESIDUAL NITRITE (•) AND INCORPORATED NITRITE (▲) LEVELS DURING INCUBATION OF BOVINE SERUM ALBUMIN (10% W/V) AND SODIUM NITRITE (200 MG/KG) AT PH 5.5 AND 20°C, 0.1 M PHOSPHATE-CITRIC ACID BUFFER (From Woolford *et al.* 1976).

The reaction of various nitrogen oxides (NO_x) with olefins has been reported to result in the formation of nitroso-nitrite derivatives (*i.e.*, nitrosites) as well

as nitroso-nitro compounds (*i.e.*, pseudonitrosites) (Klamann *et al.* 1965; Zubillaga *et al.* 1984; Ross *et al.* 1987). The potential importance of the formation of such compounds has been shown in model system studies. Lipid pseudonitrosites acted as transnitrosating agents at a temperature of 170°C and were suggested as being responsible for *N*-nitrosamine formation during bacon frying. Specifically, the pseudonitrosite of palmitodiolein was shown to be capable of nitrosating amines (Walters *et al.* 1979). Mirvish *et al.* (1983) reacted NO_2 with methyl esters of unsaturated fatty acids in a model system and demonstrated that the crude product was capable of nitrosation; nitrosation capacity generally increased with increasing unsaturation. Zubillaga *et al.* (1984) reacted olefins with NO_x to test the possibility that NO_x addition to olefins might account for antioxidant activity in cured meats. Although no antioxidant activity was observed, reaction products were formed in all experiments, but no attempts were made at identifying their structures. Ross *et al.* (1987) went a step further and reacted dinitrogen trioxide with methyl oleate. Individual products were separated by HPLC and identified by IR and MS as being nitronitroso-, dinitro-, nitronitrate- and nitro oxime additions to methyl oleate. These investigators then added these reaction products to comminuted uncured pork. Upon frying at 170°C, *N*-nitrosodimethylamine and *N*-nitrosopyrrolidine were formed in amounts similar to those produced in fried bacon; no other *N*-nitrosamines were detected. Liu *et al.* (1988) further investigated the chemistry of transnitrosation in a model system using nitro-nitroso derivatives formed from the reaction of dinitrogen trioxide with carbon-carbon double bonds of unsaturated lipids. These investigators showed that these derivatives can decompose during frying to release oxides of nitrogen which are capable of nitrosating secondary amines. Similar findings were reported by Freybler *et al.* (1993). Although not proven in a cured meat system, these results help to support the theory that nitrite reacts with unsaturated lipids in bacon to form intermediates, which are capable of nitrosating endogenous amines in uncured pork under frying conditions.

Formation of *N*-Nitrosamines. Numerous studies have shown that only a small fraction of the nitrite added to meat is, on occasion, detected as volatile *N*-nitrosamines. Many of these compounds have been shown to induce cancer in experimental animals (Preussmann and Stewart 1984). For this reason, Sen and Baddoo (1997) felt that it would be prudent to reduce the levels of both added and residual nitrite in cured meats to as low as technically feasible without compromising the protection nitrite offers meat and meat products against botulism and other harmful bacteria. Discussion of nitrite's reaction with amino compounds leading to the formation of volatile and non-volatile *N*-nitrosamines, *N*-nitrosamides and *N*-nitrosoamino acids will be dealt with in Chapter 8.

Reaction with Other Components of Meat. As already outlined, nitrite can react with various constituents of meat to give nitrosylheme complexes, gaseous products, nitrate, nitroso-reductants, protein- and lipid-derived compounds, as well as *N*-nitrosamines. It can also react with carbohydrate constituents added to meat in the form of binders, extenders and saccharides (*i.e.*, dextrose and corn syrup solids). Condensation products of glucose and amino acids in the Maillard reaction lead to the formation of Amadori compounds and their rearrangement products. Secondary amines in these products are potential sites for reaction with nitrite to form *N*-nitrosamines. Furthermore, volatile and heterocyclic compounds from the Maillard reaction have been shown to react with nitrous acid to form nitroso derivatives, such as nitrosothiazolidine (Russell 1983).

^{15}N Tracer Studies

The use of the stable isotope ^{15}N was proposed as being one of the most useful techniques for determining the fate of nitrite in meat products. Theoretically, not only can ^{15}N be accounted for in various constituents of the meat matrix, but by using labeled $Na^{15}NO_2$, a mass balance of the ingoing label with that of the ^{15}N recovered from comminuted meat products can be carried out. Sebranek *et al.* (1973) reported that the labeled nitrite added to a canned luncheon meat product changed to other forms during and after processing and that the rate of change slowed until a somewhat constant level of residual nitrite was reached. Quantitative analysis for ^{15}N was conducted on water-soluble, salt-soluble and insoluble fractions of the meat at various time intervals after processing. Two days after processing, less than half of the labeled nitrite was identified as residual nitrite. These investigators found that nitrite had reacted with Mb, non-heme protein and lipid constituents, but ^{15}N recoveries of only 72-86% could be achieved. From collected data, it was estimated that 9-12% of the added label had bound to Mb which agreed with the theoretical calculated value of 10%. Analysis of gas trapped during sample preparation showed that $^{15}N_2$ and ^{15}NO were generated. Although exact quantitation was not possible, it was estimated that 5% of the total ^{15}N added was present as these two gases. Woolford and Cassens (1977) examined the fate of labeled sodium nitrite in bacon. ^{15}N data showed that 25% of the added nitrite was incorporated into the muscle proteins of bacon while only 10% of that added had reacted with the lipid fraction of the adipose tissue. As 100% recovery of the added ^{15}N was not realized, these authors speculated that the reduction of N — NO bonds in the analytical protocol might not be 100% effective and therefore represents a loss in the ^{15}N determined. Another possibility was that the difference between the residual ^{15}N and initially added ^{15}N in the lean portions might be due to the loss of labeled gaseous and volatile compounds during various processing operations.

Emi-Miwa *et al.* (1976) examined the fate of nitrite added to whole meat cured with and without ascorbate using labeled $Na^{15}NO_2$. In the absence of ascorbate, 77% of the added nitrite was recovered from the whole meat on day 0 and 46.5% on day 7, whereas almost all of the labeled nitrite was recovered from a Mb-nitrite model system (Table 7.1). In the presence of ascorbate, a larger proportion of the added nitrite could not be explained as compared to a counterpart system devoid of ascorbate. These authors speculated that endogenous reducing compounds present in meat have the same effect as ascorbate. Fox and Nicholas (1974) reported that reduced NADH caused a 70-80% decrease in the nitrite content upon addition, followed by an increase in the concentration back to 75-85% of the original value.

From the studies carried out by Cassens and co-workers, a summary of their investigations shows that they were able to recover 70-80% of the added nitrite nitrogen and found that it was distributed as follows: 5-15% in Mb, 1-10% as nitrate, 5-20% as residual nitrite, 1-5% as gases, 5-15% bound to sulfhydryls, 1-5% bound to lipid and 20-30% bound to non-heme protein. Although nitrite reacted with various meat constituents, the majority of nitrite nitrogen was bound to non-heme protein.

Determination of Residual Nitrite in Cured Meat Products and Limitations to the Assay

Residual nitrite in cured meat products is determined by the classical Griess-Ilosvay reaction and subsequent modifications made to this assay. The reaction is based on the interaction of nitrite, as nitrous acid, with a primary aromatic amine (*i.e.*, sulfanilic acid) in acid solution to form a stable arenediazonium salt, $Ar - N^+ \equiv N$. The reagent used to determine the nitrite concentration was developed by Griess (1879) with later modifications by Ilosvay (1889). The diazonium salt is then treated with an aromatic compound bearing influential amino or hydroxyl substituents (*i.e.*, 1-naphthylamine in the Griess-Ilosvay method) to yield a brightly colored azo complex, $Ar - N = N - Ar'$, whose concentration is measured spectrophotometrically. Free nitrite in the sample is determined by comparing the absorbance of the complex to that from a standard nitrite curve. Although a wide range of amines are potential reactants for both nitrite and coupling agents, protocols have been confined to the use of a select few compounds as shown in Table 7.2 (Usher and Telling 1975).

The most commonly used coupling agent at present is *N*-(1-naphthyl)-ethylenediamine dihydrochloride (NED); this is due to its non-carcinogenic nature, *cf.* 1-naphthylamine, and speed of color development. It contains a primary amine group which is capable of being diazotised and may therefore couple with excess reagent. In order to prevent this reaction, NED can only be added to the sulfanilic acid solution after the diazonium salt has formed.

TABLE 7.1
FATE OF NITRITE IN MINCED PORK AND MYOGLOBIN-NITRITE MODEL SYSTEMS

Systems[a]	Fate of Nitrite[b]						
	Ascorbate	CCMP-N	Nitroso-thiol-N	Nitrite-N	Nitrate-N	Gaseous-N	Total-N
Meat system - day 0	0	1.8	0.9	77.0	10.0	0	89.7
	2	4.9	2.5	56.0	19.7	2.3	85.4
	20	6.2	7.1	30.0	22.7	4.5	70.5
Model system - day 0	0	0	0	100.0	0	trace	100.0
Meat system - day 7	0	3.3	1.4	46.5	13.0	4.3	68.5
	2	3.9	0.6	27.3	30.5	2.9	66.2
	20	4.7	4.5	4.8	67.6	3.4	85.0
Model system - day 7	0	0	0	96.2	3.4	1.0	100.6

[a]Meat systems contain minced pork (17.6 g) that was homogenized with 1 ml of 0.02M $Na^{15}NO_2$, ascorbate (at different levels) and 0.4 g of NaCl. Samples were cooked at 70°C for 60 min after being stored at 4°C for 0 or 7 days. Model systems consisted of 5 ml of 1 mM sperm whale myoglobin, 5 mM of $Na^{15}NO_2$, 10 ml of 0.2 M acetate buffer (pH 5.5) and 5 ml of water. Systems were cooked at 70°C for 60 min after being stored at 4°C for 0 and 7 days.
[b]Expressed as N atom % of added nitrite-N.
(Adpated from Emi-Miwa *et al.* 1976.)

TABLE 7.2
COMMON COUPLING SYSTEMS USED FOR THE COLORIMETRIC DETERMINATION OF NITRITE[a]

Investigators	Aromatic amine	Coupling reagent	Color development time (min)
Griess (1879) - Ilosvay (1889)	Sulfanilic acid	1-Naphthylamine	25-60
Bratton and Marshall (1939)	Sulfanilic acid	*N*-(1-naphthyl)ethylenediamine	10
Shinn (1941)	Sulfanilamide	*N*-(1-naphthyl)ethylenediamine	10
Follett and Ratcliff (1963)	Sulfanilic acid	1-Naphthol	34
Kamm *et al.* (1965)	1-Naphthylamine	1-Naphthylamine	120
Adriaanse and Robbers (1969)	Sulfanilic acid	1-Naphthol-7-sulfonic acid	25

[a]From Usher and Telling (1975).

Follett and Ratcliff (1963) noted that there was a tendency for the red azo dye to change color and to precipitate when the concentration of nitrite in the final solution exceeded 750 mg/kg. For retail cured meat products, this should be no problem as residual nitrite levels generally range from 5-30 mg/kg (Cassens *et al.* 1979). The time for color development when using 1-naphthylamine as the coupling agent takes anywhere from 25-60 min, whereas with NED, a rapid color is achieved for a wide range of nitrite concentrations within 10 min. The absorbance of the resultant azo dye is measured spectrophotometrically at 540 nm against a blank. The mechanism for azo dye formation from the arenediazonium salt of sulfanilic acid and the *N*-(1-naphthyl)ethylenediamine dihydrochloride coupling agent is presented in Fig. 7.2.

Perhaps the greatest limitation to the analysis is the procedure for extracting nitrite from cured meat samples. The original technique adopted by the Association of Official Analytical Chemists was described by Kerr (1925) and involved heating a 5 g portion of cured meat, diluted with 300 ml of water, for 2 h on a steam bath. After this period, 5 ml of saturated mercuric chloride was added, the flask contents cooled, diluted to volume and then filtered. A suitable aliquot was then reacted with the Griess reagent and color allowed to develop. According to present day CFIA protocols, extraction is carried out by mixing

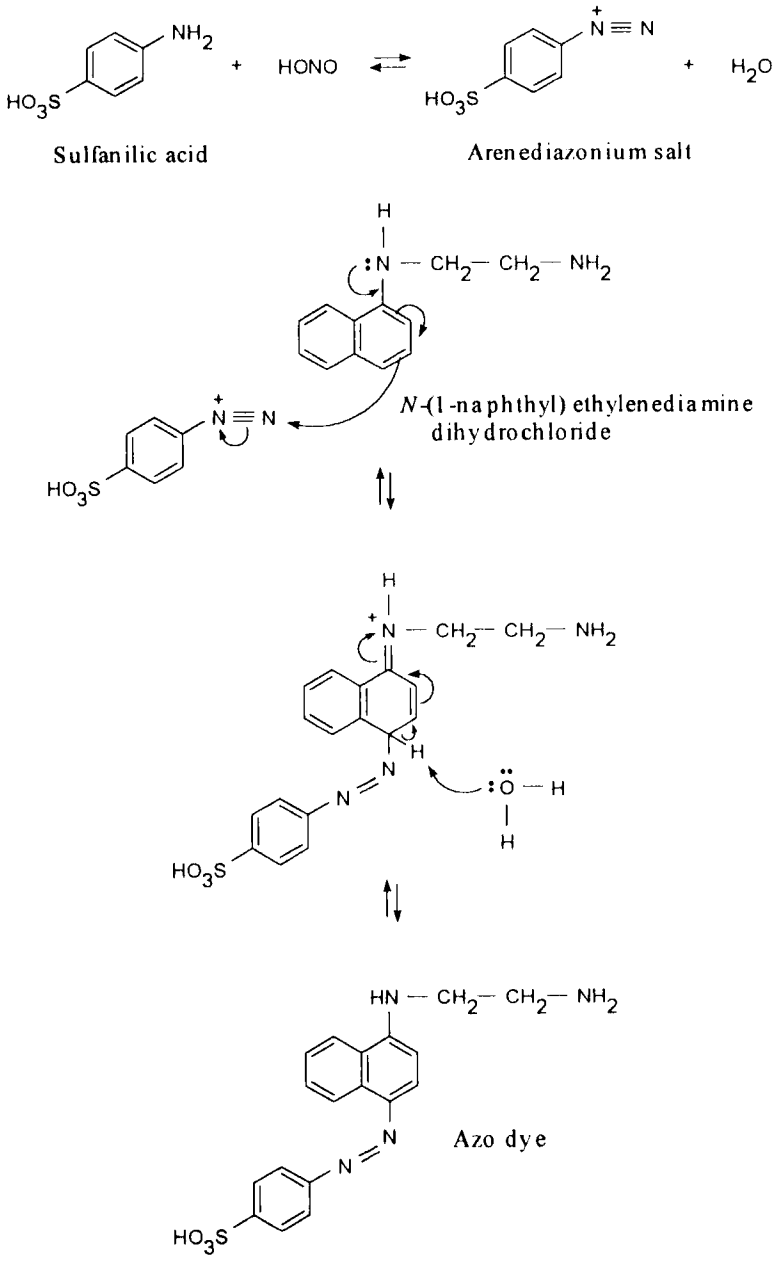


FIG. 7.2. MECHANISM OF AZO DYE FORMATION FROM THE ARENEDIAZONIUM SALT OF SULFANILIC ACID AND THE *N*-(1-NAPHTHYL)ETHYLENEDIAMINE DIHYDROCHLORIDE COUPLING AGENT

the meat sample (5g) with 15 ml of a 2% NaOH solution, then 12 ml of a 12% $ZnSO_4$ solution and finally 50 ml of distilled water. If necessary, more base is added to ensure that the pH is maintained between 7 and 8. The mixture is heated for 30 min at 50°C, allowed to cool, diluted to volume (200 ml) and then filtered. Free nitrite is that available for reaction with the modified Griess reagent after preparation and extraction of the sample. As outlined above, binding exists between nitrite and various meat components. Therefore, the severity of the sample treatment (*i.e.*, physical disruption, heat treatment, dilution, chemical treatment) may modify the amount of nitrite analyzable. A large portion of the added nitrite is protein-bound and the extent of binding is altered by numerous conditions existing within the meat, or by changes in the environment to which the product is subjected (Goutefongea *et al.* 1977). Therefore, is the nitrite being extracted from the sample residual nitrite only, or could a portion of it come from bound nitrite which is released upon sample handling? On the other hand, could a portion of the free nitrite be lost in the protein precipitation step upon addition of metal salts? Does this assay provide a tangible description of the content of residual nitrite in cured meat products?

Present Residual Nitrite Levels

Sen and Baddoo (1997) pointed out that a considerable amount of data concerning residual nitrite levels in cured meat products had been reported in the literature by Canadian and US scientists in the early to mid 1970s, but recent data were scant. Cassens (1995) reported a marked decrease ($\sim$ 80%) in residual nitrite levels of US prepared cured meat products from those determined 20 years earlier; levels in current retail products were 7 mg/kg for bacon, 6 mg/kg for sliced ham and 4 mg/kg for hot dogs. In a larger survey involving nearly 100 retail cured meat samples from various manufacturers, the overall mean for residual nitrite was 10 mg/kg, expressed as NO_2^- (Cassens 1996). He attributed the decline to various changes introduced over the years for the processing of cured products by the US meat industry. Most notable was increased use of ascorbate or erythorbate and lower nitrite addition levels.

In Canada, the trend observed by the Canadian Health Protection Branch for residual nitrite levels in cured meat products was somewhat different from that in the US (Sen and Baddoo 1997). In 1972, Panalaks *et al.* (1973) analyzed residual nitrite levels from 197 samples and found levels ranging from < 1 to 252 mg/kg with a mean of 28 mg/kg. Sen and Baddoo (1997) reported that during the period 1983-1985, nitrite levels from 659 samples ranged from < 1 to 275 mg/kg with a mean of 43.6 mg/kg; for 1993-1995, the levels from 76 samples ranged from 1 to 145 mg/kg with a mean of 30.8 mg/kg; and in 1996, the levels from 35 samples ranged from 4 to 68 mg/kg with a mean of 28 mg/kg. Table 7.3 summarizes residual nitrite levels for specific cured meat

TABLE 7.3
RESIDUAL NITRITE LEVELS IN VARIOUS CURED MEAT PRODUCTS AS DETECTED IN CROSS-CANADA SURVEYS OVER THE YEARS[a]

Products Analyzed		Residual $NaNO_2$ Levels, mg/kg	
	n	mean	range
1983 - 1985			
Bacon	52	33.7	nd - 178
Hot dog/frankfurter	109	60.9	1 - 178
Sausages	102	33.8	1 - 132
Ham	88	48.9	4 - 146
Corned beef	59	31.5	1 - 192
Meat Loaf	12	18.5	2 - 66
Bologna	39	65.5	nd - 137
Picnic hams (shoulders)	18	39.2	4 - 122
Sliced cooked meats	42	31.4	6 - 120
Uncooked cured meats (pastrami, smoked beef, spiced beef)	5	72.9	11 - 275
Salami and European-type sausages	90	37.6	3 - 174
Pepperoni	23	62.5	10 - 206
Miscellaneous	20	38.8	nd - 168
1993 - 1995			
Bacon	11	33.8	7 - 81
Hot dog/frankfurter	4	65.5	23 - 112
Sausages and Salami	31	30.2	4 - 145
Ham	7	28.6	1 - 61
Pepperoni	8	35.0	10 - 58
Miscellaneous	15	18.6	8 - 36
1996			
Bacon	6	34.0	4 - 68
Hot dog/frankfurter	8	24.0	10 - 63
Sausages and Salami	8	26.0	9 - 37
Smoked Ham	4	24.0	9 - 40
Bologna	2	33.0	31 - 35
Pepperoni	3	31.0	10 - 53
Miscellaneous	4	31.0	19 - 43

[a]Residual nitrite was determined according to the Griess colorimetric assay as described by Sen and Donaldson (1978). nd - not detected (< 1 mg/kg).
(Adapted from Sen and Baddoo 1997).

products for the periods 1983-1985, 1993-1995 and 1996 detected by the Health Protection Branch in their cross-Canada surveys. In 1996, the survey also included results from a second methodology for analyzing residual nitrite so as to verify data acquired by the classical Griess colorimetric assay. Sen *et al.* (1994) reported an HPLC-chemiluminescence detection method based on postcolumn chemical denitrosation of nitrite to NO, followed by detection of the liberated NO with a thermal energy analyzer (*n.b.*, see details in Chapter 8). This technique is *ca.* 200 times more sensitive than the colorimetric assay. Analysis of the data revealed that for residual nitrite levels below 35 mg/kg there was no statistical difference between the contents determined by both methodologies. However, results from the chemiluminescence method were consistently higher than those of the colorimetric assay when residual nitrite concentrations were greater than 35 mg/kg. Nonetheless, these findings indicate that the colorimetric method does not yield false-high results. Over the past 20-25 years, overall mean residual nitrite levels in Canadian cured meat products have decreased, but only slightly. On the other hand, the incidence of samples containing high levels of nitrite has decreased markedly during the same period.

REFERENCES

Adriaanse, A. and Robbers, J.E. 1969. Determination of nitrite and nitrate in some horticultural and meat products and in samples of soil. *J. Sci. Food Agric.* **20**, 321-325.

Bonnett, R., Chandra, S., Charalambides, A.A., Sales, K.D. and Scourides, P.A. 1980*a*. Nitrosation and nitrosylation of haemoproteins and related compounds. Part 4. Pentaco-ordinate nitrosylprotohaem as the pigment of cooked cured meat. Direct evidence from E.S.R. spectroscopy. *J.C.S. Perkin I*, 1706-1710.

Bonnett, R., Charalambides, A.A. and Martin, R.A. 1978. Nitrosation and nitrosylation of haemoproteins and related compounds. Part 1. Porphyrins and metalloporphyrins. *J.C.S. Perkin I*, 974-980.

Bonnett, R., Hursthouse, M.B., Scourides, P.A. and Trotter, J. 1980*b*. Nitrosation and nitrosylation of haemoproteins and related compounds. Part 3. Attack at the vinyl groups of protoporphyrin dimethyl ester. X-ray analysis of 8^1(E)-8^2-nitroprotoporphyrin dimethyl ester. *J.C.S. Perkin I*, 490-494.

Bonnett, R. and Nicolaidou, P. 1979. Nitrosation and nitrosylation of haemoproteins and related compounds. Part 2. The reaction of nitrous acid with the side chains of α-acylamino-acid esters. *J.C.S. Perkin I*, 1969-1974.

Bratton, A.C. and Marshall, E.K. Jr. 1939. A new coupling component for sulfanilamide determination. *J. Biol. Chem.* **128**, 537-550.

Cassens, R.G. 1995. Current content of residual nitrite in cured meats at the retail market. In *Proceedings of the 41st International Congress of Meat Science and Technology, Volume II*, San Antonio, TX, pp. 344-345.

Cassens, R.G. 1996. Residual nitrite in commercial cured meats in the USA: 1995. In *Book of Abstracts, 1996 IFT Annual Meeting*, New Orleans, LA, June 22-26, Abstract 26F-1 p. 61.

Cassens, R.G., Greaser, M.L., Ito, T. and Lee, M. 1979. Reactions of nitrite in meat. *Food Technol.* **33**(7), 46-57.

Cassens, R.G., Ito, T., Lee, M. and Buege, D. 1978. The use of nitrite in meat. *Bioscience* **28**, 633-637.

Cassens, R.G., Sebranek, J.G., Kubberod, G. and Woolford, G. 1974. Where does the nitrite go? *Food Prod. Dev.* **8**(10), 50, 52, 54, 56.

Cassens, R.G, Woolford, G., Lee, S.H. and Goutefongea, R. 1977. Fate of nitrite in meat. In *Proceedings of the Second International Symposium on Nitrite in Meat Products*, Zeist, The Netherlands, September 7-10, 1976, ed. B.J. Tinbergen and B. Krol. Wageningen Centre for Agricultural Publishing and Documentation, Wageningen, The Netherlands, pp. 95-100.

Cheah, K.S. 1976. Formation of nitrosylmyoglobin in bacon involving lactate dehydrogenase. *J. Food Technol.* **11**, 181-186.

Emi-Miwa, M., Okitani, A. and Fujimaki, M. 1976. Comparison of the fate of nitrite added to whole meat, meat fractions and model systems. *Agric. Biol. Chem.* **40**, 1387-1392.

Fiddler, R.N. 1977. Collaborative study of modified AOAC method of analysis for nitrite in meat and meat products. *J. Assoc. Off. Anal. Chem.* **60**, 594-599.

Folch, J., Lees, M. and Stanley, G.H.S. 1957. A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* **226**, 497-509.

Follett, M.J. and Ratcliff, P.W. 1963. Determination of nitrite and nitrate in meat products. *J. Sci. Food Agric.* **14**, 138-144.

Fox, J.B. Jr. Fiddler, R.N. and Wasserman, A.E. 1981. Initial reaction intermediates in the oxidation of ascorbic acid by nitrous acid. *J. Food Prot.* **44**, 28-32.

Fox, J.B., Jr. and Nicholas, R.A. 1974. Nitrite in meat. Effect of various compounds on loss of nitrite. *J. Agric. Food Chem.* **22**, 302-306.

Freybler, L.A., Gray, J.I., Asghar, A., Booren, A.M., Pearson, A.M. and Buckley, D.J. 1993. Nitrite stabilization of lipids in cured pork. *Meat Sci.* **33**, 85-96.

Fujimaki, M., Emi, M. and Okitani, A. 1975. Fate of nitrite in meat-curing model systems composed of myoglobin, nitrite and ascorbate. *Agric. Biol. Chem.* **39**, 371-377.

Gilbert, J., Knowles, M.E. and McWeeny, D.J. 1975. Formation of *C*- and *S*-nitroso compounds and their further reactions. *J. Sci. Food Agric.* **26**, 1785-1791.

Glidewell, C. and Glidewell, S.M. 1993. The fate of nitrite in food processing: isolation of dinuclear and tetranuclear iron-sulphur nitrosyl complexes from cysteine and methionine sources. *Food Chem.* **46**, 225-230.

Goutefongea, R., Cassens, R.G. and Woolford, G. 1977. Distribution of sodium nitrite in adipose tissue during curing. *J. Food Sci.* **42**, 1637-1641.

Gray, J.I., Skrypec, D.J., Mandagere, A.K., Booren, A.M. and Pearson, A.M. 1983. Further factors influencing *N*-nitrosamine formation in bacon. In *N-Nitroso Compounds: Occurrence, Biological Effects and Relevance to Human Cancer*, ed. I.K. O'Neill, R.C. Von Borstel, C.T. Miller, J. Long and H. Bartsch. IARC Scientific Publication No. 57. International Agency for Research on Cancer, Lyon, France, pp. 301-309.

Griess, P. 1879. Bemerkungen zu der Abhandlung der H.H. Weselsky und Benedikt "Über einige Azoverbindungen." *Ber. Deut. Chem. Ges.* **12**, 426-428.

Herring, H.K. 1973. Effect of nitrite and other factors on the physico-chemical characteristics and nitrosamine formation in bacon. In *Proceedings of the Meat Industry Research Conference*, American Meat Institute Foundation, Arlington, VA, pp. 47-60.

Hotchkiss, J.H., Vecchio, A.J. and Ross, H.D. 1985. *N*-Nitrosamine formation in fried-out bacon fat: Evidence for nitrosation by lipid bound nitrite. *J. Agric. Food Chem.* **33**, 5-8.

Ilosvay, M.L. 1889. L'acide azoteux dans la salive et dans l'air exhalé *Bull. Soc. Chim. de Paris* **49**, 388-391.

Kamm, L., McKeown, G.G. and Morison Smith, D. 1965. New colorimetric method for the determination of the nitrate and nitrite content of baby foods. *J. Assoc. Off. Agric. Chem.* **48**, 892-897.

Kerr, R.H. 1925. Report on meats and meat products. *J. Assoc. Off. Agric. Chem.* **8**, 696-697.

Klamann, D., Koser, W., Weyerstahl, P. and Fligge, M. 1965. Über Pseudo-nitrosite, Nitroxime und Furoxane. *Chem. Ber.* **98**, 1831-1836.

Kubberød, G., Cassens, R.G. and Greaser, M.L. 1974. Reaction of nitrite with sulfhydryl groups of myosin. *J. Food Sci.* **39**, 1228-1230.

Lee, S.H., Cassens, R.G., Winder, W.C. and Fennema, O.R. 1978. Factors affecting the formation of nitrate from added nitrite in model systems and cured meat products. *J. Food Sci.* **43**, 673-676.

Liu, R.H., Conboy, J.J. and Hotchkiss, J.H. 1988. Nitrosation by nitro-nitroso derivatives of olefins: A potential mechanism for *N*-nitrosamine formation in fried bacon. *J. Agric. Food Chem.* **36**, 984–987.

MacDougall, D.B. and Hetherington, M.J. 1992. The minimum quantity of nitrite required to stain sliced and homogenised cooked pork. *Meat Sci.* **31**, 201–210.

Malin, E.L., Greenberg, R., Piotrowski, E.G., Foglia, T.A. and Maerker, G. 1989. Deamination of lysine as a marker for nitrite-protein reactions. *J. Agric. Food Chem.* **37**, 1071–1076.

Mellet, P., Noel, P., Dumont, J.P. and Goutefongea, R. 1992. Nitrite-induced protein polymerization: Some evidence for tyrosine contribution to the formation of intermolecular bonds in bovine serum albumin treated with nitrite. *Sci. Aliments* **12**, 601–612.

Mirna, A. and Hofmann, K. 1969. Über den Verbleib von Nitrit in Fleischwaren. I. Umsetzung von Nitrit mit Sulfhydryl-Verbindungen. *Fleischwirtsch.* **49**, 1361–1364.

Mirvish, S.S., Sams, J.P. and Issenberg, P. 1983. The nitrosating agent in mice exposed to nitrogen dioxide: Improved extraction method and localization in the skin. *Cancer Res.* **43**, 2550–2554.

Möhler, K. 1971. Bilanz der Bildung des Pökelfarbstoffs im Muskelfleisch. II. Mitteilung. Hitzekatalysierte Bildung des Stickoxidkomplexes. *Lebensm. Unters. Forsch.* **147**, 123–127.

Möhler, K. 1974. Formation of curing pigments by chemical, biochemical or enzymatic reactions. In *Proceedings of the International Symposium on Nitrite in Meat Products*, Zeist, The Netherlands, September 10–14, 1973, ed. B. Krol and B.J. Tinbergen. Wageningen Centre for Agricultural Publishing and Documentation, Wageningen, The Netherlands, pp. 13–19.

Mottram, D.S., Patterson, R.L.S., Edwards, R.A. and Gough, T.A. 1977. The preferential formation of volatile *N*-nitrosamines in the fat of fried bacon. *J. Sci. Food Agric.* **28**, 1025–1029.

Newmark, H.L., Osadca, M., Araujo, M., Gerenz, C.N. and De Ritter, E. 1974. Stability of ascorbate in bacon. *Food Technol.* **28**(5), 28, 30–31, 60.

Nordin, H.R. 1969. The depletion of added sodium nitrite in ham. *Can. Inst. Food Sci. Technol. J.* **2**, 79–85.

Olsman, W.J. 1977. Chemical behavior of nitrite in meat products - 1. The stability of proteinbound nitrite during storage. In *Proceedings of the Second International Symposium on Nitrite in Meat Products*, Zeist, The Netherlands, September 7-10, 1976, ed. B.J. Tinbergen and B. Krol. Wageningen Centre for Agricultural Publishing and Documentation, Wageningen, The Netherlands, pp. 101–109.

Olsman, W.J. and Krol, B. 1972. Depletion of nitrite in heated meat products during storage. In *Proceedings of Meat Research Workers, 18th Meeting, Vol. II*, Guelph, ON, Canada, pp. 409–415.

Panalaks, T., Iyengar, J.R. and Sen, N.P (1973). Nitrate, nitrite, and dimethylnitrosamine in cured meat products. *J. Assoc. Off. Anal. Chem.* **56**, 621–625.

Philpot, J.S.L. and Small, P.A. 1938. The action of nitrous acid on *p*-cresol and tyrosine. *Biochem. J.* **32**, 534–541.

Preussmann, R. and Stewart, B.W. 1984. *N*-Nitroso carcinogens. In *Chemical Carcinogens, Second Edition, Volume 2,* ed. C.E. Searle. ACS Monograph 182. Am. Chem. Soc., Washington, DC, pp. 643–828.

Ross, H.D., Henion, J., Babish, J.G. and Hotchkiss, J.H. 1987. Nitrosating agents from the reaction between methyl oleate and dinitrogen trioxide: Identification and mutagenicity. *Food Chem.* **23**, 207–222.

Russell, G.F. 1983. Nitrite interactions in model Maillard browning systems. In *The Maillard Reaction in Foods and Nutrition,* ed. G.R. Waller and M.S. Feather. ACS Symposium Series 215. Am. Chem. Soc., Washington, DC, pp. 83–90.

Sebranek, J.G., Cassens, R.G., Hoekstra, W.G., Winder, W.C., Podebradsky, E.V. and Kielsmeier, E.W. 1973. ^{15}N tracer studies of nitrite added to a comminuted meat product. *J. Food Sci.* **38**, 1220–1223.

Sen, N.P. and Baddoo, P.A. 1997. Trends in the levels of residual nitrite in Canadian cured meat products over the past 25 years. *J. Agric. Food Chem.* **45**, 4714–4718.

Sen, N.P., Baddoo, P.A. and Seaman, S.W. 1994. Rapid and sensitive determination of nitrite in foods and biological materials by flow injection or high-performance liquid chromatography with chemiluminescence detection. *J. Chromatogr. A* **673**, 77–84.

Sen, N.P. and Donaldson, B. 1978. Improved colorimetric method for determining nitrate and nitrite in foods. *J. Assoc. Off. Anal. Chem.* **61**, 1389–1394.

Shinn, M.B. 1941. Colorimetric method for determination of nitrite. *Ind. Engng. Chem. Analyt. Edn.* **13**, 33–35.

Usher, C.D. and Telling, G.M. 1975. Analysis of nitrate and nitrite in foodstuffs: A critical review. *J. Sci. Food Agric.* **2**, 1793–1805.

Walters, C.L., Casselden, R.J. and Taylor, A.McM. 1967. Nitrite metabolism by skeletal muscle mitochondria in relation to haem pigments. *Biochim. Biophys. Acta* **143**, 310–318.

Walters, C.L., Hart, R.J. and Perse, S. 1979. The possible role of lipid pseudonitrosites in nitrosamine formation in fried bacon. *Z. Lebensm. Unters. Forsch.* **168**, 177–180.

Walters, C.L. and Taylor, A.McM. 1964. Nitrite metabolism by muscle *in vitro*. *Biochim. Biophys. Acta* **86**, 448–458.

Woolford, G. 1974. Fate of nitrite in meat curing and the determination of nitrosamines. Ph.D. Thesis, University of Surrey, Guildford, UK.

Woolford, G., Casselden, R.J. and Walters, C.L. 1972. Gaseous products of the interaction of sodium nitrite with porcine skeletal muscle. *Biochem. J.* **130**, 82P–83P.

Woolford, G. and Cassens, R.G. 1977. The fate of sodium nitrite in bacon. *J. Food Sci.* **42**, 586–589, 596.

Woolford, G., Cassens, R.G., Greaser, M.L. and Sebranek, J.G. 1976. The fate of nitrite: Reaction with protein. *J. Food Sci.* **41**, 585–588.

Zubillaga, M.P., Maerker, G. and Foglia, T.A. 1984. Antioxidant activity of sodium nitrite in meat. *J. Am. Oil Chem. Soc.* **61**, 772–776.

CHAPTER 8

POTENTIAL HEALTH CONCERNS ABOUT NITRITE

In the late 1960s and early 1970s nitrite usage in cured meats became the source of some very serious concerns, so much so, that the U.S. government even considered a total ban on its usage in meat products. Cassens (1990) elegantly recounts the events leading up to the *N*-nitrosamine scare in the U.S., the scientific data on the issue at the time, and government and industry's response to the situation. Today the hysteria over the *N*-nitrosamine problem has all but disappeared and fewer investigations into nitrite's role in meat products can be found in the scientific literature. Nonetheless, reports on the presence of *N*-nitrosamines in meat products still pop up in scientific journals now and then (Sen and Baddoo 1997). So the question becomes, has the problem been resolved satisfactorily or has a bandage merely been placed over the wound?

Despite all of its desirable effects, nitrite can react, under certain conditions, with amines and amino acids in meat producing *N*-nitrosamines in some thermally processed cured products. *N*-Nitrosodimethylamine (NDMA) and *N*-nitrosopyrrolidine (NPYR) are key examples of such reaction products and have been found to be carcinogenic, mutagenic and teratogenic in experimental animals. Because of their possible link to the incidence of various cancers in man, considerable attention has been focused on the presence of *N*-nitrosamines in nitrite cured meat products (Magee and Barnes 1967; Gray and Randall 1979; Newberne 1979; Preussmann and Stewart 1984; Preussmann and Eisenbrand 1984; Sen 1986). Greater than 90% of the more than 300 *N*-nitroso compounds that have been tested in animal species including higher primates caused cancer (Preussmann and Stewart 1984; Tricker and Preussmann 1991), but no known case of human cancer has ever been shown to result from exposure to *N*-nitroso compounds. Much indirect evidence suggests a possible link to the incidence of various cancers in man, and therefore, the presence of *N*-nitroso compounds in foods is regarded as an etiological risk factor (Oshima *et al.* 1984; Sen 1986; Raoul *et al.* 1997). Doll and Peto (1981) estimated that 35% of all cancer in humans is of dietary origin. Peters *et al.* (1994) reported that children who ate more than 12 nitrite-cured hot dogs per month showed an increased risk in developing childhood leukemia. Although the scientists cautioned that their findings were preliminary, they suggested that it might be prudent for parents to consider reducing ingestion of hot dogs for themselves, as well as their children, where consumption frequencies are high. On the other hand, all humans excrete the non-carcinogenic amino acid derivative *N*-nitrosoproline in

their urine. This finding and other studies clearly demonstrate that *N*-nitroso compounds are formed within the human body (Loeppky 1994). Therefore, it is important to ask whether *N*-nitrosamines are human carcinogens.

Chemistry of Nitrosation

N-Nitroso compounds are usually divided into two broad categories, namely *N*-nitrosamines derived from secondary amines containing dialkyl, alkylaryl and diaryl substituents, and *N*-nitrosamides derived from *N*-alkylureas, *N*-alkylcarbamates and simple *N*-alkylamides (Tricker and Kubacki 1992). *N*-Nitrosamides can also be formed from cyanamides (Mirvish *et al.* 1973), guanidines, amidines (Walser *et al.* 1974), hydroxylamines, hydrazones and hydrazides (Smith 1966). *N*-Nitrosamides are not as stable as *N*-nitrosamines due to the joining of two very electropositive functional groups (NNO and CO). Nitrite can also react with phenols and other aromatics to yield diazonium salts and diazoquinones. Such reaction products are direct mutagens and carcinogens, but require two or three nitrite molecules for each aromatic compound (Wakabayashi *et al.* 1989).

The study of the chemistry of aliphatic *N*-nitrosamines began in 1863 when Geuther obtained *N*-nitrosodiethylamine by the reaction of diethylamine hydrochloride with sodium nitrite (Crosby and Sawyer 1976). Since then, the chemistry of secondary amine nitrosation has been extensively reviewed by numerous researchers (Mirvish 1975; Douglass *et al.* 1978; Challis and Challis 1982; Williams 1983). Briefly, *N*-nitrosamines are formed by the nitrosation of organic amines (notably secondary amines), decarboxylated amino acids and other amino substances found in muscle tissue such as urea or amides. Although the nitroso moiety (— NO) can react with nitrogen, carbon or sulfur atoms, it is the *N*-nitroso product that has attracted the greatest attention because of the carcinogenicity of numerous compounds containing it. Thus, *N*-nitrosamines are classified as from the family "*N*-nitroso compounds" because all share the N — N = O functional group.

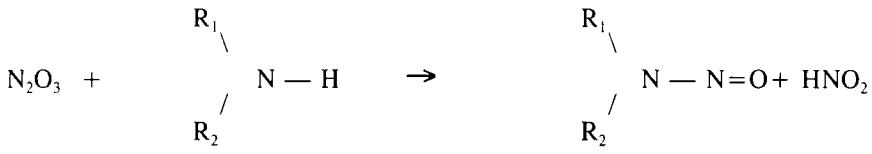
Nitrite itself is not the main nitrosating species, rather it is one of nitrite's derivatives. Nitrite is the conjugate base of a weak acid, nitrous acid (*i.e.*, HNO_2) whose pKa is 3.36.

$$HNO_2 \rightleftharpoons H^+ + NO_2^-$$

The classical method for producing a nitrosating agent for a chemical reaction is by treating nitrite with a strong acid to give nitrous acid. Consequently, the acidity of the aqueous medium will determine the relative proportions of the nitrosating species (Ridd 1961). The reaction between a secondary amine and nitrous acid requires both reactants to be in the undissociated form and proceeds

most readily at a pH around 3.5. At lower pH values, the increasing protonation of the amine tends to suppress nitrosation, while at higher pH values, the concentration of nitrite as nitrous acid decreases. Because the pH of meat and fish (*i.e.*, usually 5.5-6.5) is well above the pKa of HNO_2, its concentration in cured muscle foods is low, somewhere between 0.1 and 1.0% of the added nitrite. It is believed that the principal reactive species is the anhydride of HNO_2, dinitrogen trioxide (N_2O_3).

$$2HNO_2 \rightleftharpoons N_2O_3 + H_2O$$



The rate of nitrosation reactions is given by the product $k_1*[R_1R_2NH]*[HNO_2]^2$ in which the rate constant is independent of pH. Primary amines are not considered to be precursors of *N*-nitrosamines, as nitrosation reactions generally proceed *via* diazotization and nucleophilic replacement of the amino group. However, nitrosation of the simplest aliphatic amine, namely methylamine, results in a complex mixture of products that includes NDMA (Tricker and Kubacki 1992). Most likely NDMA is derived from creatine, a recognized constituent of muscle, through its breakdown to sarcosine, followed by the decarboxylation of its *N*-nitroso derivative (Walters 1992). More complex primary aliphatic amines yield elimination, substitution and rearrangement products (Mende *et al.* 1989).

Nitrosation reactions can be catalyzed or hindered by many factors. For example, anions such as iodide, bromide, chloride, thiocyanate, acetate, phthalate and weak acids exert a catalytic effect (Ridd 1961). On the other hand, any compound that can react with nitrite including primary amines, sulfhydryl compounds, certain phenols and tannins will inhibit the formation of *N*-nitroso compounds. Of these types of compounds, the most important one in meat products is ascorbate (Tricker and Kubacki 1992).

Carcinogenicity of *N*-Nitroso Compounds

N-nitroso compounds may have a significant role in human carcinogenesis. The ubiquity of their precursors leads to the ready formation of *N*-nitrosamines and other *N*-nitroso compounds in the environment. Therefore, there is a continuing need to understand the extent and mechanisms of *N*-nitroso compound formation as well as the mechanisms of their bioactivation and detoxification.

Recent discoveries have shown several pathways for the endogenous formation of *N*-nitroso compounds. Ingested or endogenous nitrogenous substances can react with nitrous acid in the stomach or be nitrosated elsewhere by nitrosating agents arising from the endogenous formation of NO or the bacterial reduction of nitrate (Loeppky and Michejda 1994). Although there is some understanding of the mechanism of carcinogenicity for *N*-nitrosamines, little is known about the carcinogenicity of non-volatile *N*-nitroso compounds; yet some *N*-nitrosodipeptides display mutagenic activity *in vitro* (Walker 1990). In the case of cured meats, however, the greatest concerns are for the *N*-nitrosamines that are formed during meat processing operations and when the product is cooked (*e.g.*, fried) before consumption.

N-Nitrosamines constitute a family of potent carcinogens that are formed readily from a diverse set of nitrogen-containing compounds, and nitrite or its derivatives (*i.e.*, nitrogen in a formal 3+ oxidation state). Although the carcinogenic properties of *N*-nitrosamines and other *N*-nitroso compounds have been known for over 35 years, the biochemistry and chemistry of these compounds is sufficiently complex that many of the answers sought by researchers have been slow to emerge. Careful dose response studies have shown that greater than 90% of the more than 300 *N*-nitrosamines tested for carcinogenicity have a high degree of potency, and NDMA was found to be carcinogenic in more than 20 animals (Loeppky 1994). More importantly, no species seems to be exempt from the carcinogenicity of these compounds.

N-Nitrosamines have the interesting characteristic of being relatively organ specific carcinogens. In rodents, they induced tumors in the liver, kidney, esophagus, oral and nasal cavities, lung, trachea, urinary bladder, pancreas and thyroid. *N*-Nitrosamides induced tumors in the glandular stomach, small intestine, brain, peripheral nervous system, bone and skin. They have also caused acute myelocytic leukemia as well as T and B cell lymphoma (Mirvish 1991). A list of some carcinogenic *N*-nitrosamines is provided in Table 8.1, together with some of the targets for their carcinogenicity (Watson 1993). Furthermore, *N*-nitrosamines are most effective as carcinogens in experimental animals when applied in repeated small doses as compared with larger single applications. This situation is akin to the exposure of humans to traces of carcinogens on a day-to-day basis (Walters 1992).

Investigation of the mechanisms of carcinogenesis by *N*-nitroso compounds began with studies by Magee and co-workers on the properties of NDMA as an alkylating agent. The main difference between the action of *N*-nitrosamines and *N*-nitrosamides is that *N*-nitrosamines require enzymatic activation for conversion to carcinogens whereas *N*-nitrosamides can act directly. *N*-Nitrosamines are hydroxylated (*i.e.*, activated) at positions adjacent to the NO moiety by cytochrome P450 isozymes to yield α-hydroxy derivatives (Mirvish 1995; Shu and Hollenberg 1996). After activation, these derivatives lose aldehydes to

TABLE 8.1
SPECIFIC ORGANS TARGETED BY *N*-NITROSAMINES

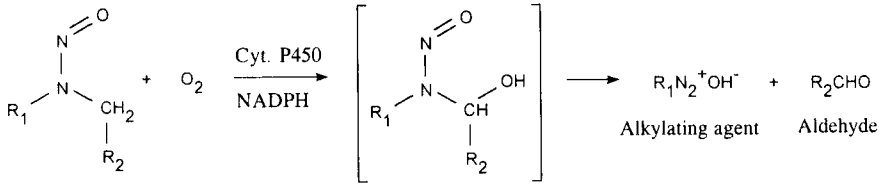
Some *N*-nitrosamines	Carcinogenic[1]	Main target organs
N-nitrosodiethylamine	yes	Liver, kidney, lung, oesophagus, forestomach
N-nitrosodi-*n*-propylamine	yes	Liver, lung, oesophagus
N-nitrosodi-*n*-butylamine	yes	Liver, bladder, forestomach, oesophagus, lung
N-nitrosopyrrolidine	yes	Liver, lung
N-nitrosopiperidine	yes	Liver, GI tract, respiratory tract
N-nitrosomethylbenzylamine	yes	GI tract
N-nitrosodiethanolamine	yes	Liver, respiratory tract
N-nitrosoproline	no	--

[1]After oral exposure in rats and/or mice.
(From Watson 1993).

form monoalkylnitrosamines which yield an agent that can alkylate DNA bases and other macromolecules in various organs. Mutations result that eventually give rise to tumors and cancers (see Fig. 8.1) (Shuker and Bartsch 1994). *N*-Nitrosamides, which already have oxygen bonded to a carbon atom adjacent to the N — N = O functional group, decompose directly to yield alkylating agents (Mirvish 1991). Thus, a general requirement for carcinogenicity to appear in *N*-nitrosamines is a free α-carbon at which hydroxylation can take place in the metabolic bioactivaiton process (Massey *et al.* 1991). Many *N*-nitrosamines undergo complex metabolism in animals and several intermediate products are formed. These intermediates themselves might be carcinogenic to different organs, perhaps explaining partially the variety of organ-specific effects among these structurally-related carcinogens (Lijinsky 1994).

DNA studies have shown that exposure of animals to *N*-nitroso carcinogens gave rise to tumors in some, but not all, of the organs in which methylated DNA was detected. This suggests that alkylation of DNA itself is not sufficient to lead to the formation of tumors in the absence of other factors, which at present are unknown. Further evidence of this observation comes from cyclic *N*-nitrosamines which comprise nearly 25% of the known *N*-nitroso compounds that have been tested for carcinogenicity. These cyclic *N*-nitrosamines are structurally restricted from forming an alkylating agent, but have shown a

similar carcinogenic effect in different species to those of their acyclic analogs. Yet those that have been examined thus far have produced very little or undetectable quantities of DNA alkylation in tissues and organs of experimental animals. This suggests that actions of the cyclic *N*-nitrosamines other than alkylation of DNA are of prime importance in inducing tumors.



N-Nitrosamine

FIG. 8.1. FORMATION AND DECOMPOSITION OF AN α-HYDROXY *N*-NITROSAMINE (From Loeppky, 1994).

Detection of *N*-Nitrosamines

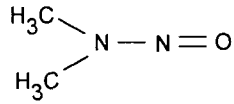
N-Nitrosamines are both volatile and non-volatile in nature. Due to difficulties inherent in extraction/concentration methodologies and separation techniques for the analysis of non-volatile *N*-nitroso compounds, fewer reports can be found than those of volatile counterparts. Yet having said this, in 1969 Sen *et al.* published one of the first articles that critically examined the techniques employed to detect volatile *N*-nitrosamines in foods. These authors found that many of the previously published methods were unreliable or otherwise inadequate. On account of these unconfirmable reports appearing in the scientific literature, the International Agency for Research on Cancer in 1971 adopted a policy for the improvement of techniques so as to assess the "true content" of *N*-nitrosamines in foods and beverages (Bogovski 1972). Although improvements in extraction techniques, alkali flame ionization detectors, Coulson electrolytic conductivity detectors and coupling of gas chromatographs to mass spectrometers were made, it was not until the development of the thermal energy analyzer (TEA) that reliable volatile *N*-nitrosamine results could be obtained.

Volatile *N*-Nitrosamines (VNAs)

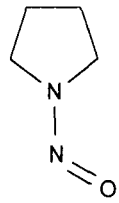
Various studies have confirmed the presence of volatile *N*-nitrosamines (VNAs) in cured products from red meat species, but there appears to be discrepancies as to both the qualitative and quantitative nature of findings reported in the literature. The term VNA is usually designated for all *N*-

nitrosated derivatives of simple low molecular weight dialkylamines and cyclic compounds which can be isolated in good yields (> 70%) from food matrices by aqueous distillation (*i.e.*, steam, atmospheric or vacuum) and analyzed by gas chromatography without derivatization (Tricker and Kubacki 1992). Examples of VNAs isolated from cured meats include simple dialkyl compounds, such as NDMA and *N*-nitrosodiethylamine (NDEA), as well as some relatively low-molecular weight cyclic compounds, such as NPYR and *N*-nitrosothiazolidine. Many factors, such as mode of cooking, cooking temperature and time, nitrite concentration, salt concentration, pH and the presence and concentration of ascorbate affect the potential for *N*-nitrosamine formation (Sen *et al.* 1979; 1985). The VNAs in bacon have received a great deal of attention. Trace levels (*ca.*, 1 μg/kg) of NDMA are occasionally detected in cured meat products, whereas NPYR is found consistently in fried bacon at levels up to 20 μg/kg (Gough *et al.* 1976). Although the origin of NPYR has not been conclusively established, its formation is dependent on frying temperature. Decarboxylation of *N*-nitrosoproline (NPRO) formed by the reaction of proline with nitrite has been proposed as the major route of NPYR formation. Some of the common VNAs which have been isolated from cured meat products are depicted in Fig. 8.2.

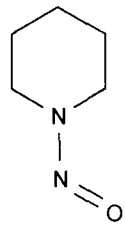
As stated above, the simplest and most effective method for the determination of minute quantities of VNAs (at the μg/kg level) in food and animal feed involves the use of a gas chromatograph (GC) coupled to a thermal energy analyzer (TEA) detector after the VNAs have been isolated from the meat matrix. Samples to be analyzed for VNAs are divided into three categories: (1) solid or semi-solid foods containing a relatively small amount of fat and oil (< 2%: *e.g.*, fish paste products, salted and semi-dried fish of lower oil content, fermented vegetables); (2) samples containing large amounts of fat and oil (> 2%: *e.g.*, salted and fresh fish and shellfish, cured meat products, dairy products); and (3) beverages or liquid foods (> 90% moisture content: *e.g.*, beers, wines, vinegar, fermented fish sauce). The most commonly used method is based on a vacuum distillation prior to liquid-liquid extraction and concentration steps followed by gas chromatography (Mavelle *et al.* 1991). The main advantage of the distillation technique is that it serves as an excellent clean-up step for separating VNAs from the bulk of the food matrix in one fraction, thereby eliminating the need to develop separate clean-up procedures for individual compounds (Sen and Kubacki 1987). However, since cured meats contain residual nitrite, efforts must be made to ensure that the nitrosation of constituent amines does not occur and lead to artifactual formation during VNA isolation. This is achieved by the addition of sulfamic acid, an efficient scavenger of nitrite. The GC is then used as a tool to separate the isolated VNAs and the TEA is employed for their detection.



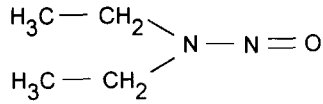
N-Nitrosodimethylamine (NDMA)



N-Nitrosopyrrolidine (NPYR)



N-Nitrosopiperidine (NPIP)



N-Nitrosodiethylamine (NDEA)

FIG. 8.2. CHEMICAL STRUCTURES OF SOME VOLATILE *N*-NITROSAMINES

As an alternative to the classical distillation approach for isolating *N*-nitrosamines, Pensabene and co-workers developed extraction methods involving solid-phase extraction (SPE) (Pensabene *et al.* 1992; Pensabene and Fiddler 1994) and supercritical fluid extraction (Fiddler and Pensabene 1996). Raoul *et al.* (1997) modified the SPE technique described by Pensabene and co-workers so that it no longer required large quantities of solvents, and would employ a commercial cartridge necessary for standardization and reproducibility of the

method for multiple analyses. Results showed that eight volatile and semivolatile *N*-nitrosamines (*i.e.*, NDMA, NPYR, NDEA, *N*-nitrosodi-*n*-propylamine [NDPA], *N*-nitrosoethylmethylamine [NEMA], *N*-nitrosomorpholine [NMOR], and *N*-nitrosopiperidine [NPIP]) could be analyzed by the SPE method without affecting the sensitivity, which was 0.3 μg/kg for all volatile *N*-nitrosamines except the internal standard (1.7 μg/kg).

Thermal Energy Analyzer (TEA)

Originally, a nitrogen-selective alkali flame ionization detector was used to detect separated *N*-nitrosamines (Palframan *et al.* 1973). This was followed by the Coulson electrolytic conductivity detector, which offered a much higher selectivity compared to the alkali flame ionization detector. Unfortunately, these GC detectors are non-specific to *N*-nitroso compounds, and are therefore only useful for screening purposes; confirmation by GC-MS is still mandatory. In the mid-1970s, Fine and Rounbehler (1975) developed a new type of detector, the TEA or thermoluminous analyzer, which is highly sensitive and specific for all N — NO bonds. Thus, a major advance in the analytical technique for *N*-nitrosamine detection was achieved when Thermo Electron of Waltham, Massachusetts, introduced the TEA. This unit was a watershed in *N*-nitrosamine research because it became possible to screen a large number of samples (*e.g.*, 12 or more per day per analyst) with only a minimum of preparation (Fig. 8.3).

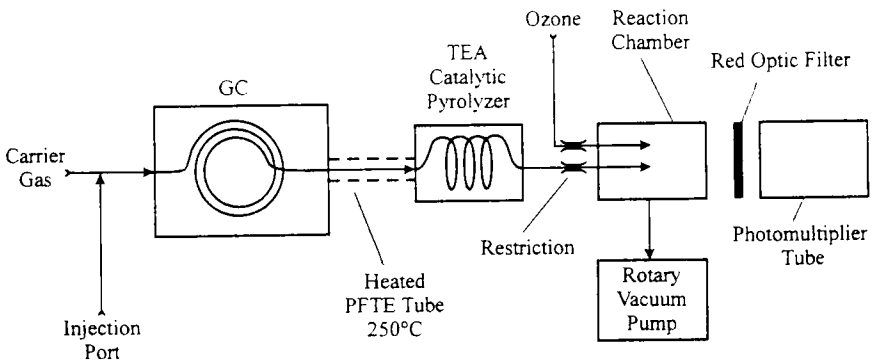
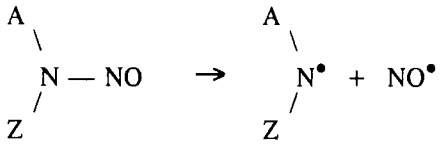


FIG. 8.3. SCHEME FOR THE GAS CHROMATOGRAPH-THERMAL ENERGY ANALYZER INTERFACE
(From Fine and Rounbehler 1975).

As an analytical tool, the selectivity of the instrument for detecting sub μg/kg quantities of *N*-nitroso compounds in complex biological materials and foodstuffs without the elaborate clean-up procedures was far beyond anything else available (Hotchkiss 1987). With present instrumentation, the high specificity and sensitivity associated with TEA enables less than 50 pg of *N*-nitrosamine to be detected following GC separation (Raoul *et al.* 1997). Mass spectrometry is still necessary to confirm positive findings of trace levels of *N*-nitrosamines (both VNA and NVNC) isolated from complex matrices such as meat. Either high- or low-resolution mass spectrometry can be used effectively for identification purposes. Gas chromatography-mass spectrometry not only allows accurate determination of the concentration of a particular *N*-nitrosamine in complex substrates, but also serves as an invaluable tool in the characterization of unknown compounds.

The TEA was designed and developed for the sole purpose of analyzing *N*-nitroso compounds with high sensitivity and selectivity. It is a gas phase technique with the essential chemical reactions occurring in the gas phase and/or at the solid-gas interface (Fine *et al.* 1975). In simplistic terms, the TEA is a modified chemiluminescence detector that relies on thermal cleavage of the N — NO bond (*i.e.*, the weakest bond in *N*-nitroso compounds) in the presence of a selective catalyst (*i.e.*, WO_3 and $W_{20}O_{58}$) to release NO (*n.b.*, in diagram below, A and Z may be any organic radical; Fine and Rounbehler 1975; Fine *et al.* 1975).



The pyrolyzer inlet is constructed in a manner similar to a GC injection port, with provision for preheated carrier gas, a septum and heaters for maintaining the temperature in the range of 275-300°C. The GC effluent is introduced directly into the TEA where the *N*-nitroso compound is flash pyrolyzed. In order to prevent condensation or adsorption of non-volatile components, the TEA pyrolyzer is coupled directly to the GC column. However, if this is not possible, a heated teflon poly(tetrafluoroethylene) (PTFE) line maintained above 200°C has been used (Fine and Rounbehler 1975). It should also be pointed out that although many compounds can pyrolyze in this temperature range, only a compound possessing a labile nitroso group will release NO.

The organic radical formed upon pyrolysis will either decompose further or rearrange itself to yield a stable product which, together with the solvent vapor and the NO, is swept through a capillary restriction into an evacuated reaction chamber. This chamber is maintained at a reduced pressure by a rotary vacuum

pump. Nitric oxide is then oxidized using ozone, generated by a high voltage electric discharge in oxygen, under reduced pressure to give an electronically excited NO_2^* molecule. The excited NO_2^* rapidly decays back to its ground state with the emission of radiation in the near infrared-region of the spectrum (between 0.6 to 0.8 μ).

$$NO^\bullet + O_3 \rightarrow NO_2^* + O_2$$

$$NO_2^* \rightarrow NO_2 + h\nu$$

This radiation in the near-infrared region of the spectrum is detected by a photomultiplier tube in conjunction with a red optic filter and then amplified electronically. The intensity of the emission is proportional to the concentration of NO, and hence to that of the *N*-nitroso compound. The red filter is used to eliminate light of wavelengths shorter than 0.6 μ which may be present from such potential interfering chemiluminescent reactions of ozone with carbon monoxide, ethylene and other compounds. In the wavelength region between 0.6 and 3.0 μ, photomultiplier tubes have poor spectral response characteristics and only a small part of the distribution out to 0.8μ is useful. Thus, by exploiting a combination of a red cut-off filter and poor photomultiplier tube characteristics, only the narrow region between 0.6 and 0.8μ is monitored (Fine *et al.* 1975). Because of the unique series of chemical reactions involved, the analyzer is sufficiently selective to be used for screening purposes. Almost theoretical yields of NO are obtained from the most simple VNAs by this procedure.

There are two limitations to this technique. Firstly, *N*-nitroso compounds must be volatile or made volatile (*i.e.*, derivatization) for GC separation and secondly, they must yield NO upon pyrolysis. Unlike *N*-nitrosamines, *N*-nitrosamides and related compounds typically rearrange upon thermolysis to yield molecular nitrogen instead of NO, and are only weakly detected by the TEA (Conboy and Hotchkiss 1989). Thus, the TEA is not sensitive enough to be useful as a detector for *N*-nitrosamides.

Non-Volatile *N*-Nitroso Compounds (NVNCs)

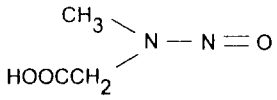
Unlike VNAs, the presence of non-volatile *N*-nitroso compounds (NVNCs) has not been widely reported, perhaps because their non-volatile character does not easily facilitate their isolation from foodstuffs and has hindered development of adequate analytical techniques. As the name implies, NVNCs tend to be higher in molecular weight, or more polar, and hence possess relatively low vapor pressures. According to Tricker and Kubacki (1992), they are defined as *N*-nitroso compounds which are not amenable to isolation *via* distillation and/or analysis by gas chromatography without prior derivatization. Hundreds of

NVNCs may be present in cured meats because any substance with an —NH— group can conceivably form a NVNC (Sen and Kubacki 1987). An assessment of the overall concentration of all *N*-nitroso compounds (*i.e.*, volatile and non-volatile) in cured meats was obtained using a chemical denitrosation/ chemiluminescence detection procedure described by Walters *et al.* (1978). Although this procedure provides no information on the levels of individual *N*-nitrosamines and may be subject to interference from some non-nitroso compounds, the technique nonetheless showed that the concentration of total *N*-nitroso compounds in cured meats is in the range of 0.5-5.0 mg/kg (Massey *et al.* 1986). Comparison of these levels to those of VNAs and *N*-nitrosoamino acids, the most predominant NVNCs, suggests that a large majority of *N*-nitroso compounds in cured meats are of unknown identity.

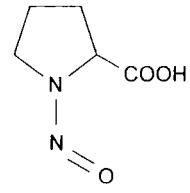
Judging from the wide variety of complex secondary amines occurring in nature, a large number of high-molecular-weight *N*-nitroso compounds are to be expected. As a result, such compounds would be non-volatile, and therefore not amenable to GC analysis or to clean-up by steam or vacuum distillation. Alternative methods of analysis are required for these *N*-nitroso compounds. As described above, techniques for the determination of μg/kg levels of volatile *N*-nitrosamines in foods are well known and their reliability has been established by international collaborative studies (Scanlan and Reyes 1985); however, development of analytical protocols for non-volatile *N*-nitrosamines has been slow and restricted primarily to *N*-nitrosoamino acids; the most predominant being NPRO, *N*-nitrosothiazolidine-4-carboxylic acid (NTCA) and *N*-nitrososarcosine (NSAR) (Fig. 8.4) (Sen and Kubacki 1987; Fiddler *et al.* 1995). Besides these, a wide variety of other NVNCs, such as the hydroxylated *N*-nitrosamines, *N*-nitroso sugar amino acids, *N*-nitrosamides (*e.g.*, *N*-nitrosoureas, *N*-nitrosoguanidines and *N*-nitrosopeptides), and *N*-nitroso derivatives of certain pesticides (*e.g.*, *N*-nitrosocarbaryl, *N*-nitrosoatrazine and *N*-nitrosoglyphosate), could occur in foods or form in the human stomach from ingested nitrite and the corresponding amine or amide precursors present in foods (Sen and Kubacki 1987). The chemical structures of some of these compounds are shown in Fig. 8.5.

Volatile derivatives of NVNCs prepared by methylation or silylation are made to permit direct analysis by GC with TEA detection of nitric oxide and confirmation by GC-MS (Sen and Kubacki 1987). Attempts have also been made to couple the TEA to HPLC units for the detection of NVNCs. In this arrangement, the eluent is passed into the pyrolyzer of the TEA at a high temperature so that it is vaporized and the N — N bond cleaved. The vaporized mobile phase is condensed in cold traps prior to detection of NO by its chemiluminescence. On account of the limited capacity of suitable cold traps, the analysis time is restricted to *ca.* 30 min. As aforementioned, *N*-nitrosamides are subject to rearrangement within the catalytic converter leading to the evolution

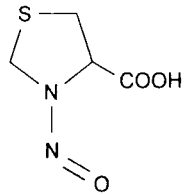
of N_2 rather than the cleavage of NO; hence, the sensitivity of the TEA towards such compounds is markedly reduced (Walters 1992). Yet, this approach has particular advantages in the search for unidentified compounds and in the analysis of any polar compounds which are not amenable to analysis by GC after formation of derivatives.



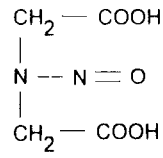
N-Nitrososarcosine (NSAR)



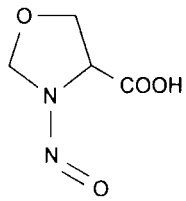
N-Nitrosoproline (NPRO)



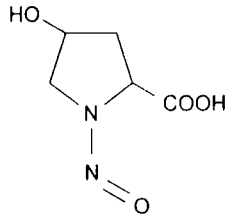
N-Nitrosothiazolidine-4-carboxylic acid (NTCA)



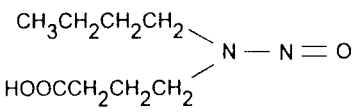
N-Nitrosoiminodiacetic acid



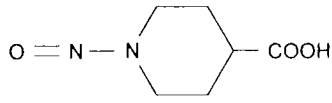
N-Nitrosooxazolidine-4-carboxylic acid (NOCA)



N-Nitroso-4-hydroxy-proline (NHPRO)

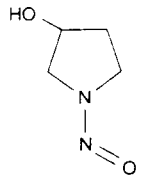


N-Nitroso-*N*-(3-carboxypropyl)-*n*-butylamine

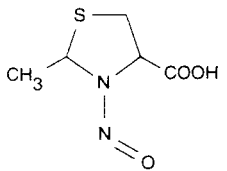


N-Nitrosoisonipecotic acid

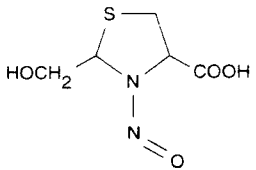
FIG. 8.4. CHEMICAL STRUCTURES OF SOME *N*-NITROSAMINO ACIDS



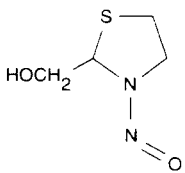
N-Nitroso-3-hydroxy-pyrrolidine



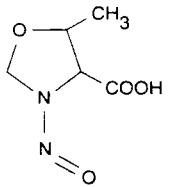
N-Nitroso-2-methyl-thiazolidine-4-carboxylic acid



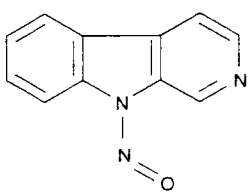
N-Nitroso-2-hydroxymethyl-thiazolidine-4-carboxylic acid



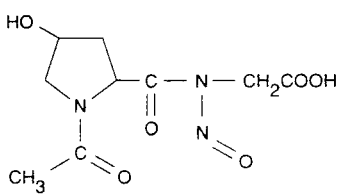
N-Nitroso-2-hydroxymethyl-thiazolidine



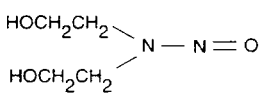
N-Nitroso-5-methyloxazo-lidine-4-carboxylic acid



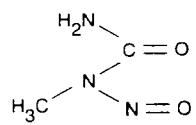
N-Nitrosonorharman



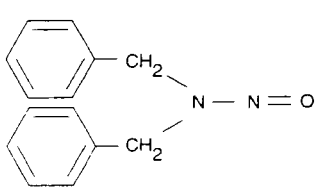
N-Nitroso-D-fructose-L-histidine



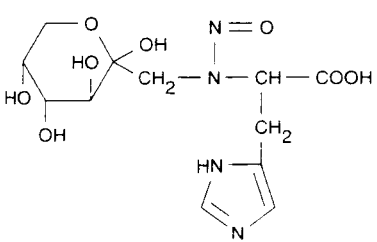
N-Nitrosodiethanolamine



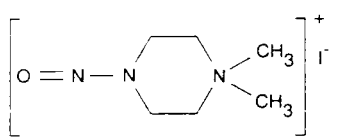
N-Nitroso-*N*-methylurea



N-Nitrosodibenzylamine



N-(*N*-Acetylprolyl)-*N*-nitrosoglycine



N-Nitroso-*N*',*N*'-dimethyl-piperazinium iodide

FIG. 8.5. CHEMICAL STRUCTURES OF SOME NON-VOLATILES *N*-NITROSO COMPOUNDS

Because of a lack of information on the identity of NVNCs to be encountered in cured meat products, several attempts have been made to determine the total content of *N*-nitroso compounds (*i.e.*, sum of all volatile and non-volatile *N*-nitrosamines). The difference between the total *N*-nitroso content from that determined for VNAs gives an estimate of the NVNCs present in a sample. The methods reported for the determination of total *N*-nitroso content of foods fall into two categories. The first deals with photolysis of the compounds by UV light to nitrous acid followed by colorimetric determination of the liberated nitrite. Daiber and Preussmann (1964) used UV light to selectively cleave the N — NO bond to liberate nitrite, which could be detected colorimetrically after addition of Griess reagents. This technique takes advantage of the fact that *N*-nitrosamines are photolabile. Eisenbrand and Preussmann (1970) modified this approach by first chemically denitrosating samples with HBr and then reacting the released nitrite with Griess reagents. In the second method, *N*-nitroso compounds are cleaved chemically by treatment with HBr in a non-aqueous environment under conditions in which the liberated nitrosyl halide is broken down to NO. The released NO is conveyed in a stream of nitrogen into a chemiluminescence analyzer for determination in a similar manner to that of the TEA.

Walters *et al.* (1978, 1983) developed a test assay using HBr in hot glacial acetic acid and subsequent detection of liberated NO by chemiluminescence. The main advantage of this method is that food or its extract can be analyzed directly without the need to isolate *N*-nitrosamines. Samples are first treated with excess sulfamic acid and allowed to sit for a period to ensure that all residual nitrite is completely destroyed. An ethyl acetate aliquot of the product is then added to a distillation flask and refluxed in the presence of HBr and glacial acetic acid. The amount of NO released (A) is determined by a chemiluminescence analyzer. A second aliquot of the test sample is added to a flask containing 0.1% (w/v) HCl and glacial acetic acid, and the released NO is determined (B). Under the latter conditions, *N*-nitroso compounds are stable and gas evolution results from the decomposition of other background compounds. Thus, the difference (A-B) represents NO derived solely from *N*-nitroso compounds within the sample being examined. Unfortunately, this technique has several disadvantages, the most critical of which is its inability to ensure that the response is from *N*-nitroso compounds (Massey *et al.* 1984*b*). Since the total release of NO is determined, it is not possible to identify individual *N*-nitroso compounds, as NO may also be liberated from other *S*-, *C*- or *O*-nitroso species present in the sample or nitrolic acids. Recognition of the non-specificity of this "total" *N*-nitroso method led several investigators to modify the original Walters' protocol and to add "apparent" to the term "total *N*-nitroso compounds" [ATNC] (Bavin *et al.* 1982; Massey *et al.* 1984*a*,*b*; Tricker *et al.* 1985, 1986; Castegnaro *et al.* 1987;

Johnson *et al.* 1987*a*,*b*, 1988; Pignatelli *et al.* 1987; Fiddler *et al.* 1995). Despite its shortcomings, this technique has provided some valuable information on the possible *N*-nitroso compounds in bacon. Massey *et al.* (1984*a*, 1986) noted that the total *N*-nitroso content in raw and fried bacon samples ranged from 470-6000 and 360-2400 μg/kg, respectively.

Havery (1990) reported an improved detection method for the determination of non-volatile *N*-nitrosamines. A postcolumn system is employed to denitrosate the *N*-nitrosamine with potassium iodide in an aqueous mobile phase. The technique can be used without a liquid chromatography column to estimate the ATNC content of samples. Reanalysis with a column can then assist in identifying the compounds responsible for the NO response. The Havery method has some potential advantages over other techniques in that it has the ability to detect labile *N*-nitrosamines. Fiddler *et al.* (1995) modified this method and then analyzed 73 samples of uncooked cured-meat products including frankfurters, bacon and ham. They found that 50 samples analyzed contained less than 1 mg/kg ATNC, expressed as *N*-nitrosoproline equivalents (Table 8.2). A mean value of 0.3 mg/kg ATNC was obtained for frankfurters; no significant difference was noted among the frankfurters made with different meats.

TABLE 8.2
APPARENT TOTAL *N*-NITROSO COMPOUNDS' CONTENT OF CURED MEAT PRODUCTS

Product	*n*	Range, mg/kg[a]	Mean[a]	Number of samples in range[b]			
				ND-1.0	1.1-5.0	5.1-10	> 10
Frankfurters	23	n.d. - 0.8	0.27	23	-	-	-
Bacon	10	n.d. - 1.0	0.50	10	-	-	-
Salami (fermented)	8	0.2 - 2.5	1.31	3	5	-	-
Dried beef	5	0.3 - 3.2	1.17	3	2	-	-
Corned beef (canned)	10	3.9 - 24.8	7.88	-	3	5	2
Ham (canned)	6	0.2 - 1.6	0.79	5	1	-	-
Ham (netted)	4	0.8 - 1.1	0.93	3	1	-	-
Other	7	0.2 - 2.4	1.22	3	4	-	-

[a] Calculation based on response of *N*-nitrosoproline.
[b] n.d., not detected; -, $n = 0$.
(From Fiddler *et al.* 1995)

It should be noted that this ATNC method or others described give no information about individual *N*-nitrosamines; however, all provide data that may be useful in identifying products that have the potential for containing high concentrations of *N*-nitroso compounds (Fiddler *et al.* 1995). As aforementioned, Massey *et al.* (1991) reported concentrations of ATNC in fried bacon ranging from 360 to 2,400 μg (N — NO)/kg; this range is equivalent to 1.2 - 7.9 mg/kg when the calculation is based on the *N*-nitrosoproline response. Yet, the total *N*-nitroso compound levels in bacon were higher than combined concentrations of simple volatile *N*-nitrosamines, *N*-nitrosoamino acids, and other *N*-nitrosamines that currently can be analyzed. On average, only 16% of the total ANTC was accounted for by the sum of the individual *N*-nitrosamines analyzed.

Presence of *N*-Nitrosamines in Cured Muscle Foods

The formation and occurrence of trace quantities of volatile *N*-nitrosamines in various cured meat products has been well-documented over the years. Those commonly reported are NDMA, NPYR, NDEA and NPIP. A number of variables affect the *N*-nitrosamine levels detected in cured meat products. These include residual and in-going nitrite levels, preprocessing procedures and conditions, smoking, method of cooking, temperature and time, lean-to-adipose tissue ratio, and the presence of catalysts and/or inhibitors (Hotchkiss and Vecchio 1985). In general, the levels detected are in the parts per billion range (50-300 μg/kg) and at times sporadic (Walker 1990; see Table 8.3). For example, Sen *et al.* (1979) reported that of 64 samples tested, 39 were negative (*i.e.*, < 0.1 μg/kg) both before and after thermal processing. Fried bacon was a problem item as it consistently contained NPYR and NDMA. In the raw stage, bacon is generally free of *N*-nitrosamines but after high-heat frying, *N*-nitrosamines are found almost invariably. Sen *et al.* (1979) reported that all the fried bacon samples and the cooked-out bacon fats analyzed in their study were positive for *N*-nitrosamines (mainly NPYR and NDMA), although at reduced levels from an earlier investigation (Sen *et al.* 1977). Almost twenty years after this study, NPYR and NDMA are commonly detected in fried bacon samples (Fiddler and Pensabene 1996). Glória *et al.* (1997) reported on the volatile *N*-nitrosamine content determined in 75 samples of traditionally produced pork bacon and 37 samples of 4 bacon-like products after frying. *N*-Nitrosopyrrolidine was detected in every sample of traditional bacon analyzed with a mean value of 6.7 μg/kg and a range of 0.7-25 μg/kg. As expected, the content of NDMA was lower with a mean value of 0.95 μg/kg; it was detected in all but one sample with an upper range of 3.0 μg/kg. The data reported in this study further illustrates the downward trend of volatile *N*-nitrosamines in fried bacon and the meat industry's efforts at curbing their formation. It is also possible that under

such processing conditions (*i.e.*, high-heat frying), bacon cannot be regarded as the only "risk" product in terms of volatile *N*-nitrosamine formation.

TABLE 8.3
MAJOR MUSCLE FOOD SOURCES OF DIETARY *N*-NITROSAMINES[a]

Muscle Food Product	Concentration of *N*-Nitrosamines (μg/kg)			
	NDMA	NDEA	NPYR	NPIP
Bacon (fried)	n.d. - 30	n.d. - 1	n.d. - 200	n.d. - 1
Cured meats	n.d. - 4	n.d. - 4	n.d. - 25	n.d. - 2
Smoked meats	n.d. - 3	n.d. - 7.9	n.d. - 0.1	n.d. - 0.1
Sausages:				
Frankfurter	n.d. - 84	-	-	-
Mettwurst	+	+	n.d. - 105	n.d. - 60
Liver sausage	n.d. - 35	n.d. - 25	n.d. - 80	-
Salami	n.d. - 80	-	-	-
Bologna	-	n.d. - 25	20 - 105	-
Fish:				
Oriental salt-dried	40 - 9000	1200 - 21000	-	-
Smoked nitrate or nitrite cured	4 - 26	-	-	-
Fresh, smoked or salted, UK	1 - 9	-	-	-
Crude salted, Hong Kong	up to 400	-	-	-
Cooked fish and fish products	n.d. - 45	n.d. - 14	n.d. - 6	-

[a] Abbreviations are: *N*-nitrosodimethylamine, NDMA; *N*-nitrosodiethylamine, NDEA; *N*-nitrosopyrrolidine, NPYR; *N*-nitrosopiperidine, NPIP; not detected, n.d.; and detected but not quantitated, +.
(Adapted from Walker 1990)

In the late 1980s, some unusual carcinogenic volatile *N*-nitrosamines, notably *N*-nitrosodi-*n*-butylamine (NDBA), were detected in cured meat products that had been packaged in elastic rubber nettings. This was particularly odd because the amine precursors from which these *N*-nitrosamines were derived are not normally present in meat. Sen *et al.* (1987; 1988) theorized and demonstrated that these *N*-nitrosamines formed as a result of the interaction of nitrite in cured meats with amine additives (*e.g.*, zinc dibutyl- and diethylthiocarbamate) in the rubber nettings. These amines were used as accelerators during vulcaniza-

tion of the rubber. These authors reported finding up to 29 μg/kg of NDBA and 2.4 μg/kg NDEA (N-nitrosodiethylamine) in 16 cured meat products processed in elastic nettings containing rubber. Furthermore, these investigators showed that netted hams had the highest concentration of NDBA in the outermost 5 mm of the surface where there is direct contact with the netting, that this *N*-nitrosamine could also form in the product, and that it could migrate to deeper levels. Similar pork products packaged in cotton nettings or plastic wrappings were devoid of these *N*-nitrosamines. In the 1990s, findings by Sen *et al.* (1993), Pensabene and Fiddler (1994) and Fiddler *et al.* (1997) showed that the principal *N*-nitrosamine now detected in netted hams was *N*-nitrosodibenzylamine (NDBzA) and not NDBA. This suggested that the rubber used for netting had been reformulated with a different accelerator, zinc dibenzyl- instead of dibutyl-dithiocarbamate, so as not to produce the carcinogenic NDBA. Fiddler *et al.* (1997) analyzed the outermost layer from 59 commercial hams for 11 volatile *N*-nitrosamines including NDBA and NDBzA. *N*-Nitrosodibenzylamine was the principal *N*-nitrosamine detected; it was present in 32 (*i.e.*, 54%) of the ham samples at the 10-100 μg/kg range and it exceeded 100 μg/kg in 18 (*i.e.*, 30%) samples, with the highest at 512.2 μg/kg. Fiddler *et al.* (1998) then reported that nettings made with rubber treated with zinc diisobutyldithiocarbamate can produce *N*-nitrosodiisobutylamine (NDiBA) in netted hams. The amine from the vulcanizing agent precursor is sterically hindered to some extent and therefore does not nitrosate as readily as the dibenzyldithiocarbamate precursor. The NDiBA values on the outer surface of netted hams ranged from 4.6 to 33.5 μg/kg (mean 16.1 μg/kg) compared to 52.3 to 739.9 μg/kg NDBzA (mean 191.2 μg/kg) in the control hams.

For many years fish has been cured either directly or by indirect means. One indirect way has been through salting with unrefined salt, as described in Chapter 2. On the other hand, nitrite has been deliberately added to certain types of smoked fish, and NO_x-type gases from incomplete combustion of natural gas or propane have served as nitrosating agents. However, in the mid-1960s, addition of nitrite and nitrate to meat and fish received a great deal of attention due to the presence of potentially carcinogenic *N*-nitrosamines in the thermally processed products. Ironically, it was an incident dealing with fish meal in Norway in the late 1950s which was the first suspicion that foods might be contaminated with *N*-nitroso compounds. Domestic animals that had been fed a fodder mixture containing fish meal prepared from nitrite-preserved herring were dying after a period from severe liver disorders (Kowalski and Cybulski 1974). *N*-Nitrosodimethylamine was later isolated and shown to be responsible for the hepatotoxic disorders in the animals, and it was proposed that the added nitrite had reacted with dimethylamine (DMA) and trimethylamine (TMA), endogenous in the fish meal, to form *N*-nitroso derivatives. These findings raised

questions regarding the "general risk" of using nitrite as a food preservative and stimulated researchers to investigate the occurrence of *N*-nitrosamines in human foods, especially those to which nitrite had intentionally been added (Hotchkiss 1987). Since then, numerous studies have confirmed the presence of *N*-nitrosamines in cured meats; however, discrepancies exist throughout the literature as to both the type and quantities of *N*-nitrosamines reported. Many factors such as mode of cooking, cooking temperature and time, nitrite concentration, salt concentration, pH and presence and concentration of ascorbate affect the potential for *N*-nitrosamine formation (Sen *et al.* 1979; 1985).

For most cured meat products in Canada, nitrite levels have been reduced to a maximum allowable level of 200 mg/kg, except for bacon which was lowered even further to 120 mg/kg, and addition of sodium ascorbate, a *N*-nitrosamine blocking agent, is required at a minimum level of 500 mg/kg (Canadian Food and Drugs Act and Regulations 1981). Such actions have resulted in a decrease in the concentration of volatile *N*-nitrosamines (*e.g.*, NDMA and NPYR) detected in thermally processed products, but not their total elimination. However, in the case of fish and seafood, reduced nitrite addition levels do not work as well at curbing *N*-nitrosamine formation. In order for *N*-nitrosamines to form, a nitrosating agent and an amine or its precursor, are required. Compared to meat, fish has substantially higher levels of amines and thus the likelihood of *N*-nitrosamine formation in smoked and cured fish products is greater.

The concentration of amines in fish depends on various factors such as species, age, environment, bacterial flora and storage conditions (Iyengar *et al.* 1976). Tissues from a variety of marine fish contain relatively high concentrations (20-300 mg/kg) of methylamine derivatives (Kowalski and Cybulski 1974). Often the breakdown of trimethylamine *N*-oxide (TMAO), an osmoregulator in fish, to TMA is used as an indicator of fish freshness. In fish of the gadoid family (*e.g.*, haddock, cod, pollock, hake and whiting), the action of endogenous enzymes will break TMAO down to DMA and formaldehyde. These enzymes operate even when fish is iced. The DMA generated can be nitrosated directly to form NDMA (Fig. 8.6; Pensabene and Fiddler 1988; Pensabene *et al.* 1991).

Addition of nitrite to fish and seafood products, as a means of preservation, is prohibited in countries besides Canada or severely restricted to specific items such as treating of fish roe. In the U.S., the U.S. Department of Agriculture, Food Safety and Inspection Service (FSIS), has been petitioned to amend the standard of identity of cooked sausage and to permit the addition of up to 15% fish protein as an optional ingredient in the form of surimi or minced fish with red meat or poultry (Pensabene and Fiddler 1988). In this way, fish protein from underutilized and non-utilized species can be incorporated as a partial

substitute for meat into cured products such as frankfurters, salami and bologna. Unlike soy protein, surimi has an elastic and chewy texture because of its unique gel-forming characteristics resulting from the high concentration of myofibrillar protein present (Fiddler *et al.* 1992). Not only would a higher value product be formed, but there would be an increase in the nutritional and sensory quality of the fabricated products. The FSIS raised concerns over the possible presence of *N*-nitrosamines, particularly NDMA, in nitrite cured-products. Brooker (1985) had reported that higher levels of NDMA were found in hybrid fish-meat compared to all-meat (control) frankfurters; however, Pensabene and Fiddler (1988) questioned the possibility of artifactual NDMA formation in this study as a result of the method of analysis employed.

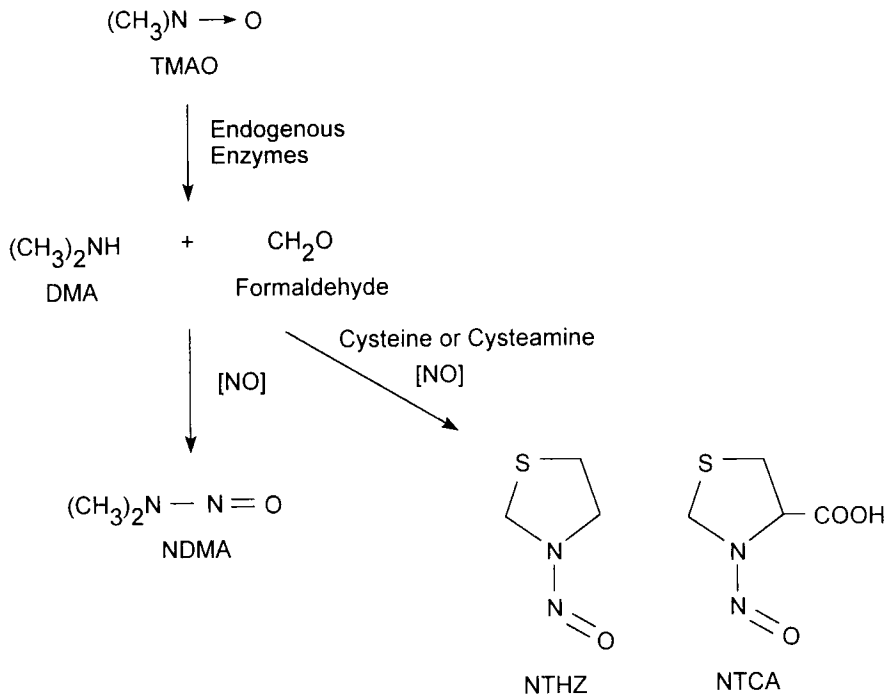


FIG. 8.6. BREAKDOWN OF TRIMETHYLAMINE *N*-OXIDE (TMAO) TO DIMETHYLAMINE (DMA) AND POSSIBLE REACTIONS LEADING TO THE FORMATION OF *N*-NITROSODIMETHYLAMINE (NDMA), *N*-NITROSOTHIAZOLIDINE (NTHZ) AND *N*-NITROSOTHIAZOLIDINE-4-CARBOXYLIC ACID (NTCA).

Fiddler *et al.* (1991) described a method for analysis of minced fish-meat and surimi-meat frankfurters for DMA, TMA and TMAO using a headspace-gas chromatographic technique. While DMA can be nitrosated directly to NDMA, both TMA and TMAO have also been shown to from NDMA. Washing of the mince or surimi manufacture should help to decrease TMAO, TMA and DMA levels and thus the chance of NDMA formation. As illustrated in Fig. 8.7, a dramatic reduction in the concentration of these amines in frankfurters

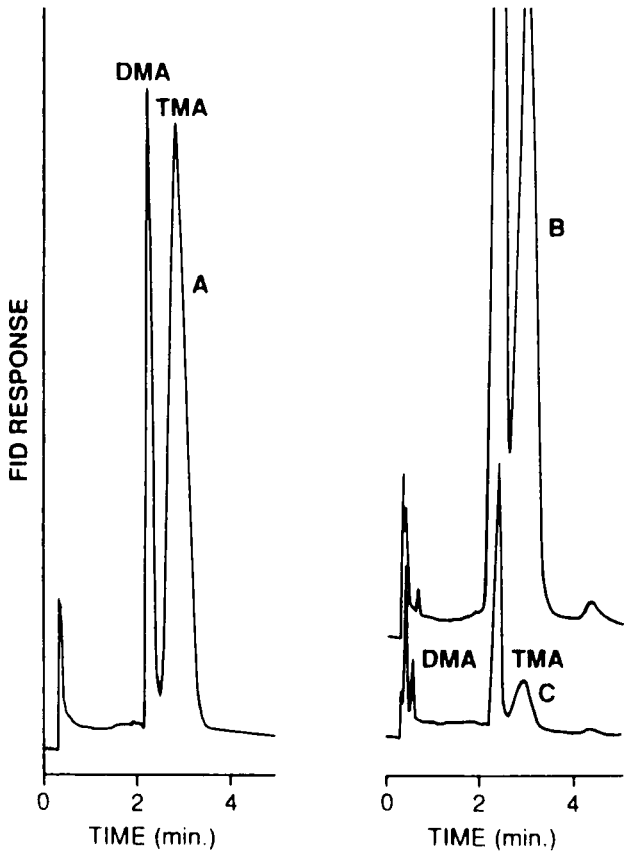


FIG. 8.7. CHROMATOGRAMS OF DIMETHYLAMINE (DMA) AND TRIMETHYLAMINE (TMA): (A) STANDARDS (10 MG/KG); (B) FRANKFURTER SAMPLE CONTAINING 50% ALASKA POLLOCK - UNWASHED MINCE; AND (C) FRANKFURTER SAMPLE CONTAINING 50% ALASKA POLLOCK - WASHED MINCE
(From Fiddler *et al.* 1991).

substituted with 50% Alaska pollock was observed. When comparing chromatogram B (*i.e.*, DMA and TMA levels in a frankfurter sample containing 50% unwashed Alaska pollock mince) and chromatogram C (*i.e.*, DMA and TMA levels in a frankfurter sample containing 50% washed Alaska pollock mince) to that of chromatogram A (*i.e.*, a standard containing 10 mg/kg DMA and TMA), washing of the mince had a significant effect on lowering DMA and TMA concentrations from 191 and 11.4 mg/kg to 4.5 and 0.9 mg/kg, respectively. Although not shown in the chromatogram, TMAO levels for the unwashed mince decreased from 1,112 mg/kg to 207 mg/kg in the washed mince frankfurter samples.

Other studies by Pensabene *et al.* (1991) revealed that the content of NTCA and NTHZ in Alaska pollock surimi-meat frankfurters was similar to or lower than those found in an all-meat control, even at 50% substitution. *N*-Nitrosothiazolidine-4-carboxylic acid and NTHZ are reaction products between formaldehyde and cysteine or its decarboxylated derivative cystamine, respectively, after nitrosation. Occurrence of these *N*-nitrosamines has also been reported in other smoked and cured all-meat products (Sen *et al.* 1986; Fiddler *et al.* 1989).

In the case of Atlantic menhaden (*Brevoortia tyrannus*) mince, the darker color of its surimi compared to that of Alaska pollock makes it unsuitable for fabrication of shellfish analog products; however, the surimi would serve well as a partial substitute for meat in processed products (Hale and Bimbo 1988). Menhaden is a member of the herring family and although NDMA is not a concern in fresh herring, its presence has been detected in salted and nitrate-pickled herring products (Pedersen and Meyland 1981; Sen *et al.* 1985). Table 8.4 shows amine and NDMA contents for menhaden-meat frankfurter formulations (*i.e.*, mince or surimi) before and after thermal processing (Fiddler *et al.* 1993). At the 15% substitution level, NDMA concentrations of 0.8, 0.3 and 0.4 μg/kg were detected for broiled unwashed mince-, washed mince- and surimi-frankfurters, respectively. Fiddler *et al.* (1993) reported that the repeatability of the analytical method was 0.5 μg/kg; hence, employment of any of the three forms of Atlantic menhaden in frankfurters at the 15% degree of substitution would yield little or no detectable NDMA. At 50% substitution, a significant increase in the NDMA concentration for broiled frankfurters that had been prepared with unwashed mince (*i.e.*, 3.1 μg/kg) was observed.

Actions Taken by the Meat Industry

Despite the concern regarding *N*-nitrosamine formation, the meat industry is committed to the use of nitrite in cured products because there is no approved alternative available. In the late 1970s, the Canadian government stated that it would phase out the use of nitrite if safe and effective alternatives became

TABLE 8.4
NDMA, DMA, TMA AND TMAO IN ATLANTIC MENHADEN MINCE AND SURIMI-MEAT FRANKFURTERS[a]

Form	Amines (mg/kg)						NDMA (μg/kg)	
	DMA		TMA		TMAO			
50% Substitution								
Mince								
Unwashed	4.9	[8.2]	9.6	[20]	76	[84]	0.28	[3.1]
Washed	1.1	[1.9]	1.4	[2.4]	9.5	[11]	0.06	[0.8]
Surimi	1.3	[1.7]	2.1	[3.3]	2.1	[11]	0.03	[0.9]
15% Substitution								
Mince								
Unwashed	2.4	[2.9]	3.5	[5.9]	30	[32]	0.13	[0.8]
Washed	1.9	[1.3]	1.2	[1.2]	5.1	[4.8]	0.05	[0.3]
Surimi	0.8	[1.1]	0.7	[2.0]	2.8	[4.4]	0.03	[0.4]

[a] Adapted from Fiddler *et al.* (1993). Values in each column represent amine or NDMA levels in the raw and [broiled] products. Abbreviations are: *N*-nitrosodimethylamine, NDMA; dimethylamine, DMA; trimethylamine, TMA; and trimethylamine *N*-oxide, TMAO.

available (Pim 1979). At the same time, the increased awareness of the dangers of nitrite prompted changes in the Canadian Food and Drugs Act and Regulations (1981) for nitrite and nitrate usage in preserved meats. This had not been totally unexpected since the rate of *N*-nitrosamine formation in meat is directly proportional to the square of the residual nitrite concentration (Mirvish 1970). In 1975, the Health Protection Branch (HPB) of Health Canada reduced the permissible levels of sodium nitrite in bacon from 200 mg/kg to 150 mg/kg, but many processors opted to use an even lower addition level of 120 mg/kg. The HPB also eliminated the addition of nitrate salts to curing formulations from bacon at this time (Sen *et al.* 1977). Similar regulatory changes for nitrite were adopted in the U.S. by the USDA. In 1978, the USDA initiated a bacon monitoring program (Angelotti 1978) under which fried bacon found to contain NPYR at levels of 10 ppb and above was in violation. Furthermore, manufacturers were not permitted to market their product until subsequently produced bacon was found to be in compliance (Glória *et al.* 1997). Undoubtedly, these regulatory changes have resulted in a considerable decrease in the NPYR levels in bacon. Reported mean values of NPYR in fried bacon were 63 μg/kg in 1973 (Fazio *et al.* 1973) and 21 μg/kg in 1982 (Sen and Seaman 1982). It should be pointed out that the necessity for studying the formation and occurrence of *N*-nitrosamines in cured meats and other food systems stems from the absolute

nature of the Food and Drug Regulations of the USDA and other regulatory bodies in Canada and Europe. These regulations deny the use of any food additive which itself is carcinogenic or produces carcinogens in food. Therefore, it is only reasonable that usage of nitrite in cured meats be reduced or phased-out when effective and safe substitutes are found.

Early kinetic research by Mirvish (1970) revealed that the rate of *N*-nitrosamine formation in meat was first order with respect to amine concentration and second order with respect to residual nitrite concentration. The concern over *N*-nitrosamines has led to technological changes in the meat processing industry. These include the elimination of nitrate from most curing applications to allow more complete control of curing reactions, the reduction of nitrite addition levels, particularly for bacon, and the incorporation of *N*-nitrosamine-blocking agents such as sodium ascorbate or its isomer, erythorbate, in cures.

N-Nitrosamine Inhibitors

To help alleviate the *N*-nitrosamine issue, several approaches have been considered by researchers. Because the rate, and therefore the amount of *N*-nitrosamine production depends on the square of the concentration of residual nitrite in meats (Williams 1983; Anon. 1987), a reduction in the level of nitrite added has proven to be an effective measure in reducing the risk of *N*-nitrosamine formation. However, total elimination of *N*-nitrosamines in cured meats is impossible because the precursors (*i.e.*, nitrite, amines and amino acids) remain. There are several scavengers of nitrite which aid in suppressing *N*-nitrosation; ascorbic acid, sodium ascorbate and erythorbate have been the preferred compounds to date. Ascorbic acid inhibits *N*-nitrosamine formation by reducing HNO_2 to give dehydroascorbic acid and NO. Because ascorbic acid competes with amines for HNO_2, *N*-nitrosamine formation is reduced. Ascorbate reacts with nitrite 240 times more rapidly than does ascorbic acid and is therefore the preferred candidate of the two. Although ascorbic acid and its derivatives react with nitrite over a range of pH values, their effectiveness is limited. Even when added at a 2,000 mg/kg level to bacon, the reduction in *N*-nitrosamine formation is only *ca.* 70% and this stems partly from a lack of solubility of ascorbate in adipose tissue (Sebranek 1979). Thus, attention has been focused on the use of lipophilic derivatives of ascorbic acid. The palmitoyl ester of ascorbic acid, L-ascorbyl palmitate (AP), and propyl gallate were found to be more effective than sodium ascorbate in reducing NPYR formation during the frying of bacon when used at a 1,000 mg/kg addition level; however, the efficacy of AP was inconsistent at times (Sen *et al.* 1976).

Other potential anti-*N*-nitrosamine agents tested include long-chain acetals of ascorbic acid, the combination of α-tocopherol and ascorbate, and the use of lactic acid. Bharucha *et al.* (1980) postulated that a good *N*-nitrosamine blocking

agent should satisfy the following requirements: (1) serve as a good NO radical trap; (2) be fat soluble; (3) be non-steam volatile; and (4) be stable up to a maximum frying temperature of about 175°C. These authors reported that long-chain acetals of ascorbic and erythorbic acids (*i.e.*, C_{16} and C_{18}) were effective blocking agents of *N*-nitrosamine formation in bacon cook-out fat (*ca.* 96%) when employed in soybean oil at a 1000 mg/kg level. The C_{16}, C_{18} and $C_{18:1}$ ascorbyl acetals did not impart a soapy aftertaste to bacon samples as did the C_{12} and C_{14} homologues. Furthermore, these authors noted that the long-chain acetals retained their efficacy in the meat even after 35 days at 3°C unlike AP, which tended to lose its activity during storage. Use of α-tocopherol as an inhibitor of *N*-nitrosamine formation in bacon was reported by Fiddler *et al.* (1978), but simultaneous application of a polysorbate emulsifier was required to aid in α-tocopherol's dispersion in the curing brine. Gray *et al.* (1982) reported that α-tocopherol-coated salts in combination with lecithin were very effective inhibitors of NPYR formation in brine-cured bacon. On the other hand, Reddy *et al.* (1982) reported that although dl-α-tocopherol-coated salts inhibited NPYR formation, their employment to dry-cured bacon caused an apparent increase in NDMA levels. Bernthal *et al.* (1986) carried out further studies to determine whether increases in NDMA levels by α-tocopherol-coated salts in dry cured bacon were genuine or artifactual. These investigators found that various levels of α-tocopherol did not contribute to the formation of NDMA nor did the lecithin used to disperse the lipophilic salt in the curing brine.

In some bacon, addition of microbes to convert sugar to lactic acid has been tested. Lactic acid build up results in a lowering of the pH level of bacon and accelerates the breakdown of nitrite. Excellent protection against botulinum toxin formation has been achieved using lactic acid producing bacteria and sucrose in nitrite-free treated bacon. Lowering of the addition level of nitrite to 40 mg/kg, together with starter lactate cultures has been approved by regulation (Thompson, 1985). Despite these results, there has been an effort to discover substitutes for nitrite.

Impact of a Nitrite Ban

If it were possible, the most reliable method to alleviate the *N*-nitrosamine problem would be the total elimination of nitrite from the curing process. Of course a whole new set of problems would develop. A large number of traditional meat products currently available to the consumer would be eliminated, and the economic implications would far outweigh the loss of these foodstuffs. Of the estimated $18 billion annual sales of packaged meats in the U.S. which include deli and non-deli items, $2.5 billion is comprised of cured ham and picnics (Anon. 1993). The sales of these products are projected to increase due to consumer demand for low fat, low calorie, sensory appealing,

and more convenient processed meat products (Fiddler *et al.* 1996). The costs associated with the elimination of nitrite from meat products are numerous (Madsen 1976) and include: (1) possible botulism; (2) reduced income/loss of cash and future markets; (3) reduced employment in farming, meat packing, distribution, and retailing; (4) loss of export markets for pork; (5) depressed trimmings market; (6) losses from closing of facilities; (7) fewer choices for consumers at meat counters; and (8) fewer convenience foods. However, if the *N*-nitrosamine problem is viewed as a major concern, this justifies research for finding a safe nitrite substitute that can preserve the characteristic properties of cured meat products (see Chapter 9).

REFERENCES

Angelotti, R. 1978. Nitrates, nitrites, and ascorbates (or isoascorbates) in bacon. *Fed. Regist.* **43**, 32136–32137.

Anonymous. 1987. Nitrate, nitrite, and nitroso compounds in foods. *Food Technol.* **41**(4), 127–136.

Anonymous. 1993. 46th Annual consumer expenditures study. *Supermarket Business* **48**(9), 65–66, 68, 70, 72, 74, 76, 78, 80, 82–84, 88.

Bavin, P.M.G., Darkin, D.W. and Viney, N.J. 1982. Total nitroso compounds in gastric juice. In *N-Nitroso Compounds: Occurrence and Biological Effects,* ed. H. Bartsch, M. Castegnaro, I.K. O'Neill and M. Okada. IARC Scientific Publications No. 41, International Agency for Research on Cancer, Lyon, France, pp. 337–344.

Bernthal, P.H., Gray, J.I., Mandagere, A.K., Ikins, W.G., Cuppett, S.L., Booren, A.M. and Price, J.F. 1986. Use of antioxidant-coated salts as *N*-nitrosamine inhibitors in dry- and brine-cured bacon. *J. Food Prot.* **49** 58–61.

Bharucha, K.R., Cross, C.K. and Rubin, L.J. 1980. Long-chain acetals of ascorbic and erythorbic acids as antinitrosamine agents for bacon. *J. Agric. Food Chem.* **28**, 1274–1281.

Bogovski, P. 1972. The importance of the analysis of *N*-Nitroso compounds in international cancer research. In *N-Nitroso Compounds. Analysis and Formation*, ed. P. Bogovski, R. Preussmann and E.A. Walker. IARC Scientific Publications No. 3. International Agency for Research on Cancer, Lyon, France, pp. 1–5.

Brooker, J.R. 1985. An evaluation of fish meat as an ingredient in hot dogs — Summary report. National Marine Fisheries Service, Washington, DC.

Canadian Food and Drugs Act and Regulations. 1981. Food additives that may be used as class I preservatives, Table XI. Last update December 1994. Issued by Department of National Health and Welfare, Ottawa, Canada, pp. 67–40B and 72A.

Cassens, R.G. 1990. Nitrite-Cured Meat. A Food Safety Issue in Perspective. Food & Nutrition Press, Trumbull, CT.

Castegnaro, M., Massey, R.C. and Walters, C.L. 1987. The collaborative evaluation of a procedure for the determination of *N*-nitroso compounds as a group. *Food Addit. Contam.* **4**, 37–43.

Challis, B.C. and Challis, J.A. 1982. *N*-Nitrosamines and *N*-nitrosoimines. In *The Chemistry of Amino, Nitroso, and Nitro Compounds and Their Derivatives*, Part 2, ed. S. Patai. John Wiley & Sons, Chichester, UK, pp. 1151–1223.

Conboy, J.J. and Hotchkiss, J.H. 1989. Photolytic interface for high-performance liquid chromatography-chemiluminescence detection of non-volatile *N*-nitroso compounds. *Analyst* **114**, 155–159.

Crosby, N.T. and Sawyer, R. 1976. *N*-Nitrosamines: A review of chemical and biological properties and their estimation in foodstuffs. *Adv. Food Res.* **22**, 1–71.

Daiber, D. and Preussmann, R. 1964. Quantitative colorimetrische Bestimmung organischer N-Nitroso-Verbindungen durch photochemische Spaltung der Nitrosaminbindung. *Z. Anal. Chem.* **206**, 344–352.

Doll, R. and Peto, R. 1981. The causes of cancer: Quantitative estimates of avoidable risks of cancer in the United States today. *J. Natl. Canc. Inst.* **66**, 1191–1308.

Douglass, M.L., Kabacoff, B.L., Anderson, G.A. and Cheng, M.C. 1978. The chemistry of nitrosamine formation, inhibition and destruction. *J. Soc. Cosmetic Chem.* **29**, 581–606.

Eisenbrand, G. and Preussmann, R. 1970. Eine neue Methode zur kolorimetrischen Bestimmung von Nitrosaminen nach Spaltung der *N*-Nitrosogruppe mit Bromwasserstoff in Eisessig. *Arzneim.-Forsch.* **20**, 1513–1517.

Fazio, T., White, R.H., Dusold, L.R. and Howard, J.W. 1973. Nitrosopyrrolidine in cooked bacon. *J. Assoc. Off. Anal. Chem.* **56**, 919–921.

Fiddler, W., Doerr, R.C. and Gates, R.A. 1991. Gas chromatographic method for determination of dimethylamine, trimethylamine, and trimethylamine oxide in fish-meat frankfurters. *J. Assoc. Off. Anal. Chem.* **74**, 400–403.

Fiddler, W. and Pensabene, J.W. 1996. Supercritical fluid extraction of volatile *N*-nitrosamines in fried bacon and its drippings: Method comparison. *J. AOAC Int.* **79**, 895–901.

Fiddler, W., Pensabene, J.W., Doerr, R.C. and Gates, R.A. 1995. Determination of extractable, apparent total *N*-nitroso compounds in cured-meat products. *J. AOAC Int.* **78**, 1435–1439.

Fiddler, W., Pensabene, J.W., Foster, J.M. and Gates, R.A. 1989. N-Nitrosothiazolidine and N-nitrosothiazolidine-4-carboxylic acid in dry-cured bacon. *J. Food Safety* **9**, 225–233.

Fiddler, W., Pensabene, J.W., Gates, R.A and Adam, R. 1998. Nitrosamine formation in processed hams as related to reformulated elastic rubber netting.

J. Food Sci. **63**, 276–278.

Fiddler, W., Pensabene, J.W., Gates, R.A., Custer, C., Yoffe, A. and Phillipo, T. 1997. *N*-Nitrosodibenzylamine in boneless hams processed in elastic rubber nettings. *J. AOAC Int.* **80**, 353–358.

Fiddler, W., Pensabene, J.W., Gates, R.A. and Foster, J.M. 1996. Investigations of nitrosamine reduction in boneless hams processed in elastic rubber nettings. *J. Muscle Foods* **7**, 389–401.

Fiddler, W., Pensabene, J.W., Gates, R.A., Hale, M. and Jahnicke, M. 1992. *N*-Nitrosodimethylamine formation in cooked frankfurters containing Alaska pollock (*Theragra chalcogramma*) mince and surimi. *J. Food Sci.* **57**, 569–571, 595.

Fiddler, W., Pensabene, J.W., Gates, R.A., Jahncke, M.L. and Hale, M.B. 1993. Atlantic menhaden (*Brevoortia tyrannus*) mince and surimi as partial meat substitutes in frankfurters: Effect on *N*-nitrosamine formation. *J. Agric. Food Chem.* **41**, 2238–2241.

Fiddler, W., Pensabene, J.W., Piotrowski, E.G., Phillips, J.G., Keating, J., Mergens, W.J. and Newmark, H.L. 1978. Inhibition of formation of volatile nitrosamines in fried bacon by the use of cure-solubilized α-tocopherol. *J. Agric. Food Chem.* **26**, 653–656.

Fine, D.H., Lieb, D. and Rufeh, F. 1975. Principle of operation of the thermal energy analyzer for the trace analysis of volatile and non-volatile *N*-nitroso compounds. *J. Chromatogr.* **107**, 351–357.

Fine, D.H. and Rounbehler, D.P. 1975. Trace analysis of volatile *N*-nitroso compounds by combined gas chromatography and thermal energy analysis. *J. Chromatogr.* **109**, 271–279.

Glória, M.B.A., Barbour, J.F. and Scanlan, R.A. 1997. Volatile nitrosamines in fried bacon. *J. Agric. Food Chem.* **45**, 1816–1818.

Gough, T.A., Goodhead, K. and Walters, C.L. 1976. Distribution of some volatile nitrosamines in cooked bacon. *J. Sci. Food Agric.* **27**, 181–185.

Gray, J.I. and Randall, C.J. 1979. The nitrite/*N*-nitrosamine problem in meats: An update. *J. Food Prot.* **42**, 168–179.

Gray, J.I., Reddy, S.K., Price, J.F., Mandagere, A. and Wilkens, W.F. 1982. Inhibition of N-nitrosamines in bacon. *Food Technol.* **36**(6), 39–45.

Hale, M.B. and Bimbo, A.P. 1988. The menhaden surimi demonstration plant: A progress report. In *Proceedings of the 12th Annual Tropical and Subtropical Fisheries Technological Conference of the Americas*. Florida Sea Grant, Gainesville, FL, pp. 270–278.

Havery, D.C. 1990. Determination of *N*-nitroso compounds by high-performance liquid chromatography with postcolumn reaction and a thermal energy analyzer. *J. Anal. Toxicol.* **14**, 181–185.

Hotchkiss, J.H. 1987. A review of current literature on *N*-nitroso compounds in foods. *Adv. Food Res.* **31**, 53–115.

Hotchkiss, J.H. and Vecchio, A.J. 1985. Nitrosamines in fired-out bacon fat and its use as a cooking oil. *Food Technol.* **39**(1), 67–73.

Iyengar, J.R., Panalaks, T., Miles, W.F. and Sen, N.P. 1976. A survey of fish products for volatile *N*-nitrosamines. *J. Sci. Food Agric.* **27**, 527–530.

Johnson, P., Pfab, J. and Massey, R.C. 1987*a*. A method for the investigation of free and protein-bound *N*-nitrosoproline in beer. *Food Addit. Contam.* **5**, 119–125.

Johnson, P., Pfab, J. and Massey, R.C. 1988. Development of methods for the characterisation of non-volatile *N*-nitroso compounds in malt. *J. Inst. Brew.* **94**, 19–22.

Johnson, P., Pfab, J., Tricker, A.R., Key, P.E. and Massey, R.C. 1987*b*. An investigation into the apparent total *N*-nitroso compounds in malts. *J. Inst. Brew.* **93**, 319–321.

Kowalski, B. and Cybulski, W. 1974. Nitrates, nitrites and N-nitrosamines in fish meal. *Bull. Vet. Inst. Puławy.* **18**, 96–101.

Lijinsky, W. 1994. Chemical structure of nitrosamines related to carcinogenesis. In *Nitrosamines and Related N-Nitroso Compounds. Chemistry and Biochemistry*, ed. R.N. Loeppky and C.J. Michejda. ACS Symposium Series 553. American Chemical Society, Washington, DC, pp. 250–266.

Loeppky, R.N. 1994. Nitrosamine and *N*-nitroso compound chemistry and biochemistry. In *Nitrosamines and Related N-Nitroso Compounds. Chemistry and Biochemistry*, ed. R.N. Loeppky and C.J. Michejda. ACS Symposium Series 553. American Chemical Society, Washington, DC, pp. 1–18.

Loeppky, R.N. and Michejda, C.J. 1994. Preface. In *Nitrosamines and Related N-Nitroso Compounds. Chemistry and Biochemistry*, ed. R.N. Loeppky and C.J. Michejda. ACS Symposium Series 553. American Chemical Society, Washington, DC, pp. xi–xii.

Madsen, H.C. 1976. Impact of the loss of nitrites on animal agriculture. In *Proceedings of the Meat Industry Research Conference*. American Meat Institute Foundation, Arlington, VA, pp. 35–40.

Magee, P.N. and Barnes, J.M. 1967. Carcinogenic nitroso compounds. *Adv. Cancer Res.* **10**, 163–246.

Massey, R.C., Bayly, J.M., Key, P.E., McWeeny, D.J. and Knowles, M.E. 1984*b*. Total *N*-nitroso group analysis of foods. II. Further studies on the precision and sensitivity of the assay. *Food Addit. Contam.* **1**, 237–244.

Massey, R.C., Key, P.E., Jones, R.A. and Logan, G.L. 1991. Volatile, non-volatile and total *N*-nitroso compounds in bacon. *Food Addit. Contam.*, **8**, 585–598.

Massey, R.C., Key, P.E., McWeeny, D.J. and Knowles, M.E. 1984*a*. The application of a chemical denitrosation and chemiluminescence detection procedure for estimation of the apparent concentration of total *N*-nitroso compounds in foods and beverages. *Food Addit. Contam.* **1**, 11–16.

Massey, R.C., McWeeny, D.J. and Knowles, M.E. 1986. Interactions of nitrites in foodstuffs. In *Interactions of Food Components*, ed. G.G. Birch and M.G. Lindley. Elsevier Applied Science Publishers, London, UK, pp. 117–130.

Mavelle, T., Bouchikhi, B. and Debry, G. 1991. The occurrence of volatile *N*-nitrosamines in French foodstuffs. *Food Chem.* **42**, 321–338.

Mende, P., Spiegelhalder, B., Wacker, C.D. and Preussmann, R. 1989. Trapping of reactive intermediates from the nitrosation of primary amines by a new type of scavenger reagent. *Food Chem. Toxicol.* **27**, 469–473.

Mirvish, S.S. 1970. Kinetics of dimethylamine nitrosation in relation to nitrosamine carcinogenesis. *J. Natl. Cancer Inst.* **44**, 633–639.

Mirvish, S.S. 1975. Formation of *N*-nitroso compounds: Chemistry, kinetics, and *in vivo* occurrence. *Toxicol. Appl. Pharmacol.* **31**, 325–351.

Mirvish, S.S. 1991. The significance for human health of nitrate, nitrite and *N*-nitroso compounds. In *Nitrate Contamination. Exposure, Consequence, and Control*, ed. I. Bogárdi, R.D. Kuzelka and W.G. Ennenga. NATO ASI Series, Series G: Ecological Sciences, Vol. 30, Springer-Verlag, Berlin, Germany, pp. 253–266.

Mirvish, S.S. 1995. Role of *N*-nitroso compounds (NOC) and *N*-nitrosation in etiology of gastric, esophageal, nasopharyngeal and bladder cancer and contribution to cancer of known exposures to NOC. *Cancer Lett.* **93**, 17–48.

Mirvish, S.S., Nagel, D.L. and Sams, J. 1973. Methyl- and ethylnitrosocyanamide. Some properties and reactions. *J. Org. Chem.* **38**, 1325–1329.

Newberne, P.M. 1979. Nitrite promotes lymphoma incidence in rats. *Science* **204**, 1079–1081.

Ohshima, H., O'Neill, I.K., Friesen, M., Pignatelli, B. and Bartsch, H. 1984. Presence in human urine of new sulfur-containing *N*-nitrosamino acids: *N*-Nitrosothiazolidine 4-carboxylic acid and *N*-nitroso 2-methylthiazolidine 4-carboxylic acid. In *N-Nitroso Compounds: Occurrence, Biological Effects and Relevance to Human Cancer*, ed. I.K. O'Neill, R.C. Von Borstel, C.T. Miller, J. Long, and H. Bartsch 1984. IARC Scientific Publications No. 57. International Agency for Research on Cancer, Lyon, France, pp. 77–85.

Palframan, J.F., MacNab, J. and Crosby, N.T. 1973. An evaluation of the alkali flame ionisation detector and the Coulson electrolytic conductivity detector in the analysis of *N*-nitrosamines in foods. *J. Chromatogr.* **76**, 307–319.

Pedersen, E. and Meyland, I. 1981. Nitrate, nitrite, and volatile nitrosamines in pickled fish prepared with addition of nitrate. *Z. Lebensm. Unters. Forsch.* **173**, 359–361.

Pensabene, J.W. and Fiddler, W. 1988. Determination of volatile *N*-nitrosamines in frankfurters containing minced fish and surimi. *J. Assoc. Off. Anal. Chem.* **71**, 839–843.

Pensabene, J.W. and Fiddler, W. 1994. Gas chromatographic/thermal energy analyzer method for *N*-nitrosodibenzylamine in hams processed in elastic

rubber netting. *J. AOAC Int.* **77**, 981–984.

Pensabene, J.W., Fiddler, W. and Gates, R.A. 1992. Solid-phase extraction method for volatile *N*-nitrosamines in hams processed with elastic rubber netting. *J. AOAC Int.* **75**, 438–442.

Pensabene, J.W., Fiddler, W., Gates, R.A., Hale, M., Jahncke, M. and Gooch, J. 1991. *N*-Nitrosothiazolidine and its 4-carboxylic acid in frankfurters containing Alaska pollock. *J. Food Sci.* **56**, 1108–1110.

Peters, J.M., Preston-Martin, S., London, S.J., Bowman, J.D., Buckley, J.D. and Thomas, D.C. 1994. Processed meats and risk of childhood leukemia (California, USA). *Can. Causes Contr.* **5**, 195–202.

Pignatelli, B., Richard, I., Bourgade, M.C. and Bartsch, H. 1987. Improved group determination of total *N*-nitroso compounds in human gastric juice by chemical denitrosation and thermal energy analysis. *Analyst*, **112**, 945–949.

Pim, L.R. 1979. Canadian government position in nitrite in cured meats. In *Additive Alert: A Guide to Food Additives for the Canadian Consumer.* Doubleday Canada Limited, Toronto, ON, pp. 87–90.

Preussmann, R. and Eisenbrand, G. 1984. *N*-Nitroso carcinogens in the environment. In *Chemical Carcinogens. Second Edition, Volume 2,* ed. C.E. Searle. ACS Monograph 182. American Chemical Society, Washington, DC, pp. 829–868.

Preussmann, R. and Stewart, B.W. 1984. *N*-Nitroso carcinogens. In *Chemical Carcinogens. Second Edition, Volume 2,* ed. C.E. Searle. ACS Monograph 182. American Chemical Society, Washington, DC, pp. 643–828.

Raoul, S., Gremaud, E., Biaudet, H. and Turesky, R.J. 1997. Rapid solid-phase extraction method for the detection of volatile nitrosamines in food. *J. Agric. Food Chem.* **45**, 4706–4713.

Reddy, S.K., Gray, J.I., Price, J.F. and Wilkens, W.F. 1982. Inhibition of *N*-nitrosopyrrolidine in dry cured bacon by α-tocopherol-coated salt systems. *J. Food Sci.* **47**, 1598–1602.

Ridd, J.H. 1961. Nitrosation, diazotisation, and deamination. *Quart. Rev. Chem. Soc. (London)* **15**, 418–441.

Scanlan, R.A. and Reyes, F.G. 1985. An update on analytical techniques for *N*-nitrosamines. *Food Technol.* **39**(1), 95–99.

Sebranek, J.G. 1979. Advances in the technology of nitrite use and consideration of alternatives. *Food Technol.* **33**(7), 58–62, 93.

Sen, N.P. 1986. Formation and occurrence of nitrosamines in food. In *Diet, Nutrition, and Cancer: A Critical Evaluation, Volume II. Micronutrients, Nonnutritive Dietary Factors, and Cancer,* ed. B.S. Reddy and L.A. Cohen. CRC Press, Inc., Boca Raton, FL, pp. 135–160.

Sen, N.P. and Baddoo, P.A. 1997. Trends in the levels of residual nitrite in Canadian cured meat products over the past 25 years. *J. Agric. Food Chem.* **45**, 4714–4718.

Sen, N.P., Baddoo, P.A. and Seaman, S.W. 1986. *N*-Nitrosothiazolidine and *N*-nitrosothiazolidine-4-carboxylic acid in smoked meats and fish. *J. Food Sci.* **51**, 821–825.

Sen, N.P., Baddoo, P.A. and Seaman, S.W. 1987. Volatile nitrosamines in cured meats packaged in elastic rubber nettings. *J. Agric. Food Chem.* **35**, 346–350.

Sen, N.P., Baddoo, P.A. and Seaman, S.W. 1993. Nitrosamines in cured pork products packaged in elastic rubber nettings: An update. *Food Chem.* **47**, 387–390.

Sen, N.P., Donaldson, B., Seaman, S., Collins, B. and Iyengar, J.R. 1977. Recent nitrosamine analyses in cooked bacon. *Can. Inst. Food Sci. Technol. J.* **10**, A13–A15.

Sen, N.P., Donaldson, B., Seaman, S., Iyengar, J.R. and Miles, W.F. 1976. Inhibition of nitrosamine formation in fried bacon by propyl gallate and L-ascorbyl palmitate. *J. Agric. Food Chem.* **24**, 397–401.

Sen, N.P. and Kubacki, S.J. 1987. Review of methodologies for the determination of nonvolatile *N*-nitroso compounds in foods. *Food Addit. Contam.* **4**, 357–383.

Sen, N.P. and Seaman, S.S. 1982. A rapid liquid-liquid extraction cleanup method for the determination of volatile N-nitrosamines in cooked-out bacon fat. *J. Agric. Food Chem.* **30**, 364–367.

Sen, N.P., Seaman, S.W., Baddoo, P.A. and Weber, D. 1988. Further studies on the formation of nitrosamines in cured pork products packaged in elastic rubber nettings. *J. Food Sci.* **53**, 731–734, 738.

Sen, N.P., Seaman, S. and Miles, W.F. 1979. Volatile nitrosamines in various cured meat products: Effect of cooking and recent trends. *J. Agric. Food Chem.* **27**, 1354–1357.

Sen, N.P., Smith, D.C., Schwinghamer, L. and Marleau, J.J. 1969. Diethylnitrosamine and other *N*-nitrosamines in foods. *J. Assoc. Off. Anal. Chem.* **52**, 47–52.

Sen, N.P., Tessier, L., Seaman, S.W. and Baddoo, P.A. 1985. Volatile and nonvolatile nitrosamines in fish and the effect of deliberate nitrosation under simulated gastric conditions. *J. Agric. Food Chem.* **33**, 264–268.

Shu, L. and Hollenberg, P.F. 1996. Identification of the cytochrome P450 isozymes involved in the metabolism of *N*-nitrosodipropyl-, *N*-nitrosodibutyl- and *N*-nitroso-*n*-butyl-*n*-propylamine. *Carcinogenesis* **17**, 839–848.

Shuker, D.E.G. and Bartsch, H. 1994. DNA adducts of nitrosamines. In *DNA Adducts: Identification and Biological Significance*, ed. K. Hemminki, A. Dipple, D.E.G. Shuker, F.F. Kadlubar, D. Segerbäck and H. Bartsch. IARC Scientific Publications No. 125. International Agency for Research on Cancer, Lyon, France, pp. 73–89.

Smith, P.A.S. 1966. *The Chemistry of Open-Chain Organic Nitrogen Compounds. Volume II.* W.A. Benjamin, New York, NY.

Thompson, K. 1985. USDA proposes to lower bacon nitrite level to 100 ppm; or 40 ppm with starter cultures. *Meat Industry*, June issue, p. 107.

Tricker, A.R. and Kubacki, S.J. 1992. Review of the occurrence and formation of non-volatile *N*-nitroso compounds in foods. *Food Addit. Contamin.* **9**, 39–69.

Tricker, A.R., Perkins, M.J., Massey, R.C. and McWeeny, D.J. 1985. *N*-Nitrosopyrrolidine formation in bacon. *Food Addit. Contam.* **2**, 247–252.

Tricker, A.R., Perkins, M.J., Massey, R.C. and McWeeny, D.J. 1986. Characterization studies on insoluble total *N*-nitroso compounds in bacon adipose connective tissue. *Food Addit. Contam.* **3**, 153–159.

Tricker, A.R. and Preussmann, R. 1991. Carcinogenic *N*-nitrosamines in the diet: occurrence, formation, mechanisms and carcinogenic potential. *Mutat. Res.* **259**, 277–289.

Wakabayashi, K., Nagao, M. and Sugimura, T. 1989. Mutagens and carcinogens produced by the reaction of environmental aromatic compounds with nitrite. *Cancer Surveys* **8**, 385–399.

Walker, R. 1990. Nitrates, nitrites and *N*-nitrosocompounds: a review of the occurrence in food and diet and the toxicological implications. *Food Addit. Contam.* **7**, 717–768.

Walser, A., Fryer, R.I., Sternbach, L.H. and Archer, M.C. 1974. Quinazolines and 1,4-benzodiazepines. LXV (1). Some transformations of chlordiazepoxide. *J. Het. Chem.* **11**, 619–621.

Walters, C.L. 1992. Reactions of nitrate and nitrite in foods with special reference to the determination of *N*-nitroso compounds. *Food Addit. Contam.* **9**, 441–447.

Walters, C.L., Downes, M.J., Edwards, M.W. and Smith, P.L.R. 1978. Determination of a non-volatile *N*-nitrosamine on a food matrix. *Analyst* **103**, 1127–1133.

Walters, C.L., Hart, R.J. and Smith, P.L.R. 1983. Analysis of total *N*-nitroso compounds as a group by denitrosation to nitric oxide, with detection using a chemiluminescence analyser. In *Environmental Carcinogens Selected Methods of Analysis. Volume 6 — N-Nitroso Compounds*, ed. R. Preussmann, G. Eisenbrand, B. Spiegelhalder, I.K. O'Neill and H. Bartsch. IARC Scientific Publications No. 45. International Agency for Research on Cancer, Lyon, France, pp. 295–308.

Watson, D.H. 1993. Nitrate, nitrite and *N*-nitrosamines. In *Safety of Chemicals In Food. Chemical Contaminants*, ed. D.H. Watson. Ministry of Agriculture, Fisheries and Food. Ellis Horwood Limited, London, UK, pp. 63–75.

Williams, D.L.H. 1983. Nitrosation mechanisms. *Adv. Phys. Org. Chem.* **19**, 381–428.

CHAPTER 9

POSSIBLE SUBSTITUTES FOR NITRITE

Kemp (1974) posed the questions: "Are we looking for substitutes that will do all the things that nitrite does, or should we be satisfied with materials that are specific for only one of the effects? If one substitute will affect color and another has bacteriostatic activity, should we use a mixture? And, if or when we find a substance that is effective, what will FDA or meat inspection officials say about it?" In an attempt to answer the first two questions, it seems clear that the possibility of finding a single compound to mimic all functions of nitrite is remote. Nitrite is unique in that this single food additive can afford to meat a characteristic cured color by means of a heat-stable nitrosyl protoheme pigment, a typical cured flavor, an extended period of refrigerated storage to cooked products without the worry of warmed-over flavor (WOF) development and bacteriostatic action against *C. botulinum* spores. Nonetheless, other food additives can reproduce the effects that nitrite bestows to meat and therefore the answer lies in the development of composite non-nitrite curing mixtures. Kemp (1974) further stated that from a sales point of view, color development is the most important function of nitrite, whereas from a health standpoint, bacteriostasis is of paramount importance. On the other hand to the food connoisseur, flavor might be the uppermost criterion, while the antioxidant effect might be the least significant attribute. However, these two effects are ultimately interrelated. A summary of substitutes which reproduce the characteristic properties of nitrite-cured meats is provided below.

Color Characteristics

Visual appearance is a key factor which influences consumers when assessing the quality and palatability of meat and meat products. Of the three major quality factors of meat, namely color, flavor and texture, color might be the most important one because if the food is unattractive in appearance, consumers may never get to enjoy its flavor or texture (Francis 1994). Numerous studies have supported the view that certain colors do, in fact, influence food acceptance (Kostyla and Clydesdale 1978). Although the color of meat may be influenced by its moisture and fat content, ultimately it is the quantity of hemoproteins present, particularly that of myoglobin (Mb) and its relationship with the environment surrounding it, which determines the meat's color (Livingston and Brown 1981; Lawrie 1991; Ledward 1992). As it has been

detailed in Chapter 3, the addition of nitrite to meat, followed by thermal processing, produces a relatively stable pink-colored pigment. The precise sequence of events resulting in the formation of cooked cured-meat pigment (CCMP) in meat is not fully understood. If nitrite were eliminated from cured meats, the result would be products with a beige or tan color, unless an acceptable colorant was employed.

Although it is important to find a substitute for nitrite that reproduces the characteristic cured-meat color, the National Academy of Sciences (NAS) noted that there were few reports of attempts to find compounds or processes that mimic the selective color fixing effect of nitrite in muscle tissue. Toxicological data on some of these potential colorants for cured meat are limited or non-existent. Various nitrogenous heterocyclic and aromatic compounds, which preserve or stabilize the red color of fresh and cured meats, have been studied and a number of patents on their application to meat systems exist. These include the use of nicotinic acid (Coleman and Steffen 1949), pyridine derivatives (Dekker 1958; Hopkins and Sato 1971), tetrazole (van den Oord and DeVries 1971) and heterocyclic compounds such as purines, pyrimidines, imidazoles, pyrazines and triazines and also derivatives of these ring systems (Tarladgis 1967). A common problem with these substances is that the color-giving product derived from each one is substantially less stable to oxidation than the nitrite-produced CCMP. Brown (1973) suggested that a compound which would react with Mb in a manner similar to that of NO might produce a color like that of NOMb. This author tested many nitrogen-containing heterocycles and found that methyl and hexyl nicotinate and *N,N*-diethylnicotinamide were able to react with Mb and form an acceptable red color. However, these pigments were less stable than the pigment of nitrite-cured meat after thermal processing. Howard *et al.* (1973) investigated a variety of nitrogenous ligands, including derivatives of pyridine, amino acids and amino acid esters, for their ability to form stable pink pigments in model and cured meat systems. Methyl and hexyl nicotinate and *N,N*-diethylnicotinamide were the most promising and produced stable pink ferrohemochromes in cooked comminuted meat mixtures. Addition of ascorbic acid or glucono-δ-lactone at 0.05% (w/w) improved the color as well as the stability of the pigment formed. Methyl nicotinate, trigonelline and *N,N*-diethylnicotinamide were also effective in combination with 10 or 20 mg/kg of sodium nitrite in forming a stable pink color in meat systems. The color of these systems was more acceptable than that of the nitrite-cured control after storage for 10 weeks at 5°C and upon exposure to air (Howard *et al.* 1973). Unfortunately, *N,N*-diethylnicotinamide and some derivatives of nicotinic acid and nicotinamide are known to have vasodilatory properties, albeit very mild ones. Smith and Burge (1987) tried to mimic cured meat color using protoporphyrin-IX, but Hunter **L**, **a**, **b** values and spectra of

pigments extracted from protoporphyrin-IX-treated systems were markedly different from those of nitrite-cured samples.

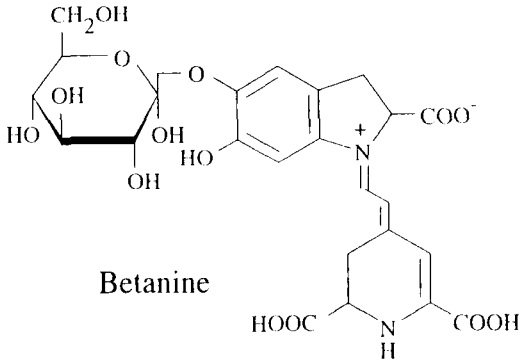
Dymicky *et al.* (1975) tested more than 300 compounds from various classes of chemicals for their ability to form hemochromes in meat slurries and emulsions at 70°C. Most of the compounds tested were nitrogenous heterocycles and imparted colors to cooked meat from beige to pink to purple. The most effective color-forming compounds were substituted pyridines and isoquinolines. Color fixation was believed to be related to the nature of the substituent and its position on the ring. The best color and most heat stable hemochromes were produced by pyridine derivatives containing carbonyl moieties at the 3-position. Therefore, 3- and 4-acyl substituted pyridines were tested for their ability to form color in nitrite-free frankfurters. Table 9.1 lists some of the compounds tested, the colors observed, and a semi-quantitative subjective evaluation of the stability of the pigments so obtained. Of the compounds investigated, 3-butyroylpyridine produced a "good" pink pigment which showed the same stability as that of the nitrite control. Fox *et al.* (1975) further reported that reductants increased the stability of the pyridine hemochrome in the frankfurters. It is important to note, however, that these studies by Fox and coworkers (Dymicky *et al.* 1975; Fox *et al.* 1975) were undertaken solely for the purpose of establishing the structure of compounds that would react with meat components to provide a satisfactory color. The effects on flavor and the toxicity of the substitutes tested were not considered.

Since the number of artificial colorants is limited, and the safety of some has been questioned, von Elbe and Maing (1973) and von Elbe *et al.* (1974*a*,*b*) investigated the use of natural pigments obtained from the red beet root (*Beta vulgaris*). This vegetable contains both red and yellow pigments belonging to a class known as betalaines (Pasch *et al.* 1975). Betalaines are quaternary ammonium amino acids that exhibit high water solubilities and are therefore readily extracted from plant tissues with water. They are divided into two subclasses known as betacyanins (red) and betaxanthines (yellow). The major betacyanin pigment in red beet is betanine (*i.e.*, comprising 75-95%; see Fig. 9.1). Because beet powder is permitted as a food colorant and is a rich source of red pigments, von Elbe *et al.* (1974*a*) used it to simulate cured meat color in cooked, smoked, and semi-dry fermented sausage products. The color of formulated products was measured using Hunter **L**, **a**, **b** values. Similar Hunter **a** and **b** values were noted in sausages containing nitrite/nitrate and those containing beet pigments. Lower Hunter **L** values were observed for pigment-treated meats denoting darker products. The color of betalain-containing sausages was more stable to light exposure during storage than the color of their nitrite/nitrate-treated counterparts, but expert tasters were able to detect subtle flavor and color differences in betalain- and nitrite-containing samples. Similar findings were reported by Dhillon and Maurer (1975), who used betalain

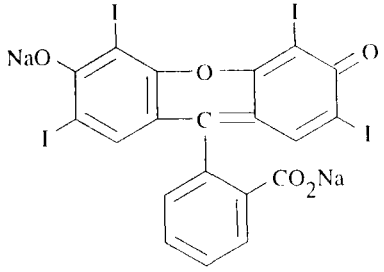
TABLE 9.1
NITROGENOUS HETEROCYCLIC COMPOUNDS TESTED FOR COLOR PRODUCTION IN NITRITE-FREE FRANKFURTERS[a]

Compound	Color Observed	Extent of Fading
Pyridine	pink	almost complete
nicotinic acid	tan	--
methylnicotinate	light pink	some
hexylnicotinate	pink	much
3-formylpyridine	pink	much
4-formylpyridine	purplish-pink	slight
3-acetylpyridine	purplish-pink	none
4-acetylpyridine	purplish-pink	none
3-propionylpyridine	purplish-pink	none
3-butyroylpyridine	pink	slight
3-valeroylpyridine	pink	much
3-caproylpyridine	pink	much
3-heptoylpyridine	pink	much
3-capryloylpyridine	pink	some
trigonelline	tan	--
p-aminobenzoic acid	tan	--
m-aminobenzoic acid	tan	--
triazine	tan	--
tetrazole	grey	--
pyrrole	tan	--
Purine	tan	--
Adenine	tan	--
Guanine	tan	--
Xanthine	tan	--
Uric acid	tan	--
Pyrimidine	pink	complete
Thymine	tan	--
Cytosine	tan	--
Uracil	tan	--
pyrazine	purple	slight
pyrazinamide	purple	slight
pyridazine	tan	--
histidine	slightly pink	none
histidine, *N*-acetyl	slightly pink	none
histamine	tan	--
histamine, *N*-acetyl	tan	--
imidazole	pink	none
4-imidazole carboxylic acid	tan	--

[a] Reported as degree of fading after 20 min of exposure to air. Slight fading was observed for the nitrite-cured frankfurter (control). (From Dymicky *et al.* 1975).



Betanine



Erythrosine

FIG. 9.1. CHEMICAL STRUCTURE OF THE RED PIGMENTS, BETANINE AND ERYTHROSINE

pigments in the production of nitrite-free summer sausages prepared with turkey meat. They reported that addition of betalain pigments at a level of 25 mg/kg produced a color resembling that of the nitrite-cured control. Although sensory scores indicated a preference for sausages formulated by the addition of betalain pigments because of a desirable color in the sausages, no flavor testing was carried out. Guerra *et al.* (1994) used red beet powder as a coloring agent in a cooked ham product. Samples were measured for color after 0, 30 and 90 days of storage and compared to a control ham containing erythrosine. Results indicated that ham color was stable for up to 60 days of storage, but after 90 days, an increase in the yellowness and a decrease in redness of the product was observed.

Besides beet powder and extracts therefrom, other natural pigments have been suggested for application as red colorants to meat and meat products. Examples include Angkak, a fermented rice pigment from *Monascus purpureus* (Fink-Gremmels *et al.* 1990; Shehata *et al.* 1998), dried radish chip extracts (Sugita *et al.* 1993), paprika pigments, β-carotene and curcumin. Bloukas *et al.* (1999) tested the effect of a number of natural colorants permitted in the European Union on the color attributes of frankfurters. Along with the natural colorant, frankfurters were prepared with and without sodium nitrite. Consumer preference studies revealed that the addition of betanin in conjunction with sodium nitrite, at a reduced level, afforded the highest score for overall acceptability of frankfurters, even though the nitrite-free counterpart faired well. In a similar study, Madsen *et al.* (1993) reported that in processed pork meat, addition of 40 mg/kg cochineal provided a color of acceptable similarity to that of a standard product containing 5.4 mg/kg erythrosine, as measured by tristimulus colorimetry, and evaluated by a sensory panel. Cochineal is obtained from the scale insect *Dactylopius coccus* Costa, and commercial preparations contain 20-50% of the coloring component carminic acid. Unfortunately, cochineal was included with reduced nitrite levels in order to provide a constant and uniform appearance to the meat product in question, and was not used solely as a replacement for nitrite. Moreover, large scale production of this pigment for application to meat and meat products would not be economically sound.

In 1982, the NAS reported that a stable uniform cured-meat color could be achieved with as little as 50 mg/kg sodium nitrite, but no suitable means of fixing color in cured meat other than reduced nitrite levels were demonstrated to be effective in products made under commercial conditions. In a recent article, Cassens (1995) reported that the basic issue of the "nitrite problem," especially as related to cured meats, is probably no further resolved at present than it was 15 years ago. Furthermore, he noted that no nitrite alternatives have been found, although much has been learned concerning the chemistry of nitrite as a curing agent. In 1975 a U.S. patent was issued to Sweet, who proposed the use of composite non-nitrite curing mixtures for duplicating the cumulative action of nitrite. His multicomponent system consisted of a red colorant (erythrosine; see Fig. 9.1), an antioxidant/chelator, an antimicrobial agent and all other curing adjuncts except for nitrite. In our laboratory, efforts toward the development of composite nitrite-free curing systems, which bestow the characteristic and desirable attributes of cooked cured-meat products without the fear of *N*-nitrosamine formation, and which may be employed at an industrial level, have been successful. Sweet's approach was employed for preparing nitrite-free products, but the colorant of choice was the natural cooked cured-meat pigment (CCMP). This nitrosylated heme pigment was preformed outside the meat matrix and then applied to meat. Palmin and coworkers had first suggested using such a pigment for improving the color of sausages (Palmin *et*

al. 1973, 1975; Palmin and Prizenko 1974). Description of the preparation of CCMP from bovine red blood cells and its derivative hemin, its handling and application to meats are described below.

Nitrite-Free Curing Using the Preformed Cooked Cured-Meat Pigment (CCMP)

The pigment is manufactured from the red blood cells (RBC) of animals (sheep, cattle, etc.), which are an industrial by-product of abattoirs, and a nitrosating agent in the presence of a reductant. The pigment can be prepared in a direct, one-step process or by an indirect method through a hemin intermediate (Shahidi *et al.* 1984; 1985; 1994*a*; Shahidi and Pegg 1991*a*,*c*). Figure 9.2 provides a flow diagram for the preparation of CCMP from hemin, isolated from bovine red blood cells, and nitric oxide (NO). Hemin can be isolated from red blood cells by the classical acetic acid method of Schalfejew (1885) or the acetic acid-acetone method of Labbe and Nishida (1957). Recovered crystals are dissolved in a dilute sodium carbonate solution and added to a "cocktail" containing sodium tripolyphosphate (STPP), sodium ascorbate and sodium acetate (see Pegg [1993] or Shahidi *et al.* [1994*a*] for experimental details). Nitric oxide is introduced to the solution producing the CCMP, which then precipitates out due to a decrease in pH. The recovered precipitate may be washed with a small volume of a dilute sodium ascorbate solution to ensure elimination of any traces of nitrite and nitrous acid from it before application to meat systems.

Application of CCMP to Meat Systems

The coloring efficacy of CCMP, as part of a composite nitrite-free package, was tested in comminuted pork. The color characteristics of these pork systems, after cooking, were examined and compared to those of uncured and nitrite-cured samples by their Hunter **L**, **a**, **b** color values (Shahidi and Pegg 1990; see Fig. 9.3). Addition of sodium nitrite to freshly comminuted pork oxidized the heme iron to the ferric state and produced the brown-colored nitrosylmetmyoglobin. Upon thermal processing, the bright pink color, typical of nitrosylmyochromogen or the CCMP, was produced. No significant ($P > 0.05$) difference in Hunter **L** and **b** values of cured pork using sodium nitrite addition levels ranging between 25 and 156 mg/kg were evident, but a significant ($P < 0.05$) increase in the Hunter **a** values was observed as greater nitrite levels were employed.

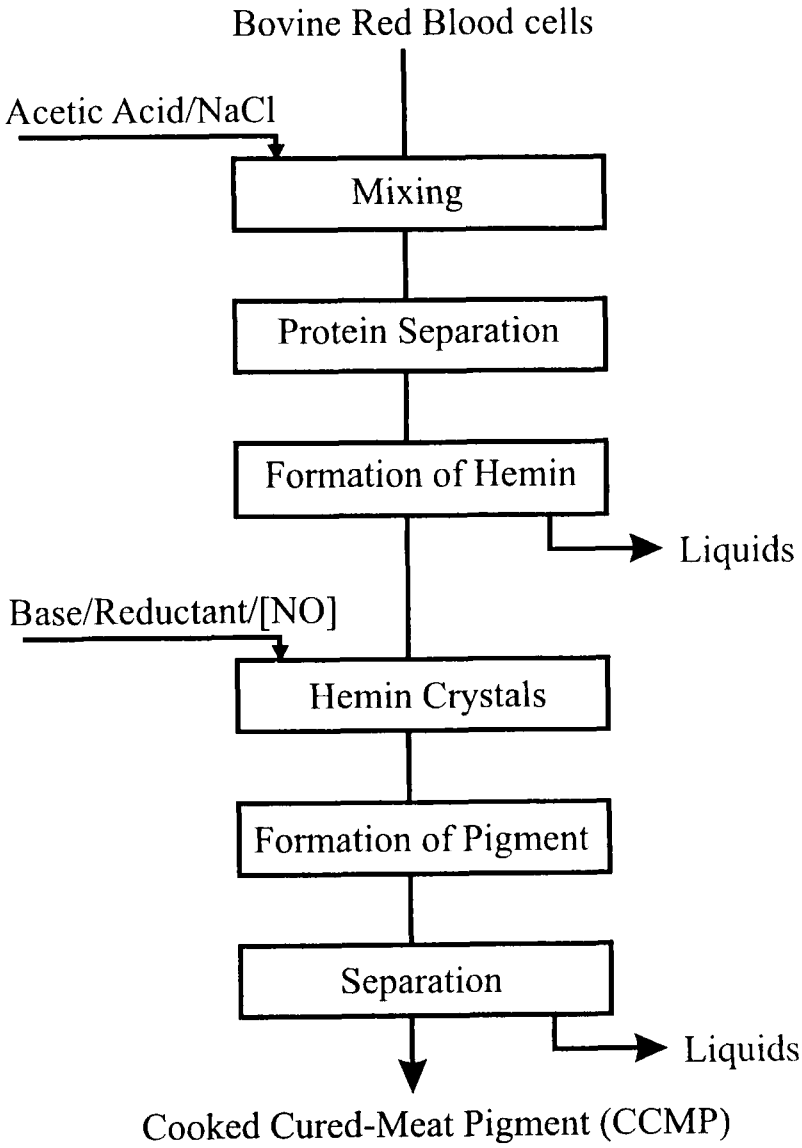


FIG. 9.2. PREPARATION OF THE COOKED CURED-MEAT PIGMENT (CCMP) FROM HEMIN ISOLATED FROM BOVINE RED BLOOD CELLS

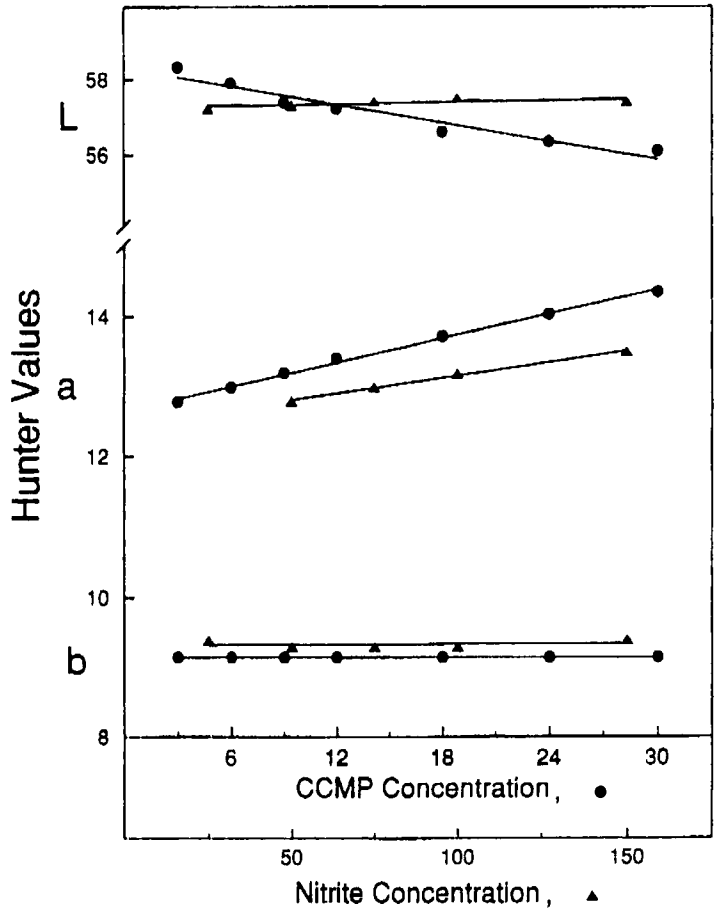


FIG. 9.3. HUNTER L, A, B VALUES OF COOKED GROUND PORK TREATED WITH THE COOKED CURED-MEAT PIGMENT (•) AND SODIUM NITRITE (▲) (From Pegg 1993).

Addition of CCMP to comminuted pork at 3-30 mg/kg levels produced a pink color after thermal processing in all cases, albeit of different intensities, that was visually similar to nitrite-treated pork systems. Although various levels of CCMP were employed, colorimetric data demonstrated that the Hunter **a** and hue angle (arctan **b/a**) values, which denote a red color, of pigment-treated pork samples at a 12-18 mg/kg level were not significantly ($P<0.05$) different from their nitrite-cured counterpart (Table 9.2). As was the case for nitrite-cured

meats, Hunter **b** values of pigment-treated pork samples were not significantly ($P > 0.05$) different from one another. As the CCMP addition level increased, a corresponding decrease in Hunter **L** values was noted unlike their nitrite-cured counterparts, thus denoting slightly darker products. Furthermore, as the concentration of CCMP increased, a significant ($P < 0.05$) increase in Hunter **a** values and a decrease in hue angle values were observed, thereby, indicating a more intense pinkish color in the products. This was presumably a consequence of an increasing concentration of nitrosylprotoporphyrin material in the meat.

In order to illustrate the importance of iron in the porphyrin matrix for proper color development of nitrite-free cured meats, protoporphyrin-IX (PP-IX) was added to comminuted pork. Smith and Burge (1987) had suggested possible use of PP-IX as a natural colorant for nitrite-free curing of meats. Addition of PP-IX to freshly comminuted pork at 60, 100, 150 and 250 mg/kg levels, imparted a purple-red color to products prior to thermal processing. However, upon cooking, PP-IX-treated meats turned dark brown as opposed to the typical pink color of nitrite-cured meat. Although a significant ($P < 0.05$) increase in Hunter **a** values of PP-IX-treated meats was observed, these samples were visually similar to that of cooked uncured meat rather than nitrite-cured or CCMP-treated meats. Pork treated with increasing levels of PP-IX also became significantly ($P < 0.05$) darker in appearance as indicated by decreasing **L** values (Table 9.3) and were visually unappealing (Pegg 1993). Use of PP-IX in comminuted meat systems does not mimic the pink color imparted to meats by nitrite or the preformed CCMP upon thermal processing. Further verification for this observation is obtained from visible spectroscopy of the pigments employed. The absorption spectrum of CCMP in a 4:1 (v/v) acetone:water solution was markedly different from that of PP-IX, but the spectrum of PP-IX was similar to the spectrum of pigments extracted from PP-IX-treated meats, after cooking (Pegg 1993). As expected, the absence of iron in the conjugated PP-IX precluded the development of the characteristic cured-meat color in the final products (Giddings 1977).

Studies have shown that the color intensity of nitrite or CCMP-treated meat products depends on the Mb content of the original meats (Shahidi and Pegg 1991*b*; Pegg 1993; Stevanović *et al.* 1997). The content of native hemoproteins in very pale, typical and dark-colored pork muscles was determined to be 0.76, 1.22 and 1.76 mg myoglobin equivalents per gram wet tissue (*i.e.*, mg Mb eq/g tissue), respectively. Of these three types of pork, the one containing 1.76 mg Mb eq/g tissue exhibited, after nitrite curing, Hunter **a** values approximately 1 and 3.5 units higher than the pork containing 1.22 and 0.76 mg Mb eq/g tissue, respectively (Table 9.4). Furthermore, hue angle values increased by 1.5 and 10 degrees, respectively, as the Mb concentrations in the samples decreased. Addition of different levels of CCMP to meats resulted in a linear increase in Hunter **a** values, but the final color of CCMP-treated pork systems depended on

the native Mb content (Pegg and Shahidi 1990). For example, addition of the preformed CCMP at a 12 mg/kg level to the pork systems resulted in Hunter a values which increased by approximately 1.5 and 2.5 units as the Mb concentration increased from 0.76 to 1.76 mg Mb eq/g tissue. Hue angle values of CCMP-treated pork samples decreased as their nitrite-cured counterparts had, but only by approximately 5 and 6 degrees, respectively. To attain a particular Hunter a value or hue angle measurement, the level of CCMP addition has to therefore be adjusted based on the Mb concentration of the muscle tissue used. Because the edible flesh of animal tissue may contain very low hemoprotein levels (*i.e.*, such as those found in some fish species) to very high contents detected in seal or whale meat, the quantity of CCMP added will have to be adjusted in order to attain an attractive cured color in the final product. Generally, it is expected that higher levels of CCMP will be required to impart optimum color to meats containing high Mb content.

TABLE 9.2
DEPENDENCE OF HUNTER COLOR VALUES OF MEAT ON CONCENTRATION OF COOKED CURED-MEAT PIGMENT (CCMP)[1]

Treatment (mg/kg)	Hunter Values[2]			Hue Angle (arctan b/a)
	L	a	b	
No additive	58.2 ± 0.5^{ab}	4.8 ± 0.1^{g}	11.9 ± 0.1^{a}	68.0 ± 0.4^{a}
$NaNO_2$, 156	57.8 ± 0.2^{abc}	13.4 ± 0.2^{bcd}	9.2 ± 0.1^{b}	34.5 ± 0.5^{cd}
CCMP, 3	58.4 ± 0.5^{a}	12.6 ± 0.2^{f}	9.1 ± 0.1^{b}	35.8 ± 0.5^{b}
CCMP, 6	57.9 ± 0.2^{abc}	12.8 ± 0.1^{ef}	9.1 ± 0.1^{b}	35.4 ± 0.4^{bc}
CCMP, 9	57.3 ± 0.3^{bcd}	13.0 ± 0.2^{def}	9.1 ± 0.1^{b}	35.2 ± 0.5^{bc}
CCMP, 12	57.1 ± 0.2^{cd}	13.2 ± 0.1^{cde}	9.1 ± 0.1^{b}	34.6 ± 0.3^{cd}
CCMP, 18	56.4 ± 0.2^{de}	13.5 ± 0.1^{bc}	9.2 ± 0.1^{b}	34.3 ± 0.3^{cd}
CCMP, 24	56.1 ± 0.4^{e}	13.8 ± 0.2^{ab}	9.1 ± 0.1^{b}	33.6 ± 0.4^{de}
CCMP, 30	55.8 ± 0.3^{e}	14.1 ± 0.2^{a}	9.1 ± 0.1^{b}	32.8 ± 0.4^{e}

[1] All samples were prepared with 20% (w/w) distilled water and 550 mg/kg sodium ascorbate.
[2] Results are means of three determinations ± standard deviation. Means sharing the same letters in a column are not significantly different ($P > 0.05$) from one another as determined by Tukey's test.

TABLE 9.3
HUNTER L, a, b VALUES OF COOKED CURED-MEAT PIGMENT- AND PROTOPORPHYRIN-IX-TREATED COOKED COMMINUTED PORK[1]

Treatment (mg/kg)	Hunter Values[2]			Hue Angle (arctan b/a)
	L	a	b	
No additives	59.1 ± 0.2[a]	5.6 ± 0.2[e]	11.8 ± 0.1[a]	64.6 ± 0.7[a]
$NaNO_2$, 156	58.2 ± 0.4[ab]	13.3 ± 0.3[a]	8.6 ± 0.2[c]	32.9 ± 0.7[e]
CCMP, 12	57.4 ± 0.6[b]	13.2 ± 0.2[a]	8.7 ± 0.2[c]	33.4 ± 0.6[e]
CCMP, 18	57.1 ± 0.6[b]	13.5 ± 0.3[a]	8.5 ± 0.2[c]	32.2 ± 0.7[e]
PP-IX, 60	52.1 ± 0.4[c]	6.8 ± 0.2[d]	9.4 ± 0.2[b]	54.1 ± 0.8[b]
PP-IX, 100	49.1 ± 0.2[d]	7.1 ± 0.2[cd]	9.3 ± 0.1[b]	52.6 ± 0.7[b]
PP-IX, 150	46.2 ± 0.6[e]	7.6 ± 0.2[bc]	7.6 ± 0.1[d]	45.0 ± 0.7[c]
PP-IX, 250	43.2 ± 0.2[f]	7.8 ± 0.2[b]	6.2 ± 0.1[e]	38.5 ± 0.7[d]

[1] All samples were prepared with 20% (w/w) distilled water and 550 mg/kg sodium ascorbate. CCMP -- cooked cured-meat pigment; PP-IX -- protoporphyrin-IX.

[2] Results are means of three determinations ± standard deviation. Means sharing the same letters in a column are not significantly different ($P>0.05$) from one another as determined by Tukey's test.

TABLE 9.4
DEPENDENCE OF HUNTER COLOR VALUES OF COOKED COMMINUTED PORK SYSTEMS ON THEIR MYOGLOBIN CONTENT[a]

Myoglobin (mg/g)	Nitrite-Cured (156 mg/kg)			Pigment-Treated (12 mg/kg)		
	L	a	Hue Angle	L	a	Hue Angle
0.76 ± 0.02	64.2 ± 0.3	10.8 ± 0.2	43.1 ± 0.7	63.3 ± 0.4	11.8 ± 0.2	39.4 ± 0.7
1.22 ± 0.06	57.8 ± 0.5	13.4 ± 0.2	34.5 ± 0.5	57.1 ± 0.2	13.2 ± 0.2	34.6 ± 0.5
1.76 ± 0.06	56.7 ± 0.7	14.2 ± 0.4	33.0 ± 0.8	55.2 ± 0.3	14.2 ± 0.3	33.5 ± 0.7

[a] All samples were prepared with 20% (w/w) distilled water and 550 mg/kg sodium ascorbate to which either sodium nitrite or the cooked cured-meat pigment was added. Myoglobin (Mb) content was determined according to Rickansrud and Henrickson (1967) and its content is reported as mg Mb equivalents/g tissue.

The effect of sodium nitrite, as the reference curing agent, and CCMP on color characteristics was compared with pork, beef, lamb, seal meat and seal surimi, mechanically deboned chicken meat and comminuted chicken breast meat, as well as cod surimi (Table 9.5). In each case, Hunter **L**, **a**, **b**, color parameters, as well as chromaticity, hue angle and overall color difference (ΔE) values obtained from Hunter data reduction, were determined. The ΔE value should be viewed with some skepticism since a wide variation in one of the Hunter parameters has a profound effect on the ΔE values between samples and the control. However, a large difference between the control and a test sample does not necessarily indicate that the color characteristics of the test sample are less appealing. Since use of ΔE values in colorimetric measurements of foods is commonplace, they have been reported in this work, but caution must be exercised as they are never judged solely on their own merit.

A close scrutiny of data presented in Table 9.5 revealed the following trends: (1) Hunter **a** values of cured samples of red meat species, seal and poultry, increased significantly ($P<0.05$) as a result of curing with nitrite; a similar increase in chromaticity and a decrease in hue angle values were noted; (2) addition of CCMP to all muscle foods resulted in an increase in Hunter **a** and a decrease in Hunter **L** values as compared to their uncured counterparts. A corresponding increase in chromaticity and a decrease in hue angle values were also noticed; (3) observed changes in Hunter **L** and **a** values, chromaticity and hue angle values depended on the concentration of added CCMP. Generally an increase in the level of CCMP added gave a parallel increase in Hunter **a** and hue angle values and a decrease in Hunter **L** and chromaticity values; (4) calculated ΔE values depended on the amount of added CCMP and type of muscle tested; and (5) no pink cured color could be duplicated in cod surimi and ΔE values increased with an increase in the concentration of added CCMP.

The above observations may be explained by consideration of the following points. An increase in Hunter **a** values as a consequence of nitrite curing occurs due to a reddening effect exerted by nitrite and a nitrosylhemochromogen formed in the products, albeit to an extent depending on the Mb content in muscles. No pink color was observed in nitrite-cured cod surimi after thermal processing. This was due to a lack of Mb in the muscle tissue, and will be explained in detail later. Increase in Hunter **a** values could be achieved to some extent by the use of varying amounts of CCMP for muscles containing some residual hemoproteins. The quantity of CCMP required for color duplication of nitrite-cured samples depended primarily on the content of Mb originally present in muscle tissues. Similar findings were reported by Stevanović *et al.* (1997) who prepared meat emulsion coagulates from white and red pork muscles. Muscle tissues richer in Mb necessitated addition of higher CCMP levels to attain the characteristic color of cooked cured meat (Table 9.6). Hunter **L** values of CCMP-treated muscle tissues were lower as compared with those of their

nitrite-cured counterparts and this decrease became more pronounced as the level of CCMP addition to muscles increased. This is not unexpected, since addition of CCMP enhances the total heme content of macerated tissues, thereby producing a darker color in products. Unlike nitrite, CCMP does not convert Mb into a nitrosyl derivative, rather it acts by imparting color to the background color from that of cooked uncured meat. Effects of CCMP and nitrite concentrations on chromaticity and hue angle values of muscle tissues followed the expected trends and depended on their apparent colors (hue angle) and intensities (chroma).

The ΔE values depended on species and type of muscle tissue examined. The ΔE values generally corresponded with differences in observed Hunter **a** values of muscles treated with CCMP and that of their nitrite-cured reference sample. A similar conclusion could also be drawn using differences in hue angle values calculated from data reduction. These differences in hue angle originated primarily from variations in Hunter **a** values since their corresponding **b** values were generally unaffected. Lack of appearance of a cured color in cod surimi as a result of nitrite curing was due to the absence of any detectable amount of hemoproteins in muscle tissues (Pegg 1993). Hunter **L**, **a**, **b** values and calculated chromaticity, hue angle and ΔE values of the cod surimi sample treated with 24 mg/kg of CCMP were very similar to those of uncured pork (Table 9.5). Absence of hemoproteins in cod surimi muscle tissues which might be needed for some sort of, yet to be defined, interaction with added CCMP could be responsible for this observation.

In a pilot-scale study, nitrite-free cured frankfurter and salami products were tested, and industry panel members were unable to distinguish the nitrite-cured control (*i.e.*, 200 mg/kg sodium nitrite) from the nitrite-free test samples (*i.e.*, 21-27 mg/kg CCMP) based on visual observation (Pegg 1993). Frankfurter and salami products contained beef, pork, some organ tissue, and mechanically deboned chicken meat in the formulations. It was only when the two sets of samples were examined under bright daylight that the nitrite-free cured sample was discovered to be slightly redder and darker in appearance (Shahidi *et al.* 1993). Colorimetry revealed that the Hunter **L** value, which denotes lightness, decreases as the level of CCMP addition to muscles increases, as opposed to that of the nitrite-cured analogue. This is not unexpected as has already been outlined; addition of CCMP enhances the total heme content of macerated tissue producing a slightly darker color in products.

Preparation of the Powdered Cooked Cured-Meat Pigment (PCCMP)

The preformed CCMP, similar to the pigment present in nitrite-cured meats, undergoes decomposition in the presence of light and air. Therefore, its stabilization/protection is crucial. Since CCMP performs well as a colorant in

TABLE 9.5.
EFFECT OF SODIUM NITRITE AND PREFORMED COOKED CURED-MEAT PIGMENT (CCMP) ON HUNTER L, a, b VALUES, CHROMACITY, HUE ANGLE AND TOTAL COLOR DIFFERENCE (ΔE) OF TREATED PORK, LAMB, BEEF, SEAL, POULTRY AND COD SURIMI SAMPLES[1]

Treatment (mg/kg)	Hunter Values					
	L	a	b	Chroma	Hue Angle	ΔE
Pork						
No additive	58.3 ± 0.3^{a}	4.9 ± 0.1^{a}	12.1 ± 0.2^{a}	13.1	68.0	7.7
$NaNO_2$ (156)	58.6 ± 0.1^{a}	12.1 ± 0.1^{b}	9.3 ± 0.1^{b}	15.3	37.6	Ref.
CCMP (8)	57.9 ± 0.3^{b}	12.2 ± 0.2^{b}	9.0 ± 0.2^{b}	15.2	36.4	0.8
CCMP (12)	56.7 ± 0.2^{bc}	12.9 ± 0.2^{c}	8.9 ± 0.1^{b}	15.7	34.6	2.1
CCMP (24)	56.3 ± 0.1^{c}	13.5 ± 0.2^{d}	8.9 ± 0.2^{b}	16.2	33.4	2.7
Lamb						
No additive	53.8 ± 0.3^{a}	5.3 ± 0.2^{a}	11.8 ± 0.1^{a}	12.9	65.8	10.5
$NaNO_2$ (156)	53.6 ± 0.2^{a}	15.3 ± 0.4^{bc}	8.6 ± 0.1^{b}	17.6	29.3	Ref.
CCMP (12)	52.7 ± 0.3^{b}	14.5 ± 0.2^{b}	8.5 ± 0.2^{b}	16.8	30.4	1.2
CCMP (24)	52.2 ± 0.4^{bc}	14.9 ± 0.3^{bc}	8.3 ± 0.1^{b}	17.1	29.1	1.5
CCMP (30)	52.0 ± 0.3^{bc}	15.3 ± 0.4^{bc}	8.3 ± 0.1^{b}	17.4	28.5	1.6
CCMP (36)	51.6 ± 0.3^{c}	15.5 ± 0.2^{c}	8.3 ± 0.2^{b}	17.6	28.2	2.0
Beef						
No additive	49.2 ± 0.5^{a}	5.7 ± 0.2^{a}	11.2 ± 0.3^{a}	12.6	63.0	12.7
$NaNO_2$ (156)	48.0 ± 0.5^{b}	18.1 ± 0.2^{b}	8.7 ± 0.1^{b}	20.1	25.7	Ref.
CCMP (12)	47.7 ± 0.3^{bc}	13.5 ± 0.2^{c}	8.8 ± 0.2^{b}	16.1	33.1	4.6
CCMP (24)	46.8 ± 0.4^{cd}	15.8 ± 0.1^{d}	8.6 ± 0.1^{b}	18.0	28.6	2.6
CCMP (36)	45.9 ± 0.3^{d}	18.0 ± 0.3^{b}	8.6 ± 0.2^{b}	20.0	25.5	2.1

TABLE 9.5 Continued

Treatment (mg/kg)	Hunter Values			Chroma	Hue Angle	ΔE
	L	a	b			
Seal Meat						
No additive	24.2 ± 0.2^{ab}	7.8 ± 0.1^{a}	8.0 ± 0.1^{a}	11.2	45.7	14.0
$NaNO_2$ (156)	24.1 ± 0.1^{b}	15.4 ± 0.2^{b}	7.5 ± 0.2^{b}	17.1	26.0	6.4
$NaNO_2$ (500)	24.1 ± 0.2^{b}	21.8 ± 0.5^{c}	7.8 ± 0.2^{ab}	23.2	14.7	Ref.
CCMP (12)	24.0 ± 0.1^{b}	12.5 ± 0.1^{d}	7.5 ± 0.2^{b}	14.6	31.0	9.3
CCMP (24)	23.8 ± 0.1^{b}	14.2 ± 0.1^{e}	7.4 ± 0.1^{b}	16.0	30.6	7.6
CCMP (36)	24.9 ± 0.5^{a}	19.8 ± 0.2^{f}	8.0 ± 0.1^{a}	21.4	22.0	2.2
CCMP (48)	23.8 ± 0.3^{b}	22.3 ± 0.3^{c}	8.0 ± 0.2^{a}	23.7	19.7	0.6
Seal Surimi						
No additive	28.9 ± 0.2^{a}	6.3 ± 0.1^{a}	8.1 ± 0.1^{a}	10.3	52.1	8.1
$NaNO_2$ (156)	28.8 ± 0.2^{a}	14.4 ± 0.2^{b}	7.3 ± 0.1^{c}	16.2	26.9	Ref.
CCMP (12)	29.3 ± 0.3^{a}	14.6 ± 0.3^{b}	7.7 ± 0.1^{b}	16.5	27.8	0.7
CCMP (24)	29.0 ± 0.2^{a}	14.8 ± 0.2^{b}	7.5 ± 0.1^{bc}	16.6	26.9	0.5
CCMP (36)	30.0 ± 0.2^{b}	15.6 ± 0.3^{c}	8.0 ± 0.1^{a}	17.5	27.1	1.8
Chicken Breast Meat						
No additive	75.8 ± 0.2^{a}	1.9 ± 0.1^{a}	12.7 ± 0.1^{a}	12.8	81.5	3.9
$NaNO_2$ (156)	74.6 ± 0.3^{b}	5.0 ± 0.1^{b}	10.6 ± 0.1^{b}	11.7	64.8	Ref.
CCMP (6)	73.9 ± 0.4^{c}	5.3 ± 0.2^{b}	9.7 ± 0.2^{c}	11.1	61.3	1.0
CCMP (12)	72.0 ± 0.5^{d}	6.4 ± 0.1^{c}	9.6 ± 0.3^{c}	11.5	56.3	3.1
CCMP (24)	69.1 ± 0.1^{e}	7.4 ± 0.1^{d}	9.6 ± 0.2^{c}	12.1	52.4	6.1

TABLE 9.5 Continued

Treatment (mg/kg)	Hunter Values			Chroma	Hue Angle	ΔE
	L	a	b			
Mechanically Deboned Chicken Meat						
No additive	51.2 ± 0.1^{a}	4.6 ± 0.1^{a}	13.8 ± 0.1^{a}	14.6	71.6	7.7
$NaNO_2$ (156)	52.9 ± 0.1^{b}	11.4 ± 0.2^{bc}	10.7 ± 0.2^{b}	15.6	43.2	Ref.
CCMP (6)	52.6 ± 0.1^{b}	11.1 ± 0.2^{b}	10.8 ± 0.1^{b}	15.5	44.2	0.4
CCMP (12)	51.5 ± 0.3^{a}	11.3 ± 0.1^{bc}	10.7 ± 0.2^{b}	15.6	43.4	1.4
CCMP (24)	51.1 ± 0.2^{a}	11.8 ± 0.3^{c}	10.6 ± 0.2^{b}	15.9	41.9	1.8
Cod Surimi						
No additive	70.9 ± 0.4^{a}	-2.0 ± 0.1^{a}	7.5 ± 0.2^{a}	7.8	89.0	0.3
$NaNO_2$ (156)	71.2 ± 0.2^{a}	-2.1 ± 0.1^{a}	7.5 ± 0.2^{a}	7.8	88.9	Ref.
CCMP (12)	63.6 ± 0.2^{b}	2.7 ± 0.2^{b}	9.2 ± 0.3^{b}	9.6	73.6	9.2
CCMP (24)	57.1 ± 0.1^{c}	5.7 ± 0.2^{c}	9.2 ± 0.1^{b}	10.8	58.2	16.2
	$[58.3 \pm 0.3]^{d}$	$[4.9 \pm 0.1]^{d}$	$[12.1 \pm 0.2]^{c}$	[13.1]	[68.0]	[15.4]
CCMP (36)	55.4 ± 0.4^{e}	8.0 ± 0.2^{e}	8.8 ± 0.2^{b}	11.9	47.7	18.8
CCMP (60)	48.3 ± 0.3^{f}	8.8 ± 0.1^{f}	9.2 ± 0.2^{b}	12.7	46.3	25.4

[1]All systems contained 20% (w/w) distilled water and 550 mg/kg sodium ascorbate. Values in parentheses indicate mg/kg concentrations of adjuncts used. Hue angle is defined as arctan(**b/a**). Results are mean values of six determinations ± standard deviation. Values in each column for each species with same symbols are not different ($P>0.05$). Hunter values in brackets are for uncured pork.
(From Shahidi & Pegg, 1991*b*).

TABLE 9.6
TOTAL HEMOPROTEIN PIGMENT CONTENT OF MUSCLE FOODS AND THE AMOUNT OF PREFORMED COOKED CURED-MEAT PIGMENT (CCMP) REQUIRED TO ACHIEVE A CURED COLOR IN PRODUCTS[a]

Species	Total Pigments (mg Mb eq/g)	CCMP (mg/kg)
Pork	1.2	8.0
Lamb	2.1	12.0
Beef	4.5	36.0
Seal	59.0	48.0
Seal Surimi	19.3	24.0
Chicken Breast Meat	0.4	6.0
Mechanically Deboned Chicken Meat	1.0	12.0
Cod Surimi	0.0	0.0

[a] All systems contained 20% (w/w) distilled water and 550 mg/kg sodium ascorbate. Total pigment content determined according to the method of Rickansrud and Henrickson (1967) and is reported as mg myoglobin equivalents/g tissue. (From Shahidi and Pegg 1991*b*).

nitrite-free meat curing systems, there was a need to develop an industrially viable scheme, suitable for commercial production. A microencapsulation technique was employed to improve the handling of the pigment for industrial application. For our purposes, starch and modified starch, cyclodextrins, dextrans, amylose, gums, and gelatin proved to be useful as encapsulating agents. Cyclodextrins have a central cavity in the molecule, and protection of CCMP against oxidation might arise from partial inclusion of the pigment in the cavity or simply by a coating mechanism. The resultant powdered cooked cured-meat pigment (PCCMP) was applied to various meat systems and it successfully duplicated the color characteristics of the nitrite-cured counterpart.

There are two key steps in manufacturing of PCCMP. The first step is preparation of CCMP from bovine red blood cells as described by Shahidi *et al.* (1994*a*). The second key step is stabilization of this pigment by an entrapping or a locking mechanism. The starting point of this process is the preparation of a solution or paste of starch, modified starches, *e.g.*, N-LOK®, maltodextrins,

Schardinger dextrins, their polymers or their derivatives or combinations, optionally with glycerin or gums, such as gum acacia. Furthermore, a reductant is added to prevent oxidative degradation of the pigment during and after the locking process. The pigment, dissolved in a basic solution containing a food-grade reductant, is added to a solution or paste of carbohydrate-based polymers to protect and physically entrap the pigment molecules; glycerin or gums, if added, serve as a binding agent. Finally, the mixture is spray-dried to obtain PCCMP. The pigment remains locked in the powder until it is released by adding water to it. Upon its dissolution in water or pickle solutions, PCCMP acts as a potent agent for color development in nitrite-free curing mixtures. Its application to a variety of meat emulsion systems consisting of poultry, pork, lamb, beef, cod, cod surimi, seal and seal surimi, as well as hybrid products containing different meat combinations at levels from 30 to 50 mg/kg has been extensively studied.

The effect of concentration of PCCMP on color intensity of treated meats has been studied. Table 9.7 summarizes typical results for PCCMP-treated meats in which combinations of wall materials consisted of 95% N-LOK, 2% STPP, 2% sodium acid pyrophosphate (SAPP) and 1% ascorbyl palmitate (AP). Results indicated that PCCMP-treated samples at 30-40 mg/kg levels resembled most closely the color of nitrite-cured meats. Higher addition levels of PCCMP significantly ($P < 0.05$) increased Hunter **a** values and decreased Hunter **L** values of treated samples, but the colors may not be visually unattractive. Nonetheless, the optimal addition level of spray-dried pigment to meat depends primarily on its original Mb content (Shahidi and Pegg 1991*b*) as well as the conditions under which encapsulation was performed. Best performance of the pigment was observed for meat systems containing a low or intermediate concentration of Mb/hemoglobin (Hb), although performance of CCMP in dark meats such as that of the seal was satisfactory. A full description of preparation of PCCMP and its application to muscle foods has been reported (Shahidi and Pegg 1991*d*).

Antioxidant Properties

In Chapter 4 it was described how nitrite acts as a strong antioxidant in cured meats and thus prevents lipid oxidation. However, nitrite is not unique in its role as a food-grade antioxidant. Shahidi (1989) proposed that any agent or combination of agents that prevents lipid oxidation, with the exception of nitrite precursors, would in principal, duplicate the antioxidant role of nitrite in the curing process, thereby preventing meat flavor deterioration (MFD) and hexanal generation. According to Shahidi (1992), this is in line with findings of other researchers and its validity was confirmed by preliminary sensory evaluations, but mutton was not included in these studies.

TABLE 9.7
EFFECT OF PIGMENT CONCENTRATION ON THE HUNTER L, a, b VALUES OF COOKED TREATED PORK SYSTEMS[1]

Treatment (mg/kg)	Hunter Values[2]		
	L	a	b
No additive, 0	59.0 ± 0.2[a]	4.7 ± 0.1[d]	11.4 ± 0.1[a]
$NaNO_2$, 156	58.4 ± 0.1[b]	11.8 ± 0.2[c]	9.1 ± 0.1[b]
CCMP, 12	57.9 ± 0.2[c]	11.7 ± 0.2[c]	9.1 ± 0.1[b]
PCCMP, 30	54.5 ± 0.2[d]	12.2 ± 0.2[bc]	9.1 ± 0.1[b]
PCCMP, 50	52.5 ± 0.1[e]	12.9 ± 0.1[a]	8.8 ± 0.1[c]

[1] All systems contained 20% (w/w) distilled water and 550 mg/kg sodium ascorbate. A payload of 1.5% (w/w) was used. Wall materials for PCCMP were N-LOK, 95%; sodium tripolyphosphate, 2%; sodium acid pyrophosphate, 2%; and ascorbyl palmitate, 1%. CCMP -cooked cured-meat pigment; PCCMP - powdered cooked cured-meat pigment.

[2] Results are mean values of six determinations ± standard deviations. Means sharing any of the same letters in a column are not significantly ($P>0.05$) different from one another.

To reproduce the antioxidative efficacy of nitrite, a number of antioxidants (Shahidi *et al.* 1987*a*), sequestrants (Shahidi *et al.* 1986) and their combinations (Shahidi *et al.* 1987*b*; 1988) were examined. Addition of antioxidants to meat and meat products resulted in the preservation of meat quality by retarding autoxidation and rancidity development, as well as discoloration and loss of nutrients. The inhibitory effect of antioxidants has been attributed to the donation of a hydrogen atom or an electron to a lipid free radical as well as possible formation of a complex between the antioxidant and the lipid molecule (Dziezak 1986). The concentration of carbonyl compounds produced in these systems from autoxidation was markedly reduced when combinations containing polyphosphates, ascorbates and low levels of an antioxidant were used. The spectrum of notable carbonyl compounds was similar to the nitrite-cured system. Among the antioxidants employed, butylated hydroxyanisole (BHA) and *tert*-butylhydroquinone (TBHQ) were the most effective, even at 30 mg/kg, for retarding oxidation during a 5-week storage at 4°C, as measured by the 2-thiobarbituric acid (TBA) test (Table 9.8). Among the food-grade sequestrants, SAPP, tetrasodium pyrophosphate (TSPP), STPP and ethylenediaminetetraacetic acid (EDTA) were the most efficacious. Sodium ascorbate and STPP by themselves retarded lipid oxidation, but together, a strong synergistic action was noted (Fig. 9.4). Yun *et al.* (1987) reported that although addition of small

quantities (*i.e.*, 30 mg/kg) of an antioxidant, such as BHA or TBHQ to the above systems, had a minor effect on the TBA values, it had a positive influence on the sensory characteristics of thermally processed meat products as noted by untrained panelists. The mixture containing sodium ascorbate (550 mg/kg) and STPP (3000 mg/kg) with or without a phenolic antioxidant (30 mg/kg) was as effective as sodium nitrite (150 mg/kg) in the presence of sodium ascorbate (550 mg/kg). Furthermore, addition of sodium nitrite to meat containing sodium ascorbate and STPP did not have any further effect in controlling lipid oxidation (Shahidi *et al.* 1987*b*).

Earlier investigations demonstrated the importance and benefits of phosphate and ascorbate addition to processed meat products. Researchers such as Chang and Watts (1949) and Tims and Watts (1958) reported that phosphate (0.5%) and ascorbic acid (0.1%) worked synergistically to prevent an increase in TBA values that developed in untreated cooked, stored pork. The mechanism by which phosphates retard lipid oxidation appeared to be related to the ability of phosphates to sequester metal ions, particularly Fe^{2+} ions. These are major prooxidants in meat systems (Love and Pearson 1974). Barbut *et al.* (1989) reported that long-chain polyphosphates were better sequestering agents for light metal ions, such as calcium and magnesium, when compared to short-chain polyphosphates for iron and copper ions. As pH increases, the chelating ability of long-chain polyphosphates also increases, while the opposite is true for short-chain polyphosphates.

Sato and Hegarty (1971) tested a variety of compounds for their ability to inhibit lipid oxidation in meat systems, as measured by TBA values. The most active compounds were the disodium salt of EDTA, STPP, sodium hexametaphosphate (SHMP), sodium citrate, sodium ascorbate, BHA and butylated hydroxytoluene (BHT), but only the last two compounds were effective at concentrations as low as 100 mg/kg. MacDonald *et al.* (1980) tested citric acid and BHT for their antioxidant activity and compared the results with those of nitrite at various concentrations. They reported that citric acid at 1,000 mg/kg and BHT at 200 mg/kg were less active than sodium nitrite at its lowest concentration of 50 mg/kg.

Shahidi and Hong (1991) reported that the addition of polyphosphates such as STPP at 3,000 mg/kg or the disodium salt of EDTA to meat systems containing prooxidants such as iron and copper ions or heme pigments resulted in a substantial decrease in their content of TBA reactive substances (TBARS). Shahidi (1992) reported on various curing adjuncts which inhibited lipid oxidation in cooked ground pork systems. Ascorbate (550 mg/kg) retarded lipid oxidation, possibly by upsetting the balance between Fe^{2+} and Fe^{3+} ions or by an oxygen scavenging mechanism (Decker and Hultin 1990), but it has been reported to have prooxidant activity in some instances (Igene *et al.* 1985). Presence of non-heme iron, tocopherols, citric acid and amino acids which are

TABLE 9.8
TBARS VALUES OF COOKED GROUND PORK TREATED WITH DIFFERENT ADDITIVES AFTER A 5-WEEK STORAGE PERIOD AT 4C

Experiment Number	Additives (mg/kg)	TBARS Value (mg malonaldehyde eq/kg)
1	Control, no additives	15.46
2	Sodium nitrite, 150	0.63
3	Butylated hydroxyanisole, 30	0.44
4	*tert*-Butylhydroquinone, 30	0.35
5	Sodium tripolyphosphate, 3000	1.86
6	Tetrasodium pyrophosphate, 3000	1.66
7	Sodium hexametaphosphate, 3000	7.71
8	(5) + Sodium ascorbate, 550	0.27
9	(6) + Sodium ascorbate, 550	0.23
10	(7) + Sodium ascorbate, 550	0.29
11	(8) + (3)	0.20
12	(8) + (4)	0.18
13	Cooked cured-meat pigment, 12	9.89
14	(11) + (13)	0.34
15	(12) + (13)	0.24
16	(14) + Sodium hypophosphite, 3000	0.28
17	(15) + Sodium hypophosphite, 3000	0.21

(From Shahidi and Pegg 1992).

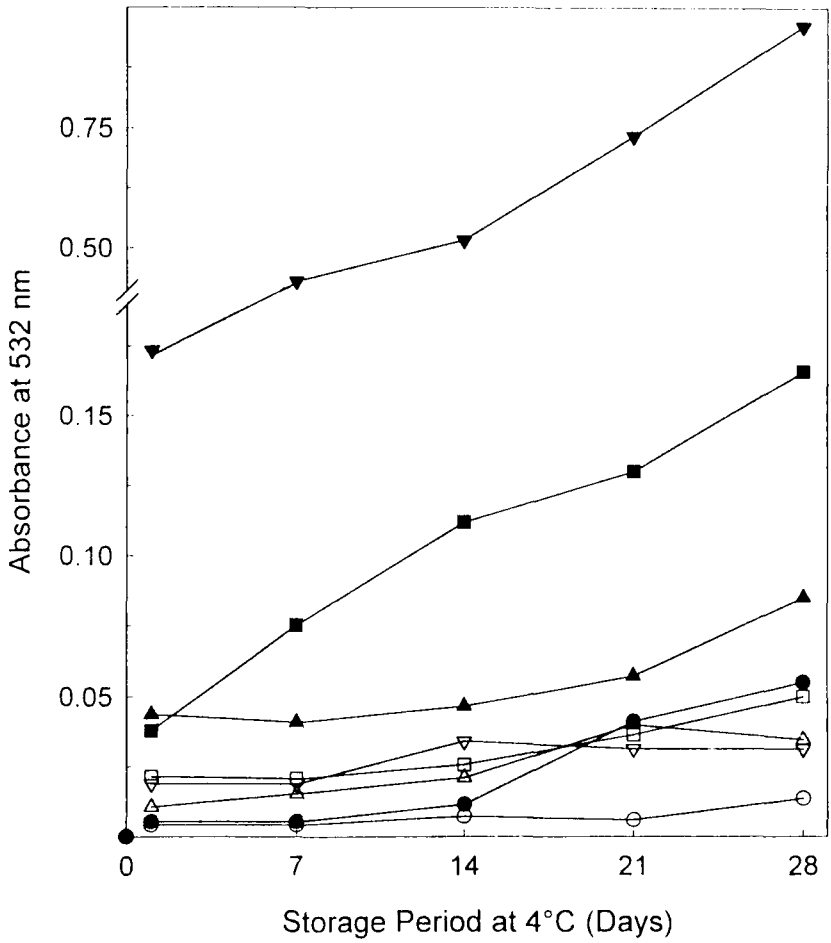


FIG. 9.4. SYNERGISTIC EFFECT OF POLYPHOSPHATES WITH SODIUM ASCORBATE IN MEAT SYSTEMS. SYMBOLS (FULL) ARE: ▼, SODIUM HEXAMETAPHOSPHATE; ■, SODIUM TRIPOLYPHOSPHATE; ▲, TETRASODIUM PYROPHOSPHATE; ●, SODIUM ACID PYROPHOSPHATE. CORRESPONDING OPEN SYMBOLS ARE FOR THE SAME POLYPHOSPHATES WITH SODIUM ASCORBATE.
(From Shahidi and Pegg 1992).

naturally present in meat may change the role of ascorbate from an antioxidant to a prooxidant. Ascorbyl palmitate and the C_{16} acetal of ascorbic acid at a 2,000 mg/kg level gave rise to strong antioxidant effects in cooked pork systems. Shahidi *et al.* (1987*a*,*b*) proposed that this activity may be due to their enhanced solubility in the fat portion of meat as compared to ascorbic acid itself.

In the food industry, there has been an ever increasing trend towards the use of natural ingredients due to greater sensitivity of consumers to synthetic additives (Bailey 1988; Shahidi and Wanasundara 1992), and especially since BHA and BHT are suspected to have carcinogenic activity (Namiki 1990; Nakatani 1992). Hence, use of naturally occurring antioxidants for retarding MFD is desirable in nitrite-free curing systems. Spices and herbs offer a potential solution as they are often added to prepared meats as condiments for coloring and seasoning. In addition, they sometimes provide a preservative effect suggesting the presence of antioxidative and antimicrobial constituents. Important fractions of spices are their volatile oils and oleoresins. The volatiles of essential oils are responsible for the characteristic aroma of the spice and their content may range from less than 1 up to 20% (Clark 1970). The oleoresin, which is the non-volatile extract, is responsible for the typical taste and pungency of the spice and usually accounts for less than 10% of the spice's mass. Research investigations have shown that constituents of spices and herbs can depress rancidity of food lipids (Nakatani 1997); however, the efficacy of these compounds depends highly upon the food system in question, their level of addition, and stability when subjected to food processing operations. Although certain spices or their fractions may possess marked antioxidant activity, their practical application to meat and meat products may be restricted due to a pungent and/or characteristic flavor imparted by the spice.

Table 9.9 summarizes results for the antioxidant activity of selected spices and their oleoresins in pork systems as percent inhibition of formation of TBARS after 3 weeks of storage at 4°C. The TBARS values for spice-containing samples were lower than those of the control, thus indicating protection to meat by these spices against lipid oxidation. Furthermore, this protection was concentration-dependent, but a saturation point might be reached after a certain amount of spice is added. This was the case for clove, where a 96% inhibition was achieved at a 500 mg/kg addition level and this protection remained unchanged even at higher spice concentrations (Shahidi *et al.* 1995). In the pork systems, clove, sage, rosemary and oregano appeared quite effective in retarding lipid oxidation as TBARS values remained at less than 1 μg/g sample over the entire storage period; ginger and thyme showed the weakest antioxidant activity in this system. The superior antioxidant activity of clove, as exhibited in the data, may arise from its high content of eugenol (3.0%) and gallic acid (1.3%). Both of these compounds are known to possess a strong antioxygenic effect at relatively low concentrations (Kramer 1985; Al-Jalay *et al.* 1987; Shahidi and Pegg 1998). The activity of rosemary is attributed mainly to carnosol, rosmanol, rosmaridiphenol, rosmariquinone, as well as carnosic and rosmaric acids (Houlihan *et al.* 1984). Oregano (*Origanum vulgare* L.), which also belongs to the same family as rosemary, contains caffeic, rosmaric and protocatechuic acids, as well as a phenyl glycoside of 2-caffeoyloxy-3-[2-(4-

hydroxybenzyl)-4,5-dihydroxy]phenylpropionic acid (Kikuzaki and Nakatani 1989) as its active components. Curcumin was found to be the active constituent in thyme (Al-Jalay *et al.* 1987), while the antioxidant activity of ginger was attributable to gingerol, shogaol, zingerone and their derivatives (Nakatani 1997).

TABLE 9.9
INHIBITION (%) OF FORMATION OF TBARS BY SPICE, SPICE OLEORESINS AND THEIR COMBINATIONS IN A GROUND PORK MODEL SYSTEM[a]

Spice/Oleoresin	Spice Addition Level (mg/kg)					
	200	500	1000	2000	Oleoresin[b]	Spice + Oleoresin[c]
Clove	56	96	96	96	79	98
Ginger	20	23	32	46	51	73
Oregano	17	35	53	85	74	88
Rosemary	6	48	60	85	48	82
Sage	14	49	74	93	69	94
Thyme	12	25	36	64	45	52

[a] % Inhibition = [1 - (Response with additive(s)/Response without additive(s))] × 100
[b] Oleoresin added at 200 mg/kg level.
[c] Spice and corresponding oleoresin added at 1000 and 200 mg/kg levels, respectively. (Adapted from Shahidi *et al.* 1995).

Deheated or low-pungency mustard flour (Alberta Industrial Mustard or AIM) is an economical meat adjunct that may be used as a spice for flavor and technological quality improvements, at levels of 1-2% (w/w), in sausage products such as wieners and bologna. AIM is "deheated" by an enzyme deactivation method and is considered as a GRAS (generally recognized as safe) product in the U.S.A. and Canada. The pungency of mustard arises from the generation of isothiocyanates from glucosinolate precursors by the action of the endogenous enzyme, myrosinase. However, AIM lacks the pungency associated with ground mustard due to inhibition of isothiocyanate production by myrosinase deactivation. AIM or its ethanolic extracts were found to effectively inhibit lipid oxidation when applied to comminuted meat products at a 1-2% level. At an addition level of 0.5%, it retarded lipid oxidation by *ca.* 65% over a 4-week storage period, but its antioxidative efficacy was concentration-

dependent (Saleemi *et al.* 1993). Based on these results, between 1.5 to 2% AIM was recommended for addition to meats in order to effectively retard their flavor deterioration in the uncured state. Shahidi *et al.* (1994*b*) separated ethanolic extracts of AIM by Sephadex LH-20 column chromatography. These authors reported that the antioxidant constituents of AIM were attributable to compounds similar to those of sinapic acid, *para*-hydroxybenzoic acid and trihydroxyphenolic compounds such as flavones or flavanones.

Application of AIM to meat products, either those cured or treated with CCMP, had no detrimental effect on color of the thermally processed products. However, AIM had a beneficial effect in enhancing the cooking yield, as reflected in water holding capacity of the treated samples (Saleemi *et al.* 1993). In this respect, its performance was equivalent or superior to that of STPP at a 3,000 mg/kg addition level. Thus, AIM together with CCMP may provide another combination for color (to be discussed below) and flavor duplication in nitrite-free curing of comminuted meat products. Similarly, our recent studies using dechlorophyllized crude tea catechins, or individual catechins, showed that CCMP along with these ingredients, at 30 or 200 mg/kg levels, could effectively duplicate the antioxidative role of nitrite in processed meats (results not shown).

Flavor Characteristics

Flavor is a complex stimulus involving characteristics such as taste, odor, texture and temperature (Gray *et al.* 1981). The National Academy of Sciences (NAS) (1982) reported that the generation of cured meat flavor is probably a composite sensation derived from the contribution of many odoriferous compounds. A positive contribution by nitrite to flavor cannot be specified in chemical terms, but the committee suggested that nitrite probably influences the flavor of cured meat by virtue of its antioxidative effects. Because the mechanism involved in the production of the characteristic cured-meat flavor is uncertain, there is no known nitrite substitute which can duplicate this flavor.

Some meat products cured without nitrite have been found to be acceptable by panelists. Taste tests conducted on bacon treated with salt, sugar, STPP, sodium ascorbate, and varying levels of nitrite showed that an acceptable bacon product could be prepared without the use of nitrite (Wasserman and Kimoto 1977). Further studies by Wasserman *et al.* (1977) and Huhtanen *et al.* (1981) revealed that no difference between the preference for nitrite-free and nitrite-cured bacon could be discerned. These results were also supported by Williams and Greene (1979). Kimoto *et al.* (1976) reported that sodium chloride was more important than nitrite to the flavor of bacon, and MacDougall *et al.* (1975) concurred by stressing the importance of sodium chloride for cured meat flavor. These authors reported that sodium chloride-free samples had almost no bacon flavor, whereas, salted, nitrite-free bacon did. On the other hand, Paquette *et*

al. (1980), who varied sodium nitrite levels in bacon samples, found that samples containing nitrite had a significantly ($P<0.05$) more desirable flavor than did nitrite-free analogs. No significant differences ($P>0.05$) in the desirability among samples containing nitrite at different concentrations were noted. Although nitrite-free cured bacon had a less desirable flavor than its nitrite-cured counterpart, it was still acceptable.

Paquette *et al.* (1980) also reported that bacon containing potassium sorbate at 2,600 mg/kg and sodium nitrite at 40 or 80 mg/kg was judged to be as desirable as that containing sodium nitrite and no potassium sorbate. No undesirable flavors were introduced by addition of the antimicrobial agent to the bacon products. Similar reports on the effect of antimicrobial agents in nitrite-free or nitrite-reduced cured meat products were complied by the NAS (1982). Data suggested that bacon processed with sodium chloride and sodium hypophosphite (SHP) at 3,000 mg/kg or bacon prepared with sodium chloride, SHP at 1,000 mg/kg, and sodium nitrite at 40 mg/kg were judged to have a flavor as desirable as that of the conventionally prepared nitrite-cured product. Bacon processed with sodium chloride alone was included as a control and was judged to have a flavor as desirable as that of the other products. Sensory data suggested that bacon treated with methyl fumarate at 1,250 mg/kg could not be distinguished from the nitrite-cured control. Hedonic scores for methyl fumarate-treated bacon and a conventional counterpart were equivalent.

For frankfurters, Simon *et al.* (1973) found that all-beef nitrite-free wieners had an equivalent flavor to nitrite-cured samples, but the flavor quality of 50% pork/50% beef wieners varied directly with the concentration of nitrite added. On the other hand, Greene and Price (1975) reported that salt was the major contributor to cured meat flavor in samples of ground pork, whereas sodium nitrite alone at a level of 200 mg/kg produced very little cured-meat flavor. Yun (1984) and Yun *et al.* (1987) evaluated combinations of ingredients which would effectively prevent lipid oxidation in cooked ground pork systems to be used in the nitrite-free curing of meat products such as frankfurters. The authors reported that sensory evaluation scores of pork systems treated with 3,000 mg/kg STPP, 550 mg/kg sodium ascorbate and 30 mg/kg of BHA or TBHQ were not significantly different ($P>0.05$) from their nitrite-cured (156 mg/kg) counterparts. Yun (1984) also observed that the concentration of volatiles identified in the distillate of cooked pork samples, notably hexanal, was significantly reduced ($P<0.05$) when samples had been pretreated with the above antioxidant/chelating agent combinations. The concentration of volatiles in these systems was depressed almost to the level of the nitrite-cured control (see Table 9.10).

The studies cited above suggest, for the most part, that it is possible to prepare nitrite-free cured-meat products without seriously compromising their flavor. If one accepts the views of Cross and Ziegler (1965) that cured-ham

flavor represents the basic flavor of meat derived from precursors other than triacylglycerols and that the different aromas of the various types of cooked meat depend on the spectrum of carbonyl compounds derived by lipid oxidation, then any agent or combination that suppresses lipid oxidation, would, in principal, duplicate the flavor of nitrite-cured meat.

TABLE 9.10
EFFECT OF CURING WITH NITRITE ON THE CONCENTRATION OF CARBONYL COMPOUNDS OF THERMALLY PROCESSED GROUND PORK

Carbonyl Compounds	Relative Concentration		
	Uncured Meat	Nitrite-Cured[a]	Nitrite-free Treated[b]
Hexanal	100	7.0	6.5
Pentanal	31.3	0.5	0.5
Heptanal	3.8	< 0.5	0.5
Octanal	3.6	< 0.5	0.5
Nonanal	8.8	0.5	0.7
2-Octenal	2.0	--	--
2-Nonenal	1.0	--	--
2-Decenal	1.1	--	< 0.1
2-Undecenal	1.4	0.5	0.5
2,4-Decadienal	1.1	--	< 0.1

[a] Sample contained 550 mg/kg sodium ascorbate.
[b] Sample contained the preformed cooked cured-meat pigment, 12 mg/kg; sodium tripolyphosphate, 3000 mg/kg; sodium ascorbate, 550 mg/kg; and *tert*-butylhydroquinone, 30 mg/kg. (From Shahidi 1989).

Antimicrobial Properties

Nitrite exerts a concentration-dependent antimicrobial effect in cured meat products, including, but not limited to, inhibition of the outgrowth of spores of putrefactive and pathogenic bacteria such as *C. botulinum* (NAS 1982). The degree of protection provided to cooked meats against microbial contamination

depends on many factors including the concentration of residual nitrite present, duration of temperature abuse and extent of contamination. Nitrite also retards microbial spoilage of cured meats by anaerobic and aerobic spore-forming bacteria. As outlined in Chapter 6, the mechanism(s) by which nitrite inhibits the outgrowth of spores and the growth of vegetative cells and microorganisms is not fully understood, but it appears that a reaction with iron-containing enzymes is involved. A better understanding of the exact mechanism(s) of the antimicrobial role of nitrite is still required.

Irrespective of future decisions on the fate of nitrite, its removal or reduction must be counter-balanced by alternatives that will assure the safety from botulinal hazards in abused products (Shahidi and Pegg 1991*a*). Furthermore, the traditional identity of cured meat products must be retained. According to Sofos and Busta (1980), any substance to be considered as an alternative to nitrite should be suitable for use in all cured meat products and should control other microorganisms of public health significance, delay product spoilage, and not interfere with beneficial microorganisms such as lactic acid-producing cultures, necessary for the manufacture of fermented meat products. The compound of choice must also be (1) at least as effective as nitrite itself, (2) safe, (3) heat stable, (4) flavorless, and (5) preferably effective at low concentrations.

Several alternatives to nitrite for its antimicrobial action have been tested. The propyl ester of *p*-hydroxybenzoic acid (*i.e.*, propylparaben) is approved for use in the casings of dry sausages to retard mold growth (Sofos and Busta 1980). Use of parabens as antimicrobial agents in nitrite-free cured meats has been suggested. Sweet (1975) used methyl- and propylparabens as antimicrobial agents in his composite nitrite-free curing system. Robach and Pierson (1978) found that these parabens were good candidates as inhibitors of toxin production by *C. botulinum* strain 10755A in microbiological media, but their effectiveness in meat against *C. botulinum* was questionable. Tanaka *et al.* (1978) showed bacteriostasis against *C. botulinum* serotypes A and B spores to be only slightly effective in frankfurters, while Deibel (1979) reported that they were ineffective in a commercial wiener system. Dymicky and Huhtanen (1979) found an increasing effectiveness of the paraben as the ester chain length increased. The undecyl ester was 3,000 times more inhibitory than its methyl counterpart, but on the whole the outlook of parabens as potential alternatives to nitrite in meat products is not very promising.

Sorbic acid and its potassium salt are known inhibitors of yeasts and molds, but knowledge on their action against bacteria is not as comprehensive (Sofos *et al.* 1979*d*). Potassium sorbate is a white crystalline compound with GRAS status. It is approved for use in dry sausages to retard the growth of molds, and the casings are dipped in a 2.5% (w/v) solution of potassium sorbate. Tompkin *et al.* (1974) reported that sorbate also delayed toxin production by *C. botulinum*

in a nitrite-free sausage product. The efficacy of potassium sorbate or sorbic acid for controlling growth of *C. botulinum* in meat products, when used either individually or in combination with reduced levels of nitrite, has been evaluated by many investigators (Ivey and Robach 1978; Tanaka *et al.* 1978; Ivey *et al.* 1978; Sofos *et al.* 1979*a*,*b*; 1980*b*). Tanaka *et al.* (1978) demonstrated that potassium sorbate addition to wieners at 2,700 mg/kg provided an anticlostridial action similar to that of 100 mg/kg nitrite. Sofos *et al.* (1979*a*,*b*,*c*; 1980*a*) reported that sorbic acid when used at a level of 2,000 mg/kg delayed *C. botulinum* toxin production in wieners to an extent similar to that of 156 mg/kg nitrite and longer than that of 80 mg/kg nitrite. These effects were pH dependent and only developed at pH values of less than 6.0. The protonated acid is more active than its anion, consequently, lowering the pH greatly enhances its anticlostridial effect. When nitrite was incorporated into the formulations at either 40 or 80 mg/kg, the bacteriostasis increased and the effective pH level was raised to 6.2 (Sofos *et al.* 1980*b*). These investigators also demonstrated that sorbic acid in wiener emulsions, with or without nitrite, inhibited clostridial spore germination. Several researchers have proposed the use of sorbate-polyphosphate combinations as antimicrobial agents. Ivey *et al.* (1978) presented data demonstrating that a mixture of potassium sorbate, STPP and SAPP was more effective against *C. botulinum* outgrowth in bacon than 120 mg/kg of added nitrite. Synergistic sorbate-polyphosphate effects were also observed in wieners by Tanaka *et al.* (1978).

In four bacon studies, combinations of 40 mg/kg nitrite and 2,600 mg/kg potassium sorbate showed an anticlostridial effect similar to or greater than that of treatments with 120 mg/kg nitrite (Ivey *et al.* 1978; Pierson *et al.* 1979*a*,*b*; Price and Stevenson 1979). In a commercial bacon trial, Sofos *et al.* (1980*b*) found that 40 mg/kg nitrite and 2,600 mg/kg sorbate were effective against clostridial outgrowth but not to the same extent as that of 120 mg/kg nitrite addition. This combination was also found to reduce *N*-nitrosamine formation in cured products from nearly 100 μg/kg to less than 5 μg/kg (Shaver 1979). Sensory data of these cured meats revealed no differences in color and flavor scores. Binstok *et al.* (1998) noted that although the combined use of sorbates and nitrite has been recommended to decrease the requirements for nitrite addition to meats, mutagenic compounds, such as ethylnitrolic acid, may be formed from the reactions between sorbates and nitrites.

Sodium hypophosphite (SHP), another GRAS substance, has been proposed for use as an antimicrobial agent in foods (Pierson *et al.* 1981; Rhodehamel and Pierson 1990). Microbiological studies indicated that a total or partial replacement of nitrite with this compound effectively inhibits production of *C. botulinum* toxins (Banner 1981). Rhodehamel (1983) found SHP to be effective in inhibiting the growth of *C. perfringens* and *C. botulinum* strains 62A, 52A and other Gram positive bacteria. Increasing sodium chloride concentrations in

the media enhanced SHP's inhibition of both *Clostridia* strains. Rhodehamel and Pierson (1990) found SHP to suppress the growth of certain Gram negative spoilage bacteria near neutral pH. In their study, the efficacy of SHP increased slightly with decreasing pH, but they reported in all other studies that SHP's effectiveness as an antimicrobial agent seemed to be independent of the pH of the media. Rhodehamel and Pierson (1990) postulated that since the pK_a (1.1) of SHP's conjugate acid, hypophosphorous acid, was much lower than those of traditionally used food acidulants such as benzoic, sorbic and propionic acids (pK_a 4-5), it is the dissociated acid anion of SHP which exhibits antimicrobial activity. Because SHP exists primarily in the dissociated form over the pH range of 5-7, this may explain why its inhibitory effect is not enhanced by decreasing pH.

At 3,000 mg/kg alone or at 1,000 mg/kg in combination with 40 mg/kg of nitrite, SHP imparted anticlostridial protection to meat products equivalent to that provided by 120 mg/kg of nitrite. Wood *et al.* (1986) evaluated the antibotulinal activity of SHP, potassium sorbate and monomethyl fumarate in nitrite-free curing systems. The treatment containing 3,000 mg/kg SHP, together with CCMP, sodium ascorbate, STPP and TBHQ, most closely resembled that of nitrite at 150 mg/kg in its ability to prevent spore outgrowth and toxin production (Table 9.11). Monomethyl fumarate at 1,250 mg/kg was slightly less effective than SHP and these additives had no adverse effect on the oxidative stability or the color of formulated pork products in model systems. Sodium hypophosphite is bland in taste and as such nitrite-free bacon containing 3,000 mg/kg of SHP had a flavor as desirable as that of its conventionally cured counterpart.

Huhtanen (1984) found methyl and ethyl esters of fumaric acid to be inhibitory to *C. botulinum* in a bacon model system, while parabens had little antibotulinal activity in frankfurters. Monomethyl and monoethyl fumarates at 1,250 mg/kg exhibited more anticlostridial activity than bacon cured with 120 mg/kg nitrite, while dimethyl and diethyl fumarates had activity only equal to that of the nitrite-cured control. These fumarate-treated meat samples were sensorially indistinguishable from that of their nitrite-cured counterparts. Wood *et al.* (1986) reported a similar finding on the efficacy of these fumarates in nitrite-free cured comminuted meats, but SHP was reported to be a superior anticlostridial agent.

Lactic acid, its sodium or potassium salts, or lactic acid-producing bacteria (LAPB) lower the pH of cured meat products and may provide microbial stability to muscle foods (Andres 1985; Anders *et al.* 1989). Because of their long association with food products, lactic acid bacteria and their metabolites possess GRAS status. Some adventitious LAPB that grow on meat cause flavor and odor defects, but conditions that favor the growth of these bacteria generally result in a dramatic extension of the storage life of chilled meats. While lactic

TABLE 9.11
EFFECT OF TREATMENT COMPOSITION ON GAS AND TOXIN PRODUCTION BY *CLOSTRIDIUM BOTULINUM* IN GROUND PORK[a]

Treatment[b]	Incubation at 27°C (days)								
	1	2	3	4	5	6	7	8	27
1. No additives	34/34 +								
2. $NaNO_2$, 150	0/36 −	11/36 +	5/18 +	8/13 +	3/5 †	2/2 +			
3. (2) + ASC	0/36 −	0/32 −	15/30 −	5/14 +	3/9 +	4/6 +	1/2 +	0/1 −	0/1 −
4. CCMP, 12	17/17 +								
5. (4) + ASC + STPP + TBHQ	12/37 +	25/25 +							
6. (4) + SHP	1/18 −	1/16 +	2/14 +	4/12 +	3/5 +	1/2+	0/1 −	0/1 −	0/1 −
7. (4) + ASC + SHP	5/17 −	0/11 −	3/11 †	6/8 +	0/2 −	0/2 −	0/2 −	0/2 −	0/2 −
8. (5) + SHP	6/35 −	3/27 †	1/24 −	6/22 +	3/16 †	5/13 +	6/8 +	1/2 +	0/1 −
9. (5) + PS	0/39 −	32/35 +	3/3 +						
10. (5) + MMF	0/37 −	1/33 −	17/31 +	9/18 +	3/7 +	3/4 +	0/1 +	0/1 +	0/1 −

[a]Number of packs showing gas production/total number of packs. Toxin present, +; toxin absent, -; †presence of toxin was not tested.
[b]Additives were: sodium ascorbate, ASC; cooked cured-meat pigment, CCMP; sodium tripolyphosphate, STPP; *tert*-butylhydroquinone, TBHQ; sodium hypophosphite, SHP; potassium sorbate, PS; and monomethyl fumarate, MMF.
(From Wood *et al.*, 1986).

acid, as such, may be used for surface treatment against microbial activity, use of lactate salts as a component of muscle foods may prove to be more beneficial. Sodium lactate can be added to meat products as a flavor enhancer and preservative; however, it is needed at a rather high level, 2-3%. Incorporation of lactic acid, preferably in an encapsulated form, or LAPB together with fermentable carbohydrates in cured meat formulations is permitted for pH reduction (Bacus 1979; Tanaka *et al.* 1978). Typical LAPB associated with meats are *Lactococcus* spp., *Lactobacillus* spp., *Leuconostoc* spp. and *Pediococcus* spp. Excellent protection against *C. botulinum* toxin formation has been achieved using these LAPB and sucrose in nitrite-free treated bacon. A lowering of the addition level of nitrite to 40 mg/kg, together with these starter cultures has been approved by regulation for use in some products (Thompson 1985).

In addition to the benefits of pH reduction, LAPB act to suppress the growth of pathogens through competitive effects and the production of antimicrobial metabolites such as hydrogen peroxide, organic acids and bacteriocins (Daly *et al.* 1973; Gilliland and Speck 1977). Bacteriocins are proteinaceous compounds that kill or inhibit closely related bacteria. However in recent years this definition has been broadened somewhat in that a number of bacteriocins have demonstrated inhibitory spectra which include unrelated genera (Scannell *et al.* 2000). The most studied bacteriocin has been nisin and it is produced by *Lactococcus* spp. Nisin is a relatively broad-spectrum, lantibiotic-type bacteriocin that is bactericidal to many Gram positive organisms, and it prevents the outgrowth of bacterial spores, notably spores of *C. botulinum* (McMullen and Stiles 1996). Nisin has proven anticlostridial activity in culture media (Scott and Taylor 1981), but due to its poor solubility above pH 6.0 and incomplete diffusion throughout bacon, it has a limited anticlostridial effect (Taylor and Somers 1985). At a level of 500 mg/kg, its extension of the shelf life of nitrite-free cooked meats is marginal, and its activity is lost during refrigerated storage to the point where it is no longer antibotulinal (Rayman *et al.* 1981). Yet, the addition of 100-250 mg/kg nisin in combination with 120 mg/kg nitrite is more effective than addition of 156 mg/kg nitrite alone (Taylor *et al.* 1985). The poor performance of nisin as a biopreservative in meat systems has resulted in the search for other bacteriocin-producing lactic acid bacteria that are more suited to growth in the chilled meat environment (McMullen and Stiles 1996). Research into the use of bacteriocins or bacteriocinogenic LAPB for meat preservation is still in its infancy, and many difficulties must be overcome before LAPB can be used commercially to extend the storage life and to enhance the safety of meats (Stiles and Hastings 1991).

Phenolic antioxidants have been known for many years to have antimicrobial activity against bacteria, molds, and viruses. The effectiveness of antioxidants and chelators as antimicrobial agents has been investigated in model and cooked

meat systems by several researchers (Pierson *et al.* 1981; Winarno *et al.* 1971). In particular, it has been reported that the addition of BHA at 50 mg/kg exerts an inhibitory effect on the growth of *C. botulinum* types A and B in comminuted meats (Pierson *et al.* 1980). BHT, TBHQ and PG were less effective agents (Robach and Pierson 1978). The anticlostridial activity of EDTA (Winarno *et al.* 1971) and various polyphosphates (Sofos 1986) was investigated, but they did not provide effective bacteriostasis. Pierson and Reddy (1982) examined the effectiveness of 15 phenolic compounds for their activity against growth of and toxin production by *C. botulinum* types A and B in comminuted pork. Some of the phenolic compounds examined included esters of *p*-hydroxybenzoic acid and gallic acid, BHA, BHT, TBHQ, 8-hydroxyquinoline and phenol derivatives. 8-Hydroxyquinoline at a concentration of 200 mg/kg, alone or in combination with sodium nitrite at 40 mg/kg, inhibited the growth and toxin production of *C. botulinum* for 60 days at 27°C.

Kanner and Juven (1980) investigated the anticlostridial activity of *S*-nitrosocysteine (SNC), a reaction product in nitrite-cured meat, in meat model systems. The activity of SNC was found to be considerably less than that of nitrite at a 156 mg/kg concentration, and it therefore may not lend itself as an antimicrobial substitute for nitrite. Such compounds may also potentially participate in transnitrosation reactions with bacterial cells and spores or even meat pigments.

Finally, the use of radiation sterilization as an established technique of microbial inactivation and as a method of food preservation has been examined. Irradiation has been used for the sterilization of spices and herbs as well as for inhibiting the sprouting of potatoes and onions (Wasik 1987). Many investigators have studied the effects of gamma irradiation on the sensory and microbiological properties of meat, poultry and seafood products (Colbey *et al.* 1961; Chipault and Mizuno 1966; Anellis *et al.* 1972; Wierbicki and Heiligman 1974; Wierbicki *et al.* 1974; Shults *et al.* 1977; Hussain *et al.* 1978; Curzio and Quaranta 1982; Urbain 1982; Piccini *et al.* 1986; Paul *et al.* 1990). Low-dose irradiation at low temperatures eliminated or reduced the undesirable effects of radiation processing and resulted in an enhancement of the quality of products. Radiation sterilization has also been found effective against the outgrowth of *C. botulinum* spores in meats cured with reduced nitrite levels. Szczawiński *et al.* (1989) and Wierbicki and Heiligman (1980) reported that bacon and ham products as well as meat model systems containing nitrite concentrations of 25-40 mg/kg were microbiologically similar to their conventionally cured-analogs, after radiation processing and upon subsequent refrigerated storage. Wierbicki and Heiligman (1974) reported that the color of irradiated meats with reduced levels of added nitrite, and without the addition of sodium nitrate, as a nitrite reservoir, faded more readily; lower preference scores were noticed in sensory studies. McCormick (1982) found that irradiated preserved meats were superior

to thermally processed meats in terms of their shelf-life without greatly altering their aroma, taste or texture. These results suggest that radiation processing may potentially be used either to substitute for the antimicrobial action of nitrite or to reduce the addition level of nitrite required for its bacteriostasis.

Cumulative Effects of Nitrite-Free Curing Mixtures

While addition of 10-40 mg/kg of sodium nitrite is sufficient to attain a cured color and flavor in processed meats (MacDougall *et al.* 1975), a nitrite concentration of 100-200 mg/kg ensures microbial stability of the products. It is the residual, free nitrite that has a dual effect; while it acts as a potent antibotulinal agent, it is also involved in the possible formation of carcinogenic *N*-nitrosamines. The bound nitrite (10-40 mg/kg) which confers color and flavor to cured meats is relatively safe and produces very little *N*-nitrosamines. Nonetheless, it would be preferred to eliminate the potential for *N*-nitrosamine formation entirely.

Several nitrite-free combinations consisting of the preformed CCMP, a sequestrant and an antioxidant, and an antimicrobial agent for the curing of meat products have been described in this Chapter. These mixtures have been found to be successful in reproducing the color, oxidative stability and flavor, as well as the antimicrobial effects of nitrite. Further details are available in a U.S. patent (Shahidi and Pegg 1993). Several antimicrobial agents in composite non-nitrite curing mixtures were used by Sweet (1975). However, recent studies on systems containing the preformed CCMP indicated that SHP at a 3,000 mg/kg level of addition was somewhat preferred (Wood *et al.* 1986). Nonetheless, all agents tested were effective only at relatively higher concentrations as compared with 100-200 mg/kg of sodium nitrite.

Taking Another Look at Nitrite

As the new century begins, we must again look objectively at nitrite. We should ask ourselves, "What has happened over the past 30-35 years since the *N*-nitrosamine issue surfaced? Has the problem been adequately resolved, or has a bandage been merely placed over the wound?" The cured meat market is a well-established sector in the North American food industry. Limits imposed by regulatory agencies in Canada and the U.S. on the level of nitrite permitted in various meat products, coupled with the responsible nitrite reductions implemented by the meat processing industry have reduced the possibility of over-curing and *N*-nitrosamine formation. This was the greatest cause of concern. Today, product innovations and food safety are what drives the meat industry. Benefits from the controlled and responsible use of nitrite overwhelms the possible risks from outbreak of botulinal food poisoning. Nonetheless because of nitrite's connection with cancer-causing *N*-nitrosamines, its use is a potential

trouble area. Hence, use of a non-nitrite cure for a niche market might be desirable and also offers a practical solution to address the needs of the industry and consumers.

REFERENCES

Al-Jalay, B., Blank, G., McConnell, B. and Al-Khayat, M. 1987. Antioxidant activity of selected spices used in fermented meat sausage. *J. Food Prot.* **50**, 25–27.

Anders, R.J., Cerveny, J.G. and Milkowski, A.L. 1989. Method for delaying *Clostridium botulinum* growth in fish and poultry. US Patent 4,798,729.

Andres, C. 1985. Encapsulated acids furnish greater flexibility in meat/poultry operations. *Food Processing*. June issue, pp. 28–29.

Anellis, A., Berkowitz, D., Swantak, W. and Strojan, C. 1972. Radiation sterilization of prototype military foods: Low-temperature irradiation of codfish cake, corned beef, and pork sausage. *Appl. Microbiol.* **24**, 453–462.

Bacus, J. 1979. Reduces nitrosamines. *Food Eng.* **51**(5), 24.

Bailey, M.E. 1988. Inhibition of warmed-over flavor, with emphasis on Maillard reaction products. *Food Technol.* **42**(6), 123–126.

Banner, R.J. 1981. Sodium hypophosphite: New nitrite replacer? *Food Eng.* **53**(10), 130.

Barbut, S., Draper, H.H. and Hadley, M. 1989. Lipid oxidation in chicken nuggets as affected by meat type, phosphate and packaging. *J. Food Prot.* **52**, 55–58, 61.

Binstok, G., Campos, C., Varela, O. and Gerschenson, L.N. 1998. Sorbate-nitrite reactions in meat products. *Food Res. Int.* **31**, 581–585.

Bloukas, J.G., Arvanitoyannis, I.S. and Siopi, A.A. 1999. Effect of natural colournats and nitrites on colour attributes of frankfurters. *Meat Sci.* **52**, 257–265.

Brown, W.D. 1973. Possible substitutes for nitrite in cured foods. In *Proceedings of the Meat Industry Research Conference*. American Meat Institute Foundation, Arlington, VA, pp. 21–27.

Cassens, R.G. 1995. Use of sodium nitrite in cured meats today. *Food Technol.* **49**(7), 72–80, 115.

Chang, I. and Watts, B.M. 1949. Antioxidants in the hemoglobin catalyzed oxidation of unsaturated fats. *Food Technol.* **3**, 332–336.

Chipault, J.R. and Mizuno, G.R. 1966. Effect of ionizing radiations on stability of fats. *J. Agric. Food Chem.* **14**, 225–229.

Clark, W.R.E. 1970. Modern trends in the application of spice. *Food Manufac.* **45**(1), 53–54.

Coleby, B., Ingram, M., Shepherd, H.J., Thornley, M.J. and Wilson, G.M. 1961. Treatment of meats with ionising radiations. VII.-Effect of low temperatures during irradiation. *J. Sci. Food Agric.* **12**, 483–490.

Coleman, H.M. and Steffen, A.H. 1949. Treatment of animal materials. US Patent 2,491,646.

Cross, C.K. and Ziegler, P. 1965. A comparison of the volatile fractions from cured and uncured meat. *J. Food Sci.* **30**, 610–614.

Curzio, O.A. and Quaranta, H.O. 1982. Delay of oxidative rancidity in irradiated hake (*Merluccius merluccius hubbsi*). *Lebensm.-Wiss. u. -Technol.* **15**, 171–172.

Daly, C., La Chance, M., Sandine, W.E. and Elliker, P.R. 1973. Control of *Staphylococcus aureus* in sausage by starter cultures and chemical acidulation. *J. Food Sci.* **38**, 426–430.

Decker, E.A. and Hultin, H.O. 1990. Nonenzymic catalysts of lipid oxidation in mackerel ordinary muscle. *J. Food Sci.* **55**, 951–953.

Deibel, R.H. 1979. Parabens. Presented at the Meat Industry Research Conference, March 29–30, American Meat Institute, Washington, DC.

Dekker, A. 1958. Meat color preserving composition. US Patent 2,863,777.

Dhillon, A.S. and Maurer, A.J. 1975. Evaluation of betalain pigments as colorants in turkey summer sausages. *Poultry Sci.*, **54**, 1272–1277.

Dymicky, M., Fox, J.B. and Wasserman, A.E. 1975. Color formation in cooked model and meat systems with organic and inorganic compounds. *J. Food Sci.* **40**, 306–309.

Dymicky, M. and Huhtanen, C.N. 1979. Inhibition of *Clostridium botulinum* by *p*-hydroxybenzoic acid *n*-alkyl esters. *Antimicrob. Agents Chemother.* **15**, 798–801.

Dziezak, J.D. 1986. Preservatives: Antioxidants. *Food Technol.* **40**(9), 94–97, 100–102.

Fink-Gremmels, J., Dresel, J. and Leistner, L. 1990. Einsatz von Monascus-Extrakten als Nitrit-Alternative bei Fleischerzeugnissen. *Mitteilungsblatt der Bundesanstalt für Fleischforschung* **29**, 191–200.

Fox, J.B., Jr., Dymicky, M., Keyser, S. and Wasserman, A.E. 1975. Reflectance spectrophotometry of pyridine hemochromes in frankfurters. *J. Agric. Food Chem.* **23**, 322–325.

Francis, F.J. 1994. Food colorants today. *World Ingred.*, Oct/Nov, 8, 9, 11.

Giddings, G.G. 1977. The basis of color in muscle foods. *CRC Crit. Rev. Food Sci. Nutr.* **9**, 81–114.

Gilliland, S.E. and Speck, M.L. 1977. Antagonistic action of *Lactobacillus acidophilus* toward intestinal and foodborne pathogens in associative cultures. *J. Food Prot.* **40**, 820–823.

Gray, J.I., MacDonald, B., Pearson, A.M. and Morton, I.D. 1981. Role of nitrite in cured meat flavor: A review. *J. Food Prot.* **44**, 302–312, 319.

Greene, B.E. and Price, L.G. 1975. Oxidation-induced color and flavor changes in meat. *J. Agric. Food Chem.* **23**, 164–167.

Guerra, M.A., Martín, M., Ramos, M. and Cerezal, P. 1994. Estabilidad del colorante de remolacha roja en polvo durante el almacenamiento refrigerado del jamón cocido. *Rev. Esp. Cienc. Tecnol. Aliment.* **34**, 684–689.

Hopkins, E.W. and Sato, K. 1971. Process for preserving the color of fresh meat. US Patent 3,597,236.

Houlihan, C.M., Ho, C-T. and Chang, S.S. 1984. Elucidation of the chemical structure of novel antioxidant, rosmaridiphenol, isolated from rosemary. *J. Am. Oil Chem. Soc.* **61**, 1036–1039.

Howard, A., Duffy, P., Else, K. and Brown, W.D. 1973. Possible substitutes for nitrite for pigment formation in cured meat products. *J. Agric. Food Chem.* **21**, 894–898.

Huhtanen, C.N. 1984. Nitrite substitutes for controlling *Clostridium botulinum*. In *Developments in Industrial Microbiology, Volume 25, Proceedings of the Fortieth General Meeting of the Society for Industrial Microbiology*, August 14–19, 1983, Sarasota, FL, pp. 349–362.

Huhtanen, C.N., Talley, F.B., Feinberg, J. and Phillips, J.G. 1981. Flavor and antibotulinal evaluation of sorbic acid-containing bacon. *J. Food Sci.* **46**, 1796–1800.

Hussain, A.M., Qureshi, M.J., Haq, I. and Chaudhry, M.A. 1978. Raduriza-tion of freshwater Rahu fish (*Labeo rohita*). *Arch. Lebensmittelhyg.* **29**, 54–57.

Igene, J.O., Yamauchi, K., Pearson, A.M. and Gray, J.I. 1985. Mechanisms by which nitrite inhibits the development of warmed-over flavour (WOF) in cured meat. *Food Chem.* **18**, 1–18.

Ivey, F.J. and Robach, M.C. 1978. Effect of sorbic acid and sodium nitrite on *Clostridium botulinum* outgrowth and toxin production in canned comminut-ed pork. *J. Food Sci.* **43**, 1782–1785.

Ivey, F.J., Shaver, K.J., Christiansen, L.N. and Tompkin, R.B. 1978. Effect of potassium sorbate on toxinogenesis by *Clostridium botulinum* in bacon. *J. Food Prot.* **41**, 621–625.

Kanner, J. and Juven, B.J. 1980. S-Nitrosocysteine as an antioxidant, color-developing, and anticlostridial agent in comminuted turkey meat. *J. Food Sci.* **45**, 1105–1108, 1112.

Kemp, J.D. 1974. Nitrate and nitrite substitutes in meat curing. *Food Prod. Dev.* **8**(8), 64, 67–68, 70.

Kikuzaki, H. and Nakatani, N. 1989. Structure of a new antioxidative phenolic acid from oregano (*Origanum vulgare L.*). *Agric. Biol. Chem.* **53**, 519–524.

Kimoto, W.I., Wasserman, A.E. and Talley, F.B. 1976. Sensory evaluation of flavor development in lean and adipose tissues of bacon. *Lebensm.-Wiss. u. -Technol.* **9**, 274–276.

Kostyla, A.S. and Clydesdale, F.M. 1978. The psychophysical relationships between color and flavor. *CRC Crit. Rev. Food Sci. Nutr.* **10**, 303–321.

Kramer, R.E. 1985. Antioxidants in clove. *J. Am. Oil Chem. Soc.* **62**, 111–113.

Labbe, R.F. and Nishida, G. 1957. A new method of hemin isolation. *Biochim. Biophys. Acta* **26**, 437.

Lawrie, R.A. 1991. Chemical and biochemical constitution of muscle. In *Meat Science, Fifth Edition*. Pergamon Press, Oxford, UK, pp. 48–81.

Ledward, D.A. 1992. Colour of raw and cooked meat. In *The Chemistry of Muscle-based Foods*, ed. D.E. Johnston, M.K. Knight and D.A. Ledward. Special Publication No. 106, The Royal Society of Chemistry, Cambridge, UK, pp. 128–144.

Livingston, D.J. and Brown, W.D. 1981. The chemistry of myoglobin and its reactions. *Food Technol.* **35**(5), 244–252.

Love, J.D. and Pearson, A.M. 1974. Metmyoglobin and nonheme iron as prooxidants in cooked meat. *J. Agric. Food Chem.* **22**, 1032–1034.

MacDonald, B., Gray, J.I. and Gibbins, L.N. 1980. Role of nitrite in cured meat flavor: Antioxidant role of nitrite. *J. Food Sci.* **45**, 893–897.

MacDougall, D.B., Mottram, D.S. and Rhodes, D.N. 1975. Contribution of nitrite and nitrate to the colour and flavour of cured meats. *J. Sci. Food Agric.* **26**, 1743–1754.

Madsen, H.L., Stapelfeldt, H., Bertelsen, G. and Skibsted, L.H. 1993. Cochineal as a colorant in processed pork meat. Colour matching and oxidative stability. *Food Chem.* **46**, 265–271.

McCormick, R.D. 1982. Sidestepping the nitrite cure in processed meats. *Prepared Foods* **6**, 97–101.

McMullen, L.M. and Stiles, M.E. 1996. Potential for use of bacteriocin-producing lactic acid bacteria in the preservation of meats. *J. Food Prot.* **Supplement**, 64–71.

Nakatani, N. 1992. Natural antioxidants from spices. In *Phenolic Compounds in Food and their Effects on Health. II. Antioxidants and Cancer Prevention*, ed. M-T. Huang, C-T. Ho and C.Y. Lee. ACS Symposium Series 507. American Chemical Society, Washington, DC, pp. 72–86.

Nakatani, N. 1997. Antioxidants from spices and herbs. In *Natural Antioxidants: Chemistry, Health Effects, and Applications*, ed. F. Shahidi. AOCS Press, Champaign, IL, pp. 64–75.

Namiki, M. 1990. Antioxidants/antimutagens in food. *CRC Crit. Rev. Food Sci. Nutr.* **29**, 273–300.

National Academy of Sciences. 1982. Alternatives to the Current Use of Nitrite in Foods. National Academy Press, Washington, DC.

Palmin, V., Fedorova, T., Prizenko, V. and Loginova, O. 1975. The manufacture of nitrosohaemochromogen and its application as meat product colorant. *Myasnaya Industriya SSSR* **2**, 40–41.

Palmin, V.V. and Prizenko, V.K. 1974. Stabilization of colour of cured meat using nitric oxide. *Izvestia Vysshikh Uchebnykh Zavedenii, Pishchevaya Tekhnologiya* No. 3, 51–53.

Palmin, V.V., Prizenko, V.K., Fedorova, T.A. and Loginova, O.A. 1973. Some aspects of colour formation in meat products. In *Proceedings of the 19th European Meeting of Meat Research Workers, Part IV*, Paris, France, pp. 1457–1472.

Paquette, M.W., Robach, M.C., Sofos, J.N. and Busta, F.F. 1980. Effects of various concentrations of sodium nitrite and potassium sorbate on color and sensory qualities of commercially prepared bacon. *J. Food Sci.* **45**, 1293–1296.

Pasch, J.H., von Elbe, J.H. and Dinesen, N. 1975. Betalaines as natural food colorants. *Food Prod. Dev.* **9**(9), 38, 42, 45.

Paul, P., Venugopal, V. and Nair, P.M. 1990. Shelf-life enhancement of lamb meat under refrigeration by gamma irradiation. *J. Food Sci.* **55**, 865–866.

Pegg, R.B. 1993. Development of Nitrite-free Meat Curing Systems. Ph.D. Thesis, Memorial University, St. John's, NF, Canada.

Pegg, R.B. and Shahidi, F. 1990. Effects of myoglobin and nitrite or pre-formed cooked cured-meat pigment concentrations on the colour of cooked meats. Presented at the 33rd Annual Conference of the Canadian Institute of Food Science and Technology, June 3–6, Saskatoon, SK. Abstract 30.

Piccini, J.L., Evans, D.R. and Quaranta, H.O. 1986. Comparison of TBA number of irradiated fish with sensory quality. *Food Chem.* **19**, 163–171.

Pierson, M.D., Ivey, F.J., Smoot, L.A. and Vantassell, K.R. 1979*a*. Potassium sorbate inhibition of *Clostridium botulinum* in bacon. Presented at the 79th Annual Meeting of the American Society for Microbiology, May 4–8, Los Angeles, CA. Abstract No. P22.

Pierson, M.D. and Reddy, N.R. 1982. Inhibition of *Clostridium botulinum* by antioxidants and related phenolic compounds in comminuted pork. *J. Food Sci.* **47**, 1926–1929, 1935.

Pierson, M.D., Rice, K.M. and Jadlocki, J.F. 1981. Sodium hypophosphite inhibition of *Clostridium botulinum* in bacon. In *Proceedings of the 27th European Meeting of Meat Research Workers, Vol. 2*, Vienna, Austria, pp. 651–654.

Pierson, M.D., Robach, M.C., Vantassell, K.R. and Smoot, L.A. 1979*b*. Sodium nitrite and potassium sorbate inhibiton of *Clostridium botulinum* as influenced by storage temperature variation. Presented at the 39th Annual Meeting of the Institute of Food Technologists, June 10–13, St. Louis, MO. Abstract No. 373.

Pierson, M.D., Smoot, L.A. and Vantassell, K.R. 1980. Inhibition of *Salmonella typhimurium* and *Staphylococcus aureus* by butylated hydroxyanisole and the propyl ester of *p*-hydroxybenzoic acid. *J. Food Prot.* **43**, 191–194.

Price, J.F. and Stevenson, K.E. 1979. Effects of sorbate and nitrite in bacon on color, flavor and *Clostridium botulinum* toxigenesis. Presented at the 39th Annual Meeting of the Institute of Food Technologists, June 10–13, St. Louis, MO. Abstract No. 372.

Rayman, M.K., Aris, B. and Hurst, A. 1981. Nisin: a possible alternative or adjunct to nitrite in the preservation of meats. *Appl. Environ. Microbiol.* **41**, 375–380.

Rhodehamel, E.J. 1983. Sodium hypophosphite inhibition of the growth of selected foodborne pathogenic and spoilage bacteria. M.S. Thesis, Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

Rhodehamel, E.J. and Pierson, M.D. 1990. Sodium hypophosphite inhibition of the growth of selected Gram negative foodborne pathogenic and spoilage bacteria. *J. Food Prot.* **53**, 56–63.

Rickansrud, D.A. and Henrickson, R.L. 1967. Total pigments and myoglobin concentration in four bovine muscles. *J. Food Sci.* **32**, 57–61.

Robach, M.C. and Pierson, M.D. 1978. Influence of para-hydroxybenzoic acid esters on the growth and toxin production of *Clostridium botulinum* 10755A. *J. Food Sci.* **43**, 787–789, 792.

Saleemi, Z.O., Wanasundara, P.K.J.P.D. and Shahidi, F. 1993. Effects of low-pungency ground mustard seed on oxidative stability, cooking yield, and color characteristics of comminuted pork. *J. Agric. Food Chem.* **41**, 641–643.

Sato, K. and Hegarty, G.R. 1971. Warmed-over flavor in cooked meats. *J. Food Sci.* **36**, 1098–1102.

Scannell, A.G., Ross, R.P., Hill, C. and Arendt, E.K. 2000. An effective lacticin biopreservative in fresh pork sausage. *J. Food Prot.* **63**, 370–375.

Schalfejew, M. 1885. Über die Darstellung des Hämins. *Ber. Deut. Chem. Ges.*, **18**, 232–233.

Scott, V.N. and Taylor, S.L. 1981. Effect of nisin on the outgrowth of *Clostridium botulinum* spores. *J. Food Sci.* **46**, 117–120, 126.

Shahidi, F. 1989. Flavor of cooked meats. In *Flavor Chemistry. Trends and Developments*, ed. R. Teranishi, R.G. Buttery and F. Shahidi. ACS Symposium Series 388. American Chemical Society, Washington, DC, pp. 188–201.

Shahidi, F. 1992. Prevention of lipid oxidation in muscle foods by nitrite and nitrite-free compositions. In *Lipid Oxidation in Food*, ed. A.J. St. Angelo. ACS Symposium Series 500. American Chemical Society, Washington, DC, pp. 161–182.

Shahidi, F. and Hong, C. 1991. Role of metal ions and heme pigments in autoxidation of heat-processed meat products. *Food Chem.* **42**, 339–346.

Shahidi, F. and Pegg, R.B. 1990. Colour characteristics of cooked cured-meat pigment and its application to meat. *Food Chem.* **38**, 61–68.

Shahidi, F. and Pegg, R.B. 1991*a*. Safety and sensory aspects of nitrite alternatives in meat curing. *Can. Chem. News* **43**(2), 12–15.

Shahidi, F. and Pegg, R.B. 1991*b*. Effect of the preformed cooked cured-meat pigment (CCMP) on color parameters of muscle foods. *J. Muscle Foods* **2**, 297–304.

Shahidi, F. and Pegg, R.B. 1991*c*. Novel synthesis of cooked cured-meat pigment. *J. Food Sci.* **56**, 1205–1208.

Shahidi, F. and Pegg, R.B. 1991*d*. Encapsulation of the pre-formed cooked cured-meat pigment. *J. Food Sci.* **56**, 1500–1504, 1518.

Shahidi, F. and Pegg, R.B. 1992. Nitrite-free meat curing systems: Update and review. *Food Chem.* **43**, 185–191.

Shahidi, F. and Pegg, R.B. 1993. Process for preparing a powdered cooked cured-meat pigment. US Patent 5,230,915.

Shahidi, F. and Pegg, R.B. 1998. Inhibition of oxidation of meat lipids with spices and their oleoresins. In *Proceedings of the 44th International Congress of Meat Science and Technology, Volume II*, Barcelona, Spain, August 30-September 4, pp. 680–681.

Shahidi, F., Pegg, R.B. and Saleemi, Z.O. 1995. Stabilization of meat lipids with ground spices. *J. Food Lipids* **2**, 145–153.

Shahadi, F., Pegg, R.B. and Sen, N.P. 1993. Novel nitrite-free processed meats. In *Impact of Food Research on New Product Development*, ed. R. Ali and P.J. Barlow. University of Karachi, Pakistan, January 24-26, pp. 221–245.

Shahidi, F., Pegg, R.B. and Sen, N.P. 1994*a*. Absence of volatile *N*-nitrosamines in cooked nitrite-free cured muscle foods. *Meat Sci.* **37**, 327–336.

Shahidi, F., Rubin, L.J., Diosady, L.L., Chew, V. and Wood, D.F. 1984. Preparation of dinitrosyl ferrohemochrome from hemin and sodium nitrite. *Can. Inst. Food Sci. Technol. J.* **17**, 33–37.

Shahidi, F., Rubin, L.J., Diosady, L.L., Kassam, N. and Li Sui Fong, J.C. 1986. Effect of sequestering agents on lipid oxidation in cooked meats. *Food Chem.* **21**, 145–152.

Shahidi, F., Rubin, L.J., Diosady, L.L. and Wood, D.F. 1985. Preparation of the cooked cured-meat pigment, dinitrosyl ferrohemochrome, from hemin and nitric oxide. *J. Food Sci.* **50**, 272–273.

Shahidi, F., Rubin, L.J. and Wood, D.F. 1987*a*. Control of lipid oxidation in cooked ground pork with antioxidants and dinitrosyl ferrohemochrome. *J. Food Sci.* **52**, 564–567.

Shahidi, F., Rubin, L.J. and Wood, D.F. 1987*b*. Control of lipid oxidation in cooked meats by combinations of antioxidants and chelators. *Food Chem.* **23**, 151–157.

Shahidi, F., Rubin, L.J. and Wood D.F. 1988. Stabilization of meat lipids with nitrite-free curing mixtures. *Meat Sci.* **22**, 73–80.

Shahidi, F. and Wanasundara, P.K.J.P.D. 1992. Phenolic antioxidants. *CRC Crit. Rev. Food Sci. Nutr.* **32**, 67–103.

Shahidi, F., Wanasundara, U.N. and Amarowicz, R. 1994*b*. Natural antioxidants from low-pungency mustard flour. *Food Res. Inter.* **27**, 489–493.

Shaver, K. 1979. Use of potassium sorbate in bacon. In *Proceedings of the Meat Industry Research Conference*. American Meat Institute Foundation, Arlington, VA, pp. 85–86.

Shehata, H.A., Buckenhüskes, H.J. and El-Zoghbi, M.S. 1998. Colour optimization of Egyptian fresh beef sausage by natural colourants. *Fleischwirtschaft Int.* No. 3, 40, 42–44.

Shults, G.W., Cohen, J.S., Howker, J.J. and Wierbicki, E. 1977. Effects of sodium nitrate and sodium nitrite additions and irradiation processing variables on the color and acceptability of corned beef briskets. *J. Food Sci.* **42**, 1506–1509.

Simon, S., Ellis, D.E., MacDonald, B.D., Miller, D.G., Waldman, R.C. and Westerberg, D.O. 1973. Influence of nitrite and nitrate curing ingredients on quality of packaged frankfurters. *J. Food Sci.* **38**, 919–923.

Smith, J.S. and Burge, D.L. Jr. 1987. Protoporphyrin-IX as a substitute for nitrite in cured-meat color production. *J. Food Sci.* **52**, 1728–1729.

Sofos, J.N. 1986. Use of phosphates in low-sodium meat products. *Food Technol.* **40**(9), 52, 54–58, 60, 62, 64, 66, 68–69.

Sofos, J.N. and Busta, F.F. 1980. Alternatives to the use of nitrite as an antibotulinal agent. *Food Technol.* **34**(5), 244–251.

Sofos, J.N., Busta, F.F., Bhothipaksa, K. and Allen, C.E. 1979*a*. Sodium nitrite and sorbic acid effects on *Clostridium botulinum* toxin formation in chicken frankfurter-type emulsions. *J. Food Sci.* **44**, 668–672, 675.

Sofos, J.N., Busta, F.F. and Allen, C.E. 1979*b*. Sodium nitrite and sorbic acid effects on *Clostridium botulinum* spore germination and total microbial growth in chicken frankfurter emulsions during temperature abuse. *Appl. Environ. Microbiol.* **37**, 1103–1109.

Sofos, J.N., Busta, F.F. and Allen, C.E. 1979*c*. Effects of sodium nitrite on *Clostridium botulinum* toxin production in frankfurter emulsions formulated with meat and soy proteins. *J. Food Sci.* **44**, 1267–1271.

Sofos, J.N., Busta, F.F. and Allen, C.E. 1979*d*. Botulism control by nitrite and sorbate in cured meats: A review. *J. Food Prot.* **42**, 739–770.

Sofos, J.N., Busta, F.F. and Allen, C.E. 1980*a*. Influence of pH on *Clostridium botulinum* control by sodium nitrite and sorbic acid in chicken emulsions. *J. Food Sci.* **45**, 7–12.

Sofos, J.N., Busta, F.F., Bhothipaksa, K., Allen, C.E., Robach, M.C. and Paquette, M.W. 1980*b*. Effects of various concentrations of sodium nitrite and potassium sorbate on *Clostridium botulinum* toxin production in commercially prepared bacon. *J. Food Sci.* **45**, 1285–1292.

Stevanović, M., Zlender, B., Abram, V. and Kumperger, U. 1997. The influence of pork muscle's type and addition of pigment CCMP on the colour of meat emulsion coagulates. In *Proceedings of 43rd International Congress of Meat Science and Technology*, Auckland, New Zealand, July 27-August 1, pp. 462–463.

Stiles, M.E. and Hastings, J.W. 1991. Bacteriocin production by lactic acid bacteria: potential for use in meat preservation. *Trends Food Sci. Technol.* **2**, 247–251.

Sugita, K., Yamauchi, K., Ohashi, T., Suiko, M. and Miura, M. 1993. Utilization of dried radish chip extracts in processing of sausages: An ingredient for a nitrite-free curing system. *J. Jap. Soc. Food Sci. Technol.* **40**, 339–347.

Sweet, C.W. 1975. Additive composition for reduced particle size meats in the curing thereof. US Patent 3,899,600.

Szczawiński, J., Szczawińska, M. and Szulc, M. 1989. Effect of irradiaton on antibotulinal efficacy of nitrite. *J. Food Sci.* **54**, 1313–1317.

Tanaka, K., Chung, K.C., Hayatsu, H. and Kada, T. 1978. Inhibition of nitrosamine formation *in vitro* by sorbic acid. *Food Cosmet. Toxicol.* **16**, 209–215.

Tarladgis, B.G. 1967. Preservation of meat color. US Patent 3,360,381.

Taylor, S.L. and Somers, E.B. 1985. Evaluation of the antibotulinal effectiveness of nisin in bacon. *J. Food Prot.* **48**, 949–952.

Taylor, S.L., Somers, E.B. and Krueger, L.A. 1985. Antibotulinal effectiveness of nisin-nitrite combinations in culture medium and chicken frankfurter emulsions. *J. Food Prot.* **48**, 234–239.

Thompson, K. 1985. USDA proposes to lower bacon nitrite level to 100 ppm; or 40 ppm with starter cultures. *Meat Industry*, June issue. p. 107.

Tims, M.J. and Watts, B.M. 1958. Protection of cooked meats with phosphates. *Food Technol.* **12**, 240–243.

Tompkin, R.B., Christiansen, L.N., Shaparis, A.B. and Bolin, H. 1974. Effect of potassium sorbate on Salmonellae, *Staphylococcus aureus*, *Clostridium perfringens*, and *Clostridium botulinum* in cooked, uncured sausage. *Appl. Microbiol.* **28**, 262–264.

Urbain, W.M. 1982. Radurization and radicidation: Meat and poultry. In *Preservation of Food by Ionizing Radiation. Volume III*, ed. E.S. Josephson and M.S. Peterson. CRC Press, Inc., Boca Raton, FL, pp. 1–11.

van den Oord, A.H.A. and DeVries, B. 1971. Preservation of meat color. US Patent 3,615,691.

von Elbe, J.H. and Maing, I.-Y. 1973. Betalains as possible food colorants of meat substitutes. *Cereal Science Today* **18**, 263–264, 316–317.

von Elbe, J.H., Klement, J.T., Amundson, C.H., Cassens, R.G. and Lindsay, R.C. 1974*a*. Evaluation of betalain pigments as sausage colorants. *J. Food Sci.* **39**, 128–132.

von Elbe, J.H., Maing, I.-Y. and Amundson, C.H. 1974*b*. Color stability of betanin. *J. Food Sci.* **39**, 334–337.

Wasik, R.J. 1987. Food irradiation. *Food Can.* **47**(6), 46.

Wasserman, A.E. and Kimoto, W. 1977. Consumer evaluation of the flavour of bacon cured with and without sodium nitrite. In *Proceedings of the Second International Symposium on Nitrite in Meat Products*, Zeist, The Netherlands, September 7–10, 1976, ed. B.J. Tinbergen and B. Krol. Wageningen Centre for Agricultural Publishing and Documentation, Wageningen, The Netherlands, pp. 73–74.

Wasserman, A.E., Kimoto, W. and Phillips, J.G. 1977. Consumer acceptance of nitrite-free bacon. *J. Food Prot.* **40**, 683–685.

Wierbicki, E. and Heiligman, F. 1974. Shelf stable cured ham with low nitrite-nitrate additions preserved by radappertization. In *Proceedings of the International Symposium on Nitrite in Meat Products*, Zeist, The Netherlands, September 10–14, 1973, ed. B. Krol and B.J. Tinbergen. Wageningen Centre for Agricultural Publishing and Documentation, Wageningen, The Netherlands, pp. 189–212.

Wierbicki, E. and Heiligman, F. 1980. Irradiated bacon without and with reduced addition of nitrite. In *Proceedings of the 26th European Meeting of Meat Research Workers, Vol 1*, Colorado Springs, CO. American Meat Science Association, Chicago, IL, pp. 198–201.

Wierbicki, E., Heiligman, F. and Wasserman, A.E. 1974. Cured meats with reduced nitrite preserved by radappertization. In *Proceedings of the 20th European Meeting of Meat Research Workers*, Dublin, Ireland, September 15–20, pp. 100–102.

Williams, J.C. and Greene, B.E. 1979. Plate waste of bacon cured with and without sodium nitrite. *J. Food Sci.* **44**, 1260, 1262.

Winarno, F.G., Stumbo, C.R. and Hayes, K.M. 1971. Effect of EDTA on the germination of and outgrowth from spores of *Clostridium botulinum* 62-A. *J. Food Sci.* **36**, 781–785.

Wood, D.S., Collins-Thompson, D.L., Usborne, W.R. and Picard, B. 1986. An evaluation of antibotulinal activity in nitrite-free curing systems containing dinitrosyl ferrohemochrome. *J. Food Prot.* **49**, 691–695.

Yun, J.J. 1984. Alternative meat curing system: Flavour and oxidative stability. M.A.Sc. Thesis, University of Toronto, Toronto, ON, Canada.

Yun, J., Shahidi, F., Rubin, L.J. and Diosady, L.L. 1987. Oxidative stability and flavour acceptability of nitrite-free meat-curing systems. *Can. Inst. Food Sci. Technol J.* **20**, 246–251.

GLOSSARY

ΔE	-	overall color difference
AIM	-	Alberta Industrial Mustard
AP	-	ascorbyl palmitate
ASC	-	sodium ascorbate
ATNC	-	apparent total *N*-nitroso compounds
BHA	-	butylated hydroxyanisole
BHT	-	butylated hydroxytoluene
CCMP	-	cooked cured-meat pigment
CFIA	-	Canadian Food Inspection Agency
CFU	-	colony forming units
DMA	-	dimethylamine
DMSO	-	dimethylsulfoxide
DNPH	-	dinitrophenylhydrazine
EDTA	-	ethylenediaminetetraacetic acid
EPR	-	electron paramagnetic resonance
FSIS	-	Food Safety and Inspection Service
GC	-	gas chromatography
GC-MS	-	gas chromatography-mass spectrometry
GDL	-	glucono-δ-lactone
GRAS	-	generally recognized as safe
Hb	-	hemoglobin
HPB	-	Health Protection Branch
HPLC	-	high performance liquid chromatography
HS	-	headspace
HS-GC	-	headspace-gas chromatography
I	-	nuclear spin quantum number
IHP	-	inositol hexaphosphate
IR	-	infrared
LAPB	-	lactic acid-producing bacteria
MAP	-	modified atmosphere packaging
Mb ($MbFe^{II}$)	-	myoglobin
MbO_2	-	oxymyoglobin
metMb ($MbFe^{III}$)	-	metmyoglobin
MFD	-	meat flavor deterioration
MMF	-	monomethyl fumarate
MS	-	mass spectrometry
n	-	principal quantum number
NADH	-	(reduced) nicotinamide adenine dinucleotide
NADPH	-	nicotinamide dinucleotide

NAS	-	National Academy of Sciences
NDBA	-	*N*-nitrosodi-*n*-butylamine
NDBzA	-	*N*-nitrosodibenzylamine
NDEA	-	*N*-nitrosodiethylamine
NDiBA	-	*N*-nitrosodiisobutylamine
NDMA	-	*N*-nitrosodimethylamine
NDPA	-	*N*-nitrosodi-*n*-propylamine
NED	-	*N*-(1-naphthyl)ethylenediamine dihydrochloride
NEMA	-	*N*-nitrosoethylmethylamine
NHPRO	-	*N*-nitroso-4-hydroxyproline
NMOR	-	*N*-nitrosomorpholine
NMR	-	nuclear magnetic resonance
NOCA	-	*N*-nitrosooxazolidine-4-carboxylic acid
NOHb	-	nitrosylhemoglobin
NOMb ($MbFe^{II}NO$)	-	nitrosylmyoglobin
NOmetMb ($MbFe^{III}NO$)	-	nitrosylmetmyoglobin
NO_x	-	various nitrogen oxides
NPIP	-	*N*-nitrosopiperidine
NPRO	-	*N*-nitrosoproline
NPYR	-	*N*-nitrosopyrrolidine
NSAR	-	*N*-nitrososarcosine
NTCA	-	*N*-nitrosothiazolidine-4-carboxylic acid
NTHZ	-	*N*-nitrosothiazolidine
NVNC	-	non-volatile *N*-nitroso compound
PCCMP	-	powdered cooked cured-meat pigment
PG	-	propyl gallate
PP-IX	-	protoporphyrin-IX
ppb	-	parts per billion
ppm	-	parts per million
PS	-	potassium sorbate
Pyr	-	pyridine
PTFE	-	teflon poly(tetrafluoroethylene)
$R^{\bullet}$	-	lipid radical
RBC	-	red blood cells
RdH	-	reductant
RH	-	relative humidity
$RO^{\bullet}$	-	alkoxy radical
$ROO^{\bullet}$	-	hydroperoxy radical
ROOH	-	lipid hydroperoxide
RSNO	-	nitrosothiol compound
S	-	magnetic spin
SAPP	-	sodium acid pyrophosphate

SHMP	-	sodium hexametaphosphate
SHP	-	sodium hypophosphite
SNC	-	*S*-nitrosocysteine
SPE	-	solid-phase extraction
STPP	-	sodium tripolyphosphate
t	-	time
T	-	temperature
TBA	-	2-thiobarbituric acid
TBARS	-	2-thiobarbituric acid reactive substances
TBHQ	-	*t*-butylhydroquinone
TEA	-	thermal energy analyzer
TLC	-	thin layer chromatography
TMA	-	trimethylamine
TMAO	-	trimethylamine *N*-oxide
TPP	-	tetraphenylporphyrin
TSPP	-	tetrasodium pyrophosphate
USDA	-	United States Department of Agriculture
USFDA	-	United States Food and Drug Administration
UV-VIS	-	ultraviolet-visible
VIS	-	visible
VNA	-	volatile *N*-nitrosamine
v/v	-	volume per volume
WOF	-	warmed-over flavor
w/v	-	weight per volume
w/w	-	weight per weight

INDEX