Handbook of Meat, Poultry and Seafood Quality

SECOND EDITION

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WILEY-BLACKWELL

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Second Edition

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WILEY-BLACKWELL
A John Wiley & Sons, Inc., Publication

This edition first published 2012 © 2012 by John Wiley & Sons Inc.
First Edition © 2007 Blackwell Publishing Ltd.

Wiley-Blackwell is an imprint of John Wiley & Sons, formed by the merger of Wiley's global Scientific, Technical and Medical business with Blackwell Publishing.

Editorial Offices
2121 State Avenue, Ames, Iowa 50014-8300, USA
The Atrium, Southern Gate, Chichester, West Sussex, PO19 8SQ, UK
9600 Garsington Road, Oxford, OX4 2DQ, UK

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Library of Congress Cataloging-in-Publication Data

Handbook of meat, poultry and seafood quality / [edited by] Leo M. L. Nollet. – 2nd ed.
p. cm.
Includes bibliographical references and index.
ISBN 978-0-470-95832-2 (hardcover : alk. paper)
1. Meat–Quality–Handbooks, manuals, etc. 2. Poultry–Quality–Handbooks, manuals, etc.
3. Seafood–Quality–Handbooks, manuals, etc. I. Nollet, Leo M. L., 1948–
TX556.M4H36 2012
363.19′29–dc23

2012004867

A catalogue record for this book is available from the British Library.

Wiley also publishes its books in a variety of electronic formats. Some content that appears in print may not be available in electronic books.

Set in 10/12pt Times by SPi Publisher Services, Pondicherry, India

1 2012

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Preface

The quality of a food is defined from two perspectives: scientific status and consumer preferences. Scientific factors affecting the quality of a food include composition, spoilage, colorants, additives, nutrients, flavorants, functional ingredients (affecting health), contamination, general safety, etc. Consumer preferences are linked directly to the human senses: sight, touch, smell, taste, and mouthfeel. Visual factors refer to color, moisture, overall appearance, etc. Tactile factors refer to sliminess, elasticity, softness, hardness, etc. Factors responsible for taste and smell cover many specific chemicals. Mouthfeel refers to texture, softness, tenderness, chewy sensation, etc. In the last 10 years or so, food quality has been defined by most professionals to include "health" and "safety." The nutrition and safety of foods has always been important, especially since the 1970s. The word "health" now includes manipulating certain chemical components in food to increase food's positive impact on our health. "Safety" now refers to a whole spectrum of new legal or recommended requirements for both fresh and processed foods. These requirements are designed to exclude or prevent undesirable agents (biological, chemical, physical, environmental, and extraneous) in our foods.

For ease of reference, we can consider that the quality of a food is the composite picture of many factors. In the last 5 to 10 years, many professional reference books have become available that explore the relationship between such factors and food quality.

The second edition of *Handbook of Meat, Poultry, and Seafood Quality* focuses especially on the different quality factors of muscle foods (beef, pork, poultry, and seafood).

The book consists of six parts. Part I, Quality Aspects of Products of Animal Origin, deals with general sensory aspects of muscle foods such as color, texture, and flavor and how to determine these parameters in muscle foods. Part II, Flavor, covers the sensory and chemical characterization of food; describes the process, savory, and off flavors and rancidity in foods; and deals with land animal products and marine animal and plant products.

Parts III to VI explore in depth quality parameters of beef (Part III), of pork (Part IV), of poultry (Part V), and seafood (Part VI). Parameters discussed in the different chapters of these four parts include shelf life, microbiological and sensory properties, packaging, and others, for fresh and for frozen muscle foods.

This work is the result of the combined efforts of more than 30 professionals from industry, government, and academia worldwide. They represent more than 11 countries with diverse expertise and background in the quality of muscle foods. An international editorial team of seven members from three countries led these experts. Each contributor or editor was responsible for researching and reviewing subjects of immense depth, breadth, and complexity. Care and attention were paramount to ensure technical accuracy for each topic. It is our sincere hope and expectation that it will serve as an essential reference on the quality of muscle foods for all professionals in government, industry, and academia.

The editorial team wishes to thank all the contributors for sharing their expertise throughout our journey. We also thank the reviewers for giving their valuable comments on how to improve the contents of each chapter. All these professionals are the ones who made this book possible. We trust that you will benefit from the fruits of their labor.

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Part One

Quality Aspects of Products of Animal Origin

1 Chemical and Biochemical Aspects of Color in Muscle Foods

José Ángel Pérez-Alvarez and Juana Fernández-López

Abstract: Color is the main aspect that defines a food's quality and that most influences consumer choice. Sensorial quality, especially color and appearance of meat, can be affected by both internal and external factors.

Chromoproteins, carotenes, and carotenoproteins are important in meat color. Carotenes are responsible for the color of beef fat, poultry meat and skin, fish, and shellfish. Of the hemoglobins present postmortem in the muscle, myoglobin is the one mainly responsible for color. Cytochromes are metalloproteins with a heme group with a role in meat coloration. The principal role of fat is in the brightness of meat products.

The color of meat may be altered by several factors, including exposure to light, microbial growth, rancidity, and exposure to oxygen.

Keywords: Muscle-based food color, carotenes, hemoproteins, myoglobin, cytochromes, fat color, melanosis, discoloration, premature browning, shelf life

1.1 GENERAL ASPECTS OF MUSCLE-BASED FOOD COLOR

The first impression that a consumer receives concerning a food product is established visually, and among the properties observed are color, form, and surface characteristics. Color is the main aspect that defines a food's quality, and a product may be rejected simply because of its color, even before other properties, such as aroma, texture, and taste, can be evaluated. This is why the appearance (optical properties, physical form, and presentation) of muscle-based products at the sales point is of such importance (Lanari *et al*. 2002).

Regarding the specific characteristics that contribute to the physical appearance of meat, color is the quality that most influences consumer choice (Krammer 1994). The relationship between meat color and quality has been the subject of study since the 1950s, indeed, since Urbain (1952) described how consumers had learned through experience that the color of fresh meat is bright red, and any deviation from this color (nonuniform or anomalous coloring) is unacceptable (Diestre 1992). The color of fresh meat and associated adipose tissue is, then, of great importance for its commercial acceptability, especially in the cases of beef and lamb (Cornforth 1994), and in certain countries, for example, the USA and Canada, there have been many studies to identify the factors controlling its stability. Adams and Huffman (1972) affirmed that consumers relate the color of meat to its freshness. In poultry, the consumers of

Handbook of Meat, Poultry and Seafood Quality, Second Edition. Edited by Leo M. L. Nollet.

many countries also associate meat color with the way in which the animal was raised (intensive or extensive) and fed (cereals, animal feed, etc.).

Color as a quality factor for meat can be appreciated in different ways in different countries; for example, in Denmark, pork meat color holds fifth place among qualities that affect consumers' purchase decisions (Bryhni *et al*. 2002). The sensorial quality, especially color and appearance (Brewer & Mckeith 1999), of meat can be affected by both internal and external factors. In the case of internal factors, in fish, for example, a particular problem that has been encountered in rearing some Pargus species is the darkening of the body after the capture of wild fish and during farming. During farming and marketing, the skin color (silver-red) turns dark gray (especially the tail and fins) (Kentouri *et al*. 1995; Lin *et al*. 1998). In the case of farmed salmon, too, feeding fish with carotenoid pigments is regarded as the most important management practice for marketing (Moe 1990), because without them, flesh and skin color would be less visually attractive, and therefore would be less valued as a food (Baker 2002).

Food technologists, especially those concerned with the meat industry, have a special interest in the color of food for several reasons—first, because of the need to maintain a uniform color throughout processing; second, to prevent any external or internal agent from acting on the product during its processing, storage, and display; third, to improve or optimize a product's color and appearance; and, lastly, to attempt to bring the product's color into line with what the consumer expects. Put simply, the color of meat is determined by the pigments present in it. These can be classified into four types: (1) biological pigments (carotenes and hemopigments), which are accumulated or synthesized in the organism antemortem (Lanari *et al*. 2002); (2) pigments produced as a result of damage during manipulation or inadequate processing conditions; (3) pigments produced postmortem (through enzymatic or nonenzymatic reactions) (Montero *et al*. 2001; Klomklao *et al*. 2006); and (4) pigments resulting from the addition of natural or artificial colorants (Fernández-López *et al*. 2002).

As a quality parameter, color has been widely studied in fresh meat (MacDougall 1982; Cassens *et al*. 1995; Faustman *et al*. 1996) and cooked products (Anderson *et al*. 1990; Fernández-Ginés *et al*. 2003; Fernández-López *et al*. 2003). However, dry-cured meat products have received less attention (Pérez-Alvarez 1996; Pagán-Moreno *et al*. 1998; Aleson *et al*. 2003) because in this type of product, color formation takes place during different processing stages (Pérez-Alvarez *et al*. 1997; Fernández-López *et al*. 2000); recently, a new heme pigment has been identified in this type of product (Parolari *et al*. 2003; Wakamatsu *et al*. 2004a, b). From a practical point of view, color plays a fundamental role in the animal production sector, especially in meat production (beef and poultry, basically) (Zhou *et al*. 1993; Esteve 1994; Verdoes *et al*. 1999; Irie 2001), since in many countries of the European Union (e.g., Spain and Holland) paleness receives a wholesale premium.

For fish, skin and flesh discoloration is a very important problem, especially in highly appreciated species. Since the skin and flesh color must be very vivid, many efforts have been directed at improving color, mainly through dietary control (carotene-enriched diets) (Fujita *et al*. 1983; Mori 1993). Without these pigments, the aquaculture industry would find it hard to undertake the production of some species because fish demand is driven through consumer demand for quality products (Baker 2002). In fish, consumer preference is often influenced by body pigmentation. Fish flesh color is an important quality parameter for most farmed fish, especially with salmonids (salmon, rainbow trout), (Francis 1995; Hyun *et al*. 1999), in which the pink or red color of fillets is an important feature (Sigurgisladottir *et al*. 1994; Sigurgisladottir *et al*. 1997). For example, a uniform red color in rainbow trout is considered to indicate a high-quality product and is a reason for its acceptability, while for the tuna fish industry, it is very important to avoid discoloration in fresh and processed meat and to increase its shelf life

(Goodrick *et al*. 1991; Tze *et al*. 2001). Fish nutrition has an important impact on several parameters that directly influence the quality of fish, some of which are color and appearance. The color of salmonid flesh is one of the most important quality parameters, because consumers have a preference for red- or pink-colored products in the case of salmonids. This is the reason for using carotenoids in aquaculture.

1.2 CHEMICAL AND BIOCHEMICAL ASPECTS OF MUSCLE-BASED FOOD COLOR

Of the major components of meat, proteins are the most important since they are only provided by essential amino acids, which are very important for the organism's correct functioning; proteins also make a technological contribution during processing, and some are responsible for such important attributes as color. These are the so-called chromoproteins, and they are mainly composed of a porphyrinic group conjugated with a transition metal, principally iron metalloporphyrin, which forms conjugation complexes (heme groups) (Whitaker 1972) that are responsible for color. However, carotenes and carotenoproteins (organic compounds with isoprenoid-type conjugated systems) exist alongside chromoproteins and also play an important part in meat color. There are also some enzymatic systems whose coenzymes or prosthetic groups possess chromophoric properties (peroxidases, cytochromes, and flavins) (Faust-man *et al*. 1996). However, their contribution to meat color is slight. Below, the principal characteristics of the major compounds that impart color to meat are described.

1.3 CAROTENES

Carotenes are responsible for the color of beef fat, poultry meat and skin, fish, and shellfish; in the last two cases, these are of great economic importance. The color of the fat is also important in carcass grading. Furthermore, carotenoids can be used as muscle-based food coloring agents (Verdoes *et al*. 1999). An important factor to be taken into account with these compounds is that they are not synthesized by the live animal but are obtained by assimilation (Pérez-Alvarez *et al*. 2000), for instance, in the diet. Salmonids, for example, obtain carotenes in the wild in their preys, but in intensive fish culture, carotenoids must be added to the diet. Farmed fish, especially colored fish (e.g., salmon and rainbow trout), are now a major industry. For example, Norway exports a great part of its salmon production. Carotenoid pigments have been used in aquafeed for many years in order to impart the desired flesh color in farmed salmonids (Baker 2002). Astaxanthin has been the main flesh-coloring pigment of choice in most trout- and salmon-farming industries. The type of carotene used in animal feed is very important because the fish farmer may find that pigmentation takes on a heterogeneous appearance, which is contrary to general consumer acceptance (Yanar *et al*. 2006). The preferred pigments used in the Canadian aquaculture industry are synthetic canthaxanthin (Cx) and synthetic astaxanthin (Ax) (Higgs *et al*. 1995). In fats, the fatty acid composition can affect their color. When the ratio of cis-monounsaturated to saturated fatty acids is high, the fat exhibits a greater yellow color (Zhou *et al*. 1993). In the case of the carotenes present in fish tissues, these come from the ingestion of zooplankton, algae, and crustacean wastes (Ostermeyer & Schmidt 2004), and the levels are sometimes very high. This is possible because fish have the capacity to transport and deposit this pigment to specific sites in their muscles (Baker 2002). The deposition of Ax is higher in dark muscle than in light muscle (Ingemansson *et al*. 1993). The shells of many

crustaceans, for example, lobster (*Panulirus argus*), also contain these compounds. Carotenoids have been extracted from crustacean wastes with organic solvents, but in many of the methods pigment degradation occurs (Charest *et al*. 2001).

The pigments responsible for color in fish, particularly salmonids (trout and salmon, among others), are Ax and Cx, although they are also present in tunids and are one of the most important natural pigments of marine origin. In the case of shellfish, their color depends on the so-called carotenoproteins, which are proteins with a prosthetic group that may contain various types of carotene (Minguez-Mosquera 1997), which are themselves water soluble (Shahidi & Matusalach-Brown 1998). Henmi *et al*. (1990a) reported that carotenoid–protein interaction in the salmon muscle is weak, and that Ax and Cx have a trans configuration in vivo. Henmi *et al*. (1990b) also reported that the actomyosins from salmonids showed a higher affinity for ketocarotenoids than those of other fish, except common mackerel. These authors also described correlations between the surface hydrophobicity of actomyosins and the combination of Ax and/or canthaxanthin with actomyosins. From a chemical point of view, astaxanthin or canthaxanthin bind via a beta-ionone ring to a hydrophobic binding site on actomyosin; the hydroxyl and keto end groups of the beta-end group of carotenoids intensify binding to actomyosin. Salmon actomyosin forms complexes with free Ax, astaxanthin monoester, canthaxanthin, echinenone, zeaxanthin, and beta-carotene, but not astaxanthin diester (in which a long-chain fatty acid residue may cause steric hindrance). The lipids in the actomyosin complex have no effect on the binding of carotenoids (Henmi *et al*. 1989). They are distributed in different amounts in the flesh, head, and carapace of crustaceans; for example, astaxanthin and its esters are the major carotenoids found in the extracts from different species of shrimp (*Penaeus monodon*, *Penaeus indicus*, *Metapenaeus dobsonii*, *Parapenaeopsis stylifera*) (Sachindra *et al*. 2005a), but there are different types of carotenes, depending on whether the crustaceans are marine or fresh water (Sachindra *et al*. 2005b). Another difference is that the concentration of unsaturated fatty acids in its carotenoid extracts was found to be higher than that of saturated fatty acids. In raw muscle, the main carotenoid concentration was strongly correlated with some color attributes (hue, chroma, and lightness) (Choubert *et al*. 1992). Torrisen *et al*. (1989) reported that a level of 4 mg/kg in fish fillets is regarded as a minimum acceptable carotenoid concentration in marketable-farmed salmon. Sex also affects carotene concentration: female muscles, which contain much more carotenoid, are more strongly colored than male muscles (Norris & Cunningham 2004).

As suggested by Torrisen *et al*. (1989), the rate of carotenoid deposition in salmonids is curvilinear throughout the life of the fish. As the growth rate is obviously under strong genetic control, the genetic correlation between the growth rate and color is high. It must be taken into account that carotenoids migrate from the muscle to the gonads. Carotene-type deposition in salmonid species differs; for example, Ax is more efficiently deposited than Cx in rainbow trout (Storebakken & Choubert 1991; Torrissen 1986), but this pattern is not the same for Atlantic salmon. These differences may be due to genetic background and/or environment (Baker 2002). Choubert *et al*. (1997) reported that in rainbow trout there is an unequal distribution of carotenoids so that the color of the muscle lightens from the head toward the tail and from the midline of the fish toward the dorsal and ventral external area of the fish.

From a chemical point of view, carotenoids are organic molecules that contain a conjugated carbon–carbon double bond system, which is responsible for their color. But this can be a problem during processing, because a high number of conjugated double bonds may be subject to oxidation, which can lead to discoloration of the carotenoids (Liaaen-Jensen 1971; Choubert & Baccaunaud 2006). As carotenoids are lipid soluble compounds, it might be thought that increasing dietary fat would increase carotene absorption and deposition, but this is not necessarily the case for all salmonids. The retention of carotenoids in the flesh is relatively poor,

with only 10%–18% of pigment obtained from the diet being retained (Nickell & Bromage 1998). Astaxanthin can be found in its free, mono-, or diesterified forms. In processed shrimps, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are the principal fatty acids esterified with the portion of astaxanthin linked to chitin in the carapace (Guillou *et al*. 1995). b-carotene and Ax are fat-soluble pigments found in squid oil. However, technological processes, such as refining, can remove Ax completely (Hwei & Min 1994).

In fish-derived products, the carotene content has previously been used as a quality parameter on its own; however, it has been demonstrated that this is not appropriate, and that other characteristics may influence color (Little *et al*. 1979). The carotene content and its influence on color is perhaps one of the characteristics that has received most attention (Swatland 1995). In the case of meat, especially beef, an excess of carotenes may actually lower the quality (Irie 2001), as occurs sometimes when classifying carcasses. The Japanese system for beef carcass classification identifies acceptable fats as white, slightly off-white, or slightly reddish white in color, while pink-yellowish and dark yellow are unacceptable (Irie 2001). It is precisely the carotenes that are responsible for these last two colorations. However, in other animal species, such as chicken (Castaneda *et al*. 2005), the opposite effect is observed, since a high carotene (xanthophyll) concentration is much appreciated by consumers (Esteve 1994), yellow being associated with traditional or "home-reared" feeding (Pérez-Alvarez *et al*. 2000). The use of the carotenoid canthaxanthin as a coloring agent in poultry feeds is designed to result in the desired coloration of poultry meat skins. The carotenoids used include citranaxanthin, capsanthin, and capsorubin, but Cx shows superior pigmenting properties and stability during processing and storage (Blanch 1999). To improve its color and brilliance, 0.004–0.04 wt% proanthocyanidin is added to fish feed containing carotenoids (Sakiura 2001). For rainbow trout, carotenoid concentrations could be 10.7 or 73 ppm Cx, or 47 or 53 ppm Ax.

1.4 HEMOPROTEINS

Of the hemoproteins present, postmortem in the muscle, myoglobin (Mb) is the one mainly responsible for color, since hemoglobin (Hb) arises from the red cells that are not eliminated during the bleeding process and are retained in the vascular system, basically in the capillaries (incomplete exsanguination; the average amount of blood remaining in meat joints is 0.3%) (Warris & Rodes 1977). However, the contribution of red cells to color does not usually exceed 5% (Swatland 1995). There is wide variation in the amounts of Hb from muscle tissue of bled and unbled fish. Mb content is minimal compared with the Hb content in fish light muscle and white fish whole muscle. Hb made up 65% and 56% by weight of the total heme protein in dark muscle from unbled and bled fish, respectively (Richards & Hultin 2002). Mb, on average, represents 1.5% by weight of the proteins of the skeletal muscle, while Hb represents about 0.5%, the same as the cytochromes and flavoproteins combined. Mb is an intracellular (sarcoplasmic) pigment apparently distributed uniformly within muscles (Ledward 1992; Kanner 1994). It is red in color and water soluble, and it is found in the red fibers of both vertebrates and invertebrates (Knipe 1993; Park & Morrisey 1994), where it fulfills the physiological role of intervening in the oxidative phosphorylation chain in the muscle (Moss 1992).

1.4.1 Structure of myoglobin

Structurally, Mb can be described as a monomeric globular protein with a very compact, well-ordered structure that is specifically, almost triangularly, folded and bound to a heme group (Whitaker 1972). It is structurally composed of two groups: a proteinaceous group and a heme

group. The protein group has only one polypeptidic chain composed of 140–160 amino acid residues, measuring 3.6 nm and weighing 16 900 Da in vertebrates (Lehninger 1981). It is composed of eight relatively straight segments (where 70% of the amino acids are found), separated by curvatures caused by the incorporation into the chain of proline and other amino acids that do not form alpha-helices (such as serine and isoleucine). Each segment is composed of a portion of alpha-helix, the largest of 23 amino acids and the shortest of 7 amino acids, all dextrogyrating. Mb's high helicoidal content (forming an ellipsoid of $44 \times 44 \times 25$ Å) and lack of disulfide bonds (there is no cysteine) make it an atypical globular protein. The absence of these groups makes the molecule highly stable (Whitaker 1972). Although the three-dimensional structure seems irregular and asymmetric, it is not totally anarchic, and all the molecules of Mb have the same conformation. One very important aspect of the protein part of Mb is its lack of color. However, the variations presented by its primary structure and the amino acid composition of the different animal and fish species destined for human consumption are the cause of the different colorations of meat and their stability when the meats are displayed in the same retail illumination conditions (Lorient 1982; Lee *et al*. 2003a). The heme group of Mb (as in Hb and other proteins) is, as mentioned above, a metalloporphyrin. These molecules are characterized by their high degree of coloration as a result of their conjugated cyclic tetrapyrrolic structure (Kalyanasundaram 1992). The heme group is composed of a complex, organic annular structure, protoporphyrin, to which an iron atom in ferrous state is united (Fe II). This atom has six coordination bonds, four with the flat protoporphyrin molecule (forming a flat square complex) and two perpendicular to it. The sixth bond is open and acts as a binding site for the oxygen molecule.

Protoporphyrin is a system with a voluminous flat ring composed of four pyrrolic units connected by methyl bridges (=C–). The Fe atom, with a coordination number of 6, lies at the center of the tetrapyrrole ring and is complexed to four pyrrolic nitrogens. The heme group is complexed to the polypeptidic chain (globin) through a specific histidine residue (imadazolic ring) occupying the fifth position of the Fe atom (Davidsson & Henry 1978). The heme group is bound to the molecule by hydrogen bridges, which are formed between the propionic acid side chains and other side chains. Other aromatic rings exist near and almost parallel to the heme group, which may also form pi (π) bonds (Stauton-West *et al*. 1969).

The Hb contains a porphyrinic heme group identical to that of Mb and equally capable of undergoing reversible oxygenation and deoxygenation. Indeed, it is functionally and structurally paired with Mb, and its molecular weight is four times greater since it contains four peptidic chains and four heme groups. The Hb, like Mb, has its fifth ligand occupied by the imidazole group of a histidine residue, while the sixth ligand may or may not be occupied. It should be mentioned that positions 5 and 6 of other hemoproteins (cytochromes) are occupied by R groups of specific amino acid residues of the proteins and therefore cannot bind to oxygen (O_2), carbon monoxide (CO), or cyanide (CN–), except a_3, which, in its biological role, usually binds to oxygen.

One of the main differences between fish and mammalian Mb is that fish Mb have two distinct endothermic peaks, indicating multiple states of structural unfolding, whereas mammalian Mb followed a two-state unfolding process. Changes in alpha-helix content and tryptophan fluorescence intensity with temperature are greater for fish Mb than for mammalian Mb. Fish Mb shows labile structural folding, suggesting greater susceptibility to heat denaturation than that of mammalian Mb (Saksit *et al*. 1996).

The helical contents of frozen-thawed Mb were practically the same as those of unfrozen Mb, regardless of pH. Frozen-thawed Mb showed a higher autoxidation rate than unfrozen Mb. During freezing and thawing, Mb suffered some conformational changes in the nonhelical region, resulting in a higher susceptibility to both unfolding and autoxidation (Chow *et al*.

1989). In tuna fish, Mb stability followed the order bluefin tuna (*Thunnus thynnus*), yellowfin tuna (*Thunnus albacares*), and bigeye tuna (*Thunnus obesus*); autoxidation rates were in the reverse order. The pH dependency of Mb from skipjack tuna (*Katsuwonus pelamis*) and mackerel (*Scomber scombrus*) were similar. Lower Mb stability was associated with higher autoxidation rates (Chow 1991).

1.4.2 Chemical properties of myoglobin

The chemical properties of Mb center on its ability to form ionic and covalent groups with other molecules. Its interaction with several gases and water depends on the oxidation state of the Fe of the heme group (Fox 1966), since this may be in either its ferrous (Fe II) or its ferric (Fe III) state. Upon oxidation, the Fe of the heme group takes on a positive charge (Kanner 1994) and, typically, binds with negatively charged ligands, such as nitrites, the agents responsible for the nitrosation reactions in cured meat products.

When the sixth coordination ligand is free, Mb is usually denominated deoxymyoglobin (DMb), which is purple in color. However, when this site is occupied by oxygen, the oxygen and the Mb form a noncovalent complex, denominated oxymyoglobin (OMb), which is cherry or bright red (Lanari & Cassens 1991). When the oxidation state of the iron atom is modified to the ferric state and the sixth position is occupied by a molecule of water, the Mb is denominated metmyoglobin (MMb), which is brown. There are several possible causes for MMb generation, and these may include the ways in which tunids, meat, and meat products are obtained, transformed, or stored (MacDougall 1982; Lee *et al*. 2003b; Mancini *et al*. 2003). Among the most important factors are low pH, the presence of ions, and high temperatures during processing (Osborn *et al*. 2003); the growth and/or formation of metabolites from the microbiota (Renerre 1990); the activity of endogenous reducing enzymes (Arihara *et al*. 1995; Osborn *et al*. 2003); and the levels of endogenous (Lanari *et al*. 2002) or exogenous antioxidants, such as ascorbic acid or its salts, tocopherols (Irie *et al*. 1999), or plant extracts (Xin & Shun 1993; Fernández-López *et al*. 2003; Sánchez-Escalante *et al*. 2003). The pH, which may be altered depending on postslaughter metabolism and on ingredient addition, can affect the stability of the central iron atom in Mb and Hb. At high pH, the heme iron is predominantly in the Fe21 state; low pH accelerates Fe21 conversion to Fe31 (Zhu & Brewer 2002, 2003). While oxygen can bind to Fe21 only, many other ligands (CN, nitric oxide [NO], CO) can bind to either Fe21 or Fe31 so producing a variety of colors. This change in the oxidation state of the heme group will result in the group being unable to bind with the oxygen molecule (Arihara *et al*. 1995). DMb is able to react with other molecules to form colored complexes, many of which are of great economic relevance for the meat industry. The most characteristic example is the reaction of DMb with nitrite, since its incorporation generates a series of compounds with distinctive colors: red in dry-cured meat products or pink in heat-treated products. The products resulting from the incorporation of nitrite are denominated cured, and such products are of enormous economic importance worldwide (Pérez-Alvarez 1996). The reaction mechanism is based on the propensity of nitric oxide (NO, generated in the reaction of nitrite in acid medium, readily gives up electrons) to form strong coordinated covalent bonds; it forms an iron complex with the DMb heme group independent of the oxidation state of the heme structure. The compound formed after the nitrification reaction is denominated nitrosomyoglobin (NOMb). As mentioned above, the presence of reducing agents such as hydrogen sulfide acid (H_2S) and ascorbates leads to the formation of undesirable pigments in both meat and meat products. These green pigments are called sulfomyoglobin (SMb) and colemyoglobin (ColeMb), respectively, and are formed as a result of bacterial activity and an excess of reducing agents in the medium. The formation of SMb is reversible, but that of

ColeMb is an irreversible mechanism, since it is rapidly oxidized between pH 5 and 7, releasing the different parts of the Mb (globin, iron, and the tetrapyrrolic ring).

From a chemical point of view, it should be borne in mind that the color of Mb, and therefore of the meat or meat products, depends not only on the molecule that occupies the sixth coordination site, but also on the oxidation state of the iron atom (ferrous or ferric), the type of bond formed between the ligand and the heme group (coordinated covalent, ionic, or none), and the state of the protein (native or denatured form), not to mention the state of the porphyrin of the heme group (intact, substituted, or degraded) (Pérez-Alvarez 1996).

During the heat treatment of fish flesh, the aggregation of denatured fish proteins is generally accompanied by changes in light-scattering intensity. Results demonstrate that changes in relative light-scattering intensity can be used for studying structural unfolding and aggregation of proteins under thermal denaturation (Saksit *et al*. 1998). When fatty fish meat like *Trachurus japonicus* was heat treated, the MMb content increased linearly, and the percentages of denatured Mb and apomyoglobin increased rapidly when mince was exposed to heat; however, when the temperature reached 60°C, the linearity was broken. The results indicated that MMb color stability was higher than that of Mb and that the thermal stability of heme was higher than that of apomyoglobin (Hui *et al*. 1998). Both Mb and ferrous iron accelerated the lipid oxidation of cooked, water-extracted fish meat. Ethylenediaminetetraacetic acid (EDTA) inhibited the lipid oxidation accelerated by ferrous iron, but not that accelerated by Mb. Also, with cooked, nonextracted mackerel meat, EDTA noticeably inhibited lipid oxidation. Nonheme iron catalysis seemed to be related in part to lipid oxidation in cooked mackerel meat. The addition of nitrite in combination with ascorbate resulted in a marked inhibition of lipid oxidation in the cooked mackerel meat. From these results, it was postulated that nitric oxide ferrohemochromogen, formed from added nitrite and Mb (present in the mackerel meat) in the presence of a reducing agent, possesses an antioxidant activity, which is attributable in part to its function as a metal chelator (Ohshima *et al*. 1988).

Tuna fish meat color can be improved when the flesh is treated or packaged with a modified atmosphere in which CO is included. Normally, the rate of penetration of CO or carbon dioxide (CO_2) in fish meat such as tuna, cod, or salmon, under different packaging conditions, is measured by monitoring pressure changes in a closed constant volume chamber with constant volume and temperature. Alternatively, however, the specific absorption spectrum of carboxymyoglobin (MbCO), within the visible range, can be obtained and used as an indicator of MbCO formation. Mb extracts from tuna muscle treated with CO exhibited higher absorbance at 570 than at 580 nm. Therefore, the relationship between absorbance at 570 nm and absorbance at 580 nm could be used to determine the extent of CO penetration of tuna steaks placed in a modified atmosphere in which CO was included. The penetration of CO into tuna muscle was very slow. After approximately 1–4 h, CO had penetrated 2–4 mm under the surface, and after 8 h, CO had penetrated 4–6 mm (Chau *et al*. 1997).

In products with added nitrite or nitrate, the complex nitrosylmyoglobin (MbFe[II]NO) is the main contributor to the characteristic color of cooked cured ham, and brine-cured and dry-cured meat products. Meat and meat products without nitrite/nitrate addition will normally attain a dull brown color or a gray color in heated products, which influences consumer acceptance negatively (Adamsen *et al*. 2005). In dry-cured meat products such as Parma ham produced without nitrite or nitrate addition, the characteristic bright red color (Wakamatsu *et al*. 2004a) is caused by a Zn-protoporphyrin IX (Zn-pp) complex, a heme derivative. Adamsen *et al*. (2005) showed that the use of nitrite as a curing ingredient inhibits the formation of Zn-pp. In the same work, the author described that this color compound is present in other meat products like Iberian ham, although in a lower concentration.

Virgili *et al*. (1999) reported that this color may be due to the action of low-molecular weight compounds containing electron-donating atoms, formed during maturation, in particular

basic peptides or amino acids resulting from an external proteolysis, which may play a role as Fe ligands in Mb. Wakamatsu *et al*. (2004b) reported that anaerobic conditions favor the formation of Zn-pp and that endogenous enzymes as well as microorganisms may also be involved. There are several hypotheses that try to explain the formation of this compound. Wakamatsu *et al*. (2004b) described three possible substitution patterns: (1) a nonenzymatic reaction in which Zn(II) substitutes Fe(II) under anaerobic conditions, with concomitant dissociation of the heme; (2) a bacterial enzymatic reaction, whereby bacterial growths naturally degrade the meat proteins including the pigment; and (3) an enzymatic reaction where an endogenous ferrochelatase interchanges the two metals. However, Adamsen *et al*. (2005) described this process as having the three following mechanisms to explain the metal substation: (1) a nonenzymatic enzymatic reaction driven by the binding of iron in the high chloride meat matrix; (2) a bacterial enzymatic reaction; and (3) an endogenous enzymatic reaction.

Also, spectroscopic studies of Parma ham during processing revealed a gradual transformation of muscle Mb, initiated by salting and continuing during aging. Using electron spin resonance spectroscopy, Moller *et al*. (2003) have shown that the Parma ham pigment is different from MbFe(II)NO and is not a nitric oxide complex such as that found in brine-cured ham and Spanish Serrano hams. These authors also establish that the heme moiety is present in the acetone-water extract and that Parma ham pigment is gradually transformed from a Mb derivative into a nonprotein heme complex, which is thermally stable in an acetone-water solution. Adamsen *et al*. (2003) also demonstrated that the heme moieties of Parma ham pigments have antioxidative properties. Pigments became increasingly lipophilic during processing, suggesting that a combination of drying and maturing yields a stable red color (Parolari *et al*. 2003).

1.4.3 Cytochromes

Cytochromes are metalloproteins with a prosthetic heme group, whose putative role in meat coloration is undergoing revision (Boyle *et al*. 1994; Faustman *et al*. 1996). Initially, they were not thought to play a very important role (Ledward 1984). These compounds are found in low concentrations in the skeletal muscle, and in poultry, they do not represent more than 4.23% of the total hemoproteins present (Pikul *et al*. 1986). It has now been shown that the role of cytochrome (especially its concentration) in poultry meat color is fundamental when the animal has been previously exposed to stress (Ngoka & Froning 1982; Pikul *et al*. 1986). Cytochromes are most concentrated in cardiac muscle so that when this organ is included in meat products, the heart's contribution to color, not to mention the reactions that take place during elaboration processes, must be taken into consideration (Pérez-Alvarez *et al*. 2000).

1.5 COLOR CHARACTERISTICS OF BLOOD

Animal blood is little used in the food industry because of the dark color it imparts to the products to which it is added. For solving the negative aspects of blood incorporation, specifically food-color-related problems, several different processes and means have been employed, but they are not always completely satisfactory. The addition of 12% blood plasma to meat sausages leads to pale-colored products. The addition of discolored whole blood or globin (from which the Hb's heme group has been eliminated) has also been used to address color problems. Natural red pigments can be obtained from blood without using coloring agents such as nitrous acid salts; these pigments have Zn-pp as the metalloporphyrin moiety and can be used to produce favorably colored beef products, whale meat products, and fish products (including fish pastes) (Numata & Wakamatsu 2003). There was wide variation in amounts of

Hb extracted from the muscle tissue of bled and unbled fish, and the residual level in the muscle of bled fish was substantial. Mb content was minimal as compared with Hb content in mackerel light muscle and trout whole muscle. Hb made up 65% and 56% by weight of the total heme protein in dark muscle from unbled and bled mackerel, respectively. The blood-mediated lipid oxidation in fish muscle depends on various factors, including Hb concentration, Hb type, plasma volume, and erythrocyte integrity (Richards & Hultin 2002). The presence of blood, Hb, Mb, Fe12, Fe13, or Cu12 can stimulate lipid oxidation in the fillets of icefish (Rehbein & Orlick 1990; Richards & Li 2004). Kanner *et al*. (1987) reported that Hb, Mb, copper, and iron have the potential to promote lipid oxidation in muscle foods. Since iron can be released from Hb during storage, it is difficult to ascertain whether the intact heme protein, dissociated heme, or released iron is responsible for the bulk of lipid oxidation that occurs during storage. For this reason, Svingen *et al*. (1979) used the term "low molecular weight iron" instead of "free iron" since iron binds to other low molecular weight compounds to gain solubility and hence potential reactivity. Ferrous and ferric forms of iron can promote lipid oxidation processes (Gutteridge 1986; Tadolini & Hakim 1996). Iron shows a high reactivity with reactants such as hydrogen peroxide and lipid peroxides (Kanner & Harel 1987).

Mitochondria are a source of reactive oxygen species that could confound lipid oxidation reactions due to added Hb. During fish processing (e.g., tuna fish), the loss of redness can be a good indicator that lipid oxidation processes mediated by Hb are progressing. Just after death, Hb in muscle tissue is primarily in the reduced state (i.e., oxyhemoglobin [OHb] and deoxyhemoglobin [deoxyHb]).

This mixture of OHb and deoxyHb has a red color. With increased postmortem aging, Hb autoxidizes to methemoglobin (MHb), a brown pigment. MHb is considered more prooxidative than reduced Hb due to its less tightly bound heme group and its reactivity with hydrogen peroxide and lipid peroxides to form hypervalent Hb catalysts (Everse & Hsia 1997).

From a technological point of view, during meat or fish processing, rapid chilling may alter oxygen solubility in tissues resulting in less available oxygen to oxygenate either OMb or Hb. The conversion of OMb to MMb, which is brown and unattractive, occurs under conditions of very low oxygen tension as well (Nicolalde *et al*. 2005).

Field *et al*. (1978) describe how bone marrow is high in Hb, while muscle has a high Mb content. As with other meats, its color and Hb stability depend on packaging and storage conditions. Good temperature control and modified atmosphere packaging (MAP) with high oxygen atmospheres (80%) are often used to extend both microbiological and color shelf life (Nicolalde *et al*. 2005).

1.6 FAT COLOR

From a technological point of view, fat fulfills several functions, although, regarding color, its principal role is in the brightness of meat products. Processes such as "afinado" during the elaboration of dry-cured ham involve temperatures at which fat melts so that it infiltrates the muscle mass and increases its brilliance (Sayas 1997). When the fat is finely chopped, it "dilutes" the red components of the color, thus decreasing the color intensity of the finished product (Pérez-Alvarez *et al*. 2000). However, fats do not play such an important role in fine pastes since, after emulsification, the fat is masked by the matrix effect of the emulsion so that it contributes very little to the final color. The color of fat basically depends on the feed that the live animal received (Esteve 1994; Irie 2001). In the case of chicken and ostrich, the fat has a "white" appearance (common in Europe) when the animal has been fed with "white" cereals or other ingredients not containing xanthophylls, since these are accumulated in subcutaneous fat