Myoglobin Chemistry and Meat Color

Surendranath P. Suman[1,*] and Poulson Joseph[2]

[1]Department of Animal and Food Sciences, University of Kentucky, Lexington, Kentucky 40546; email: spsuma2@uky.edu

[2]Department of Food Science, Nutrition and Health Promotion, Mississippi State University, Mississippi State, Mississippi 39762; email: poulsonjoseph@gmail.com

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*Corresponding author

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Abstract

Consumers rely heavily on fresh meat color as an indicator of wholesomeness at the point of sale, whereas cooked color is exploited as an indicator of doneness at the point of consumption. Deviations from the bright cherry-red color of fresh meat lead to product rejection and revenue loss. Myoglobin is the sarcoplasmic heme protein primarily responsible for the meat color, and the chemistry of myoglobin is species specific. The mechanistic interactions between myoglobin and multiple extrinsic and intrinsic factors govern the color of raw as well as cooked meats. The objective of this review is to provide an overview of the current research in meat color and how the findings are applied in the meat industry. Characterizing the fundamental basis of myoglobin's interactions with biomolecules in postmortem skeletal muscles is necessary to interpret the chemistry of meat color phenomena and to engineer innovative processing strategies to minimize meat discoloration–induced revenue loss to the agricultural economy.

INTRODUCTION

Of the several quality attributes of fresh meat, color is the most important one influencing purchase decisions (Mancini & Hunt 2005). At the point of sale, consumers, in general, cannot evaluate the odor or feel the texture of meat without opening the packages. Thus, a bright cherry-red color is commonly utilized as an indicator of wholesomeness in fresh meat. Surface-discolored whole-muscle cuts are ground to low-value products, such as ground beef, to salvage the cuts' interiors, which might still be red or are discarded often well before microbial safety is compromised; both practices lead to sales loss and wastage of valuable food (Faustman & Cassens 1990). It has been estimated that discoloration-induced price discounts result in more than one billion dollars in revenue loss for the United States meat industry every year (Smith et al. 2000). The impacts of discoloration-induced meat wastage on agriculture sustainability and our environment are yet to be determined.

Myoglobin and Meat Color

Myoglobin (Mb) is the sarcoplasmic heme protein primarily responsible for the color of meat obtained from a well-bled livestock carcass (Livingston & Brown 1981). The chemistry and functions of Mb in live muscles and meat can be different. In live muscle, Mb functions as the oxygen binder and delivers oxygen to the mitochondria, enabling the tissue to maintain its physiological functions (Wittenberg & Wittenberg 2003). In meats, Mb serves as the major pigment responsible for the red color. Although bleeding of food animals at harvest removes the majority of blood, some residual blood is trapped inside the arteries and veins within the large skeletal muscles, resulting in the presence of hemoglobin in meat. Pigments such as hemoglobin and cytochrome also contribute to meat color, but only to a lesser extent. The pigments other than Mb are more relevant to color in poultry, fish, and game meats than in livestock species.

Mb is a monomeric heme protein with a heme prosthetic group and a globin (protein) moiety. The globin chain consists of eight helical segments forming a coiled structure enwrapping the heme, and the ability of Mb to bind oxygen is due to the presence of heme located within the heme crevice. The globin chain confers water solubility to the heme group and protects the heme iron from external environments/oxidation so that the protein can maintain its functionality. The resonant nature of the conjugated double bonds in the heme group is responsible for the ability of Mb to absorb visible light and thus serve its function as a pigment. The heme group contains an iron atom that can exist in a reduced (ferrous/Fe^{2+}) or oxidized (ferric/Fe^{3+}) form. The iron can accept six electrons in its outer orbit and can thus form six coordinate bonds. Four of the bonds are with pyrrole groups of the heme porphyrin ring and one is with proximal histidine (position 93 in the globin chain), which connects heme to the globin chain. Additionally, another histidine residue (distal histidine at position 64 in the globin chain) is in the vicinity of the heme but not bonded with the heme. The sixth position of heme iron is available for binding with oxygen or other small ligands, such as carbon monoxide (CO) or nitric oxide (NO). The spacial arrangement of distal histidine and heme limits the size of the ligands occupying the sixth coordinate in native Mb and protects the heme by preventing its interactions with large biomolecules (Cornforth & Jayasingh 2004).

CHEMISTRY OF MEAT PIGMENTS

In postmortem skeletal muscles and processed meats, the chemistry of Mb exhibits striking differences depending on the type of packaging used and the processing technology applied. This

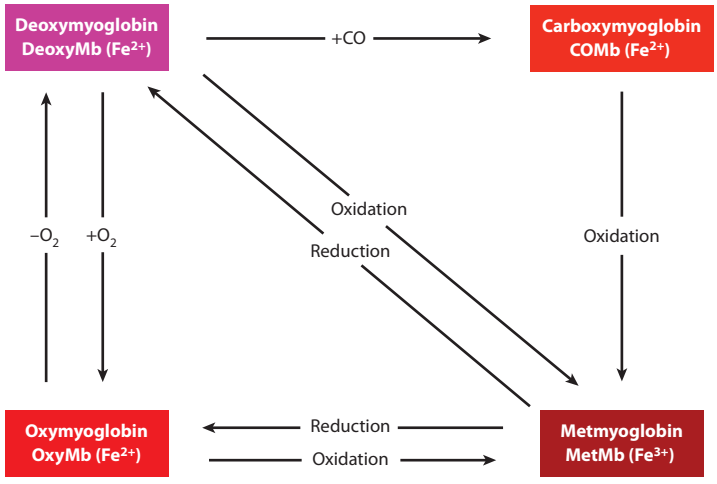

Figure 1

Myoglobin redox forms in fresh meats. (Adapted with permission from Mancini & Hunt 2005.)

leads to the variations in the color of meat and meat products, from bright cherry-red in bloomed fresh meat to dull brown in cooked meat.

Pigments in Fresh Meat

Fresh meats, also commonly referred to as raw meats, are integral grocery commodities across the world and are stored under a variety of packaging systems for retail display. In packaged fresh meats, Mb can exist in any of the four redox states (**Figure 1**) (Mancini & Hunt 2005), namely deoxymyoglobin (DeoxyMb), oxymyoglobin (OxyMb), carboxymyoglobin (COMb), and metmyoglobin (MetMb). DeoxyMb, OxyMb, and COMb are in a ferrous state. OxyMb and COMb provide bright cherry-red color critical to acceptance, and the red color of these two redox forms is indistinguishable by human eyes (Cornforth & Hunt 2008). DeoxyMb is purplish-red in color. The sixth coordinate of heme iron is occupied by oxygen in OxyMb and CO in COMb, whereas no ligand is bound with the heme iron in DeoxyMb. Saturating Mb with oxygen provides attractive cherry-red color to meat through formation of OxyMb. Mb has a greater affinity to CO than to oxygen, resulting in the increased stability of bright cherry-red COMb. Formation of brown MetMb results from the oxidation of the three ferrous forms to a ferric state and is associated with meat discoloration. MetMb has a water molecule bound at the sixth coordinate of the ferric heme and is incapable of binding oxygen.

All four forms of Mb in fresh meats are readily soluble in water and low-ionic strength buffers, and the absorbance spectra (between 500 and 600 nm) of the redox forms are different enough to be identified spectrophotometrically (Krzywicki 1982, Suman et al. 2006, Tang et al. 2004). DeoxyMb exhibits a strong absorption maximum at 557 nm, whereas MetMb exhibits a peak at 503 nm. OxyMb has large twin peaks at 542 and 582 nm (Tang et al. 2004). Interestingly, COMb also has two absorbance peaks at 543 and 581 nm (Suman et al. 2006). The absorption spectra of COMb and OxyMb are nearly identical. Nevertheless, there exist some features to distinguish them; the peak at the 580-nm region has the greatest magnitude in OxyMb, whereas the peak at the 540-nm region is the predominant one in COMb (Suman et al. 2006). The absorption spectra of the four redox forms intersect (isobestic point) at 525 nm, and the spectrophotometric

absorbance at 525 nm is employed to estimate total Mb concentration in solutions as well as from fresh meat extracts (Tang et al. 2004).

Pigments in Cooked Meat

The process of cooking results in denaturation of soluble Mb, and heat-induced Mb denaturation is responsible for the dull-brown color of cooked meats (King & Whyte 2006). Denaturation of the globin exposes the heme group and increases the susceptibility of heme to oxidation. The pigments in cooked meat are coagulated because of the unfolding of the globin chain and therefore are insoluble in aqueous solutions (Cornforth & Jayasingh 2004). Heat-induced denaturation of MetMb results in denatured globin hemichrome (ferrihemochrome), which is responsible for the dull-brown appearance of cooked meats. However, the denaturation of globin in ferrous Mb forms leads to the formation of pink-red denatured globin hemochrome (ferrohemochrome), which is oxidized to brown ferrihemochrome. Cooking-induced denaturation of COMb results in the formation of pink-red denatured globin CO-hemochrome (Nam & Ahn 2002).

Pigments in Cured Meat

The stable pink color of cured meat is formed by the reaction between Mb and nitrates/nitrites (Cassens 1997). Sea salt, historically used to preserve meat, contains nitrates, which are reduced to nitrites by bacteria, leading to the development of pink color in cured meat. In modern-day meat curing, nitrous acid is generated when nitrite comes in contact with water. The nitrous acid reacts with Mb and oxidizes it to MetMb. The bound nitrous acid is converted to heme-bound NO in MetMb. NO-MetMb is brown and under anaerobic environment is reduced to nitrosyl-Mb (red color). The nitrosyl-Mb is denatured upon cooking and is converted to nitrosyl hemochrome, which is a pink pigment (Honikel 2008). Pink-colored nitrosyl hemochrome is sensitive to the presence of light and oxygen; the bound NO dissociates on exposure to oxygen and light, and the cured color fades off. Therefore, cured products are stored and retailed under vacuum packaging in opaque films. With the increased awareness of health concerns due to nitrosamines, associated with nitrites in cured meats, research efforts have been focused on using plant/organic ingredients and natural curing processes as replacements for nitrites to achieve the characteristic pink color (Sebranek et al. 2012, Sindelar et al. 2007).

FACTORS INFLUENCING MEAT COLOR

Research to date has demonstrated that a multitude of factors (endogenous and exogenous) contribute to meat color stability and biochemistry. Controlling these factors allows manipulation of color in raw as well as cooked meats. The scientific principles behind these endogenous and exogenous factors have been exploited to develop strategies in meat processing and animal production to minimize discoloration.

Endogenous Factors

Several endogenous factors contribute to meat color, and the most prominent among them are pH, muscle source, presence of antioxidants, lipid oxidation, and mitochondrial activity (Mancini & Hunt 2005). In addition, various live animal–related factors, such as management, diet, and genetics, are also known to influence meat color (Faustman & Cassens 1990). Previous publications have extensively covered these factors, whereas in this review emphasis has been placed on certain recent fundamental concepts in color biochemistry and their applications in meat processing.

Of the several endogenous factors affecting meat color, muscle source received significant attention with the achievement of beef-muscle profiling (Von Seggern et al. 2005). Individual muscles have specific anatomical locations and physiological functions, resulting in differences in metabolism; consequently, each muscle demonstrates unique color biochemistry (Hunt & Hedrick 1977). OxyMb oxidation and discoloration in beef depend on muscle source (McKenna et al. 2005, Seyfert et al. 2007), and on the basis of color stability, beef muscles have been categorized as color-stable and color-labile. Muscles that demonstrate greater rates of oxygen consumption (O'Keeffe & Hood 1982) and lower rates of MetMb reduction (Ledward 1985) are color-labile. In contrast, muscles with greater reducing activities are the ones that are color-stable (Reddy & Carpenter 1991). Longissimus lumborum, retailed as New York Strip steak, is a color-stable muscle, whereas Psoas major is the muscle marketed as filet mignon or tenderloin and is color-labile. Biochemical investigations documented that Psoas major demonstrated greater lipid oxidation, lower MetMb-reducing activity, and lower color stability than Longissimus lumborum (Joseph et al. 2012, McKenna et al. 2005, Seyfert et al. 2007). Previous studies attributed the muscle-specific nature of meat discoloration to various enzymes (Arihara et al. 1995, Hagler et al. 1979). However, a proteomic approach (Joseph et al. 2012) indicated that antioxidant (thioredoxin, peroxiredoxin-2, and peptide methionine sulfoxide reductase) and chaperone (heat shock protein-27 kDa) proteins are overabundant in color-stable beef muscles and protect Mb from oxidation, resulting in improved color stability.

Lipid oxidation generates reactive secondary products, such as aldehydes and ketones, that are responsible for off-odors (Pearson et al. 1977). Although the role of lipid oxidation in rancidity development in meats has been well known for more than a century, its contribution to off-color phenomenon is relatively new (Faustman et al. 2010). The reactive products of lipid oxidation compromise meat color by accelerating Mb oxidation (Faustman et al. 1999). The highly reactive aldehydes are polar (Esterbauer et al. 1991) and readily diffuse to the sarcoplasm, where they covalently adduct Mb molecules. Cysteine residues in proteins are the most favorable targets for nucleophilic adduction by aldehydes; the absence of cysteine in livestock and poultry Mb (Swiss Inst. Bioinform. 2012) renders histidines as the favorable candidates (Alderton et al. 2003, Faustman et al. 1999, Lee et al. 2003, Naveena et al. 2010, Suman et al. 2007). Adduction of aldehydes at proximal and distal histidines, which coordinates the stability of heme, exposes the heme group to an oxidizing environment, leading to increased Mb oxidation (Alderton et al. 2003, Lee et al. 2003, Naveena et al. 2010, Suman et al. 2007). Therefore, strategies to inhibit lipid oxidation not only minimize rancidity but also improve color stability (Faustman et al. 2010).

Mb and mitochondrial functionalities are closely interrelated (Wittenberg & Wittenberg 2007), and the role of mitochondria on meat color stability has been examined extensively in the past decade. In live animals, Mb serves as an oxygen carrier to mitochondria in muscles (Wittenberg & Wittenberg 2007). However, mitochondria continue to metabolize oxygen in postmortem skeletal muscles, and intact mitochondria can be isolated up to 60 days postmortem (Tang et al. 2005a). The competition between mitochondria and Mb is a key component in the development of bright-red color. Mitochondria can influence color stability via oxygen consumption and MetMb-reducing activity. As a result, factors influencing mitochondrial activity can also affect meat color. Noticeably, very high and very low mitochondrial function can be detrimental to meat color stability (Sammel et al. 2002). Mitochondrial respiration influences meat color by decreasing oxygen partial pressure, and mitochondria can interact with OxyMb, resulting in the transfer of heme-bound oxygen from OxyMb to mitochondria. Both of these cellular functions promote the formation of DeoxyMb, resulting in darker meat color; low oxygen partial pressure resulting from increased mitochondrial activity maintains Mb in a deoxygenated state. In this situation, meat fails to bloom and does not form the characteristic bright cherry-red color. In contrast, decreased mitochondrial

activity at low temperatures results in better bloom compared with meat at elevated temperatures (Bendall & Taylor 1972). Furthermore, mitochondria can out-compete Mb for oxygen, resulting in deoxygenated pigment and dark-colored meat (Ramanathan et al. 2009). Mitochondrial oxygen consumption promotes an anaerobic environment that favors MetMb reduction, and Tang et al. (2005b) reported that mitochondria-mediated MetMb reduction occurs via the transfer of available electrons to MetMb by cytochrome. Furthermore, mitochondrial substrates, such as succinate (Tang et al. 2005b) and malate (Mohan et al. 2010), can also increase MetMb reduction, suggesting the potential use of these ingredients to improve meat color stability.

Exogenous Factors

A wide variety of exogenous factors commonly encountered in meat processing are known to influence the meat color. Presence of ligands, antioxidants, and prooxidants governs meat color stability, and several extensive reviews may be consulted for the details (Faustman & Cassens 1990, Mancini & Hunt 2005, McMillin 2008).

Ligands, often gases, play critical roles in determining the color of fresh meats. On exposure of fresh-cut meats to air, oxygen reacts with Mb to form OxyMb, resulting in cherry-red color. Oxygenation of Mb, commonly known as blooming in the industry, happens within 30–60 minutes and provides the consumer-preferred cherry-red color to aerobically packaged meat. Nevertheless, conventionally bloomed meat undergoes discoloration because of the formation of MetMb and has a color shelf life of less than a week (McMillin 2008). Increasing the level of oxygen through modified atmosphere packaging (MAP) contributes to an increase in color shelf life (Jakobsen & Bertelsen 2000). However, use of CO (at less than 1% level) promotes formation of stable COMb and further increases the color shelf life because of the increased affinity of Mb to CO (Sorheim et al. 1999). In the absence of air or any ligand, for instance, in the center of whole-muscle cuts, Mb exists as purplish-red DeoxyMb.

Exogenous antioxidants can promote a favorable environment for Mb by minimizing oxidation of heme and can exert a color-stabilizing effect on intact muscle cuts as well as comminuted meats. In whole-muscle cuts, antioxidants are utilized in injection-enhancement solutions, ensuring uniform delivery into the interiors. Use of food-grade antioxidants, such as erythorbate (Suman et al. 2005), rosemary (Lee et al. 2006), ascorbate (Sepe et al. 2005), lactate (Mancini et al. 2009), chitosan (Suman et al. 2011), and succinate (Mancini et al. 2011), has demonstrated improvement in color stability and color shelf life of whole-muscle cuts as well as ground meats.

MYOGLOBIN PRIMARY STRUCTURE AND COLOR STABILITY

Mbs from poultry and livestock have 153 amino acids (Swiss Inst. Bioinform. 2012). Although Mb functions are highly conserved across meat-producing livestock and poultry species, its primary structure is not. Livestock Mbs share less than 75% sequence similarity with their poultry counterparts (**Table 1**). Mbs of traditional livestock and poultry species were characterized decades ago, whereas those of several different exotic, game, and emerging meat animals were characterized recently. Mbs of several closely related meat animals have the same amino acid sequence. For instance, Mbs of beef, bison, and yak have the same sequence; red deer and white-tailed deer Mbs have 100% sequence similarity; and turkey and chicken Mbs have the same primary structure (Swiss Inst. Bioinform. 2012). Noticeably, the distal and proximal histidines are conserved in livestock and poultry Mbs. Furthermore, poultry Mbs are 300–400 Daltons heavier than red meat Mbs (**Table 2**), attributing to the substitutions of smaller amino acids with larger ones (**Figure 2**).

The primary structure of Mb dictates its tertiary structure, which in turn influences the protein's interactions with biomolecules and ultimately impacts meat color. Furthermore, it is well

Table 1 Percentage sequence similarities between red meat and poultry myoglobins

Species	Beef	Buffalo	Sheep	Goat	Pig	Chicken	Emu	Ostrich
Beef	100							
Buffalo	98.0	100						
Sheep	98.7	96.7	100					
Goat	97.4	95.4	98.7	100				
Pig	88.2	86.9	89.5	88.2	100			
Chicken	72.5	71.2	72.5	71.9	76.5	100		
Emu	69.3	68.6	69.3	68.6	73.2	90.2	100	
Ostrich	70.6	69.9	70.6	69.9	74.5	92.8	94.8	100

documented that the primary structure influences functional properties of Mb as an oxygen carrier in live muscles and as a pigment in meat. A schematic representation of the potential mechanisms through which Mb primary structure influences color is presented in **Figure 3**.

Autoxidation

Autoxidation is the process by which ferrous Mb forms (DeoxyMb and OxyMb) are oxidized to ferric MetMb, leading to brown discoloration. The influence of primary structure on autoxidation was studied in mammalian and fish Mbs (Brown & Mebine 1969); the oxidation rates were species dependent, and tuna Mb oxidized faster than the sperm whale and beef counterparts. Further studies (Kitahara et al. 1990) confirmed that the Mb autoxidation rate was influenced by the presence of oxidizable residues in the primary structure; tuna Mb contains a highly oxidizable cysteine residue, which is absent in livestock and sperm whale Mbs (Livingston & Brown 1981). The unique presence of oxidizable cysteine residue (Carbone et al. 2005) could be the potential reason behind the greater autoxidation observed in tuna Mb than the mammalian counterparts investigated. Investigations by Chow (1991) compared the autoxidation rates in three different tuna species (bigeye, bluefin, and yellowfin tuna), which shared more than 95% sequence similarity, and observed that the rate of Mb autoxidation was greatest in bigeye tuna followed by the yellowfin and bluefin varieties.

Autoxidation in red-meat Mbs from ruminants (beef, lamb, and red deer) and a non-ruminant (pork) was investigated (Gutzke & Trout 2002), and the results indicated that pork Mb, which

Table 2 Comparison of molecular mass of red meat and poultry myoglobins

Species	Molecular mass (Da)	Reference
Beef (*Bos taurus*)	16,946	Han et al. 1970
Buffalo (*Bubalus bubalis*)	17,034	Dosi et al. 2006
Sheep (*Ovis aries*)	16,923	Han et al. 1972
Goat (*Capra hircus*)	16,824	Suman et al. 2009a
Pig (*Sus scrofa*)	16,953	Rousseaux et al. 1976
Chicken (*Gallus gallus*)	17,291	Deconinck et al. 1975
Turkey (*Meleagris gallopavo*)	17,295	Joseph et al. 2010b
Emu (*Dromaius novaehollandiae*)	17,380	Suman et al. 2010a
Ostrich (*Struthio camelus*)	17,325	Enoki et al. 2008

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Sequence    1         10         20         30         40         50
Beef        GLSDGEWQLV LNAWGKVEAD VAGHGQEVLI RLFTGHPETL EKFDKFKHLK
Buffalo     GLSDGEWQLV LNAWGKVETD VAGHGQEVLI RLFTGHPETL EKFDKFKHLK
Sheep       GLSDGEWQLV LNAWGKVEAD VAGHGQEVLI RLFTGHPETL EKFDKFKHLK
Goat        GLSDGEWTLV LNAWGKVEAD VAGHGQEVLI RLFTGHPETL EKFDKFKHLK
Pig         GLSDGEWQLV LNVWGKVEAD VAGHGQEVLI RLFKGHPETL EKFDKFKHLK
Chicken     GLSDQEWQQV LTIWGKVEAD IAGHGHEVLM RLFHDHPETL DRFDKFKGLK
Emu         GLSDQEWQHV LTIWGKVESD LAGHGHEILM RLFHDLPETL DRFERFKGLT
Ostrich     GLSDQEWQQV LTIWGKVESD IAGHGHAILM RLFQDHPETL DRFEKFKGLT
                 *  **   **     *  *    *** *    ***     ** **  * *

Sequence    51        60         70         80         90         100
Beef        TEAEMKASED LKKHGNTVLT ALGGILKKKG HHEAEVKHLA ESHANKHKIP
Buffalo     TEAEMKASED LKKHGNTVLT ALGGILKKKG HHEAEVKHLA ESHANKHKIP
Sheep       TEAEMKASED LKKHGNTVLT ALGGILKKKG HHEAEVKHLA ESHANKHKIP
Goat        TGAEMKASED LKKHGNTVLT ALGGILKKKG HHEAEVKHLA ESHANKHKIP
Pig         SEDEMKASED LKKHGNTVLT ALGGILKKKG HHEAELTPLA QSHATKHKIP
Chicken     TPDQMKGSED LKKHGATVLT QLGKILKQKG NHESELKPLA QTHATKHKIP
Emu         TPDQMKASEE LKKHGVTVLT QLGKILKLKG KHEAELKPLA QTHATKHKIP
Ostrich     TPDQMKASED LKKHGVTVLT QLGKILKQKG KHEAELKPLA QTHATKHKIP
            ****  *  *      *     *  *   *   *  * ***   **  *

Sequence    101       110        120        130        140        150
Beef        VKYLEFISDA IIHVLHAKHP SDFGADAQAA MSKALELFRN DMAAQYKVLG FHG
Buffalo     VKYLEFISDA IIHVLHDKHP SDFGADAQAA MSKALELFRN EMAAQYKVLG FHG
Sheep       VKYLEFISDA IIHVLHAKHP SDFGADAQGA MSKALELFRN DMAAQYKVLG FQG
Goat        VKYLEFISDA IIHVLHAKHP SDFGADAQGA MSKALELFRN DMAAQYKVLG FQG
Pig         VKYLEFISEA IIQVLQSKHP GDFGADAQGA MSKALELFRN DMAAKYKELG FQG
Chicken     VKYLEFISEV IIKVIAEKHA ADFGADSQAA MKKALELFRN DMASKYKEFG FQG
Emu         VKYLEFISEV IIKVIAEKHS ADFGADSQAA MKKALELFRN DMASKYKEFG FQG
Ostrich     VKYLEFISEV IIKVIAEKHS ADFGADSQAA MKKALELFRN DMASKYKEFG FQG
                    **   * ***  * *     * *   *         *  **  **   *
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* The residues that are different between poultry and red-meat myoglobins.

Figure 2

Amino acid sequence of red-meat and poultry myoglobins.

is phylogenetically distant from ruminant Mb (Rousseaux et al. 1976), was less susceptible to autoxidation than its ruminant counterparts.

Buffalo meat [from water buffalo (*Bubalus bubalis*)] was introduced to the Italian meat market as a comparable alternative to beef. However, buffalo meat demonstrated a tendency to darken more rapidly than beef, thus discouraging consumers. Dosi et al. (2006) characterized the primary sequence of buffalo Mb and observed sequence differences between buffalo and beef Mbs at three positions (19, 117, and 141). Although the homology between these two ruminant Mbs was 98%, this study reported that the presence of negatively charged residues (Abov117Dbuf in helix G

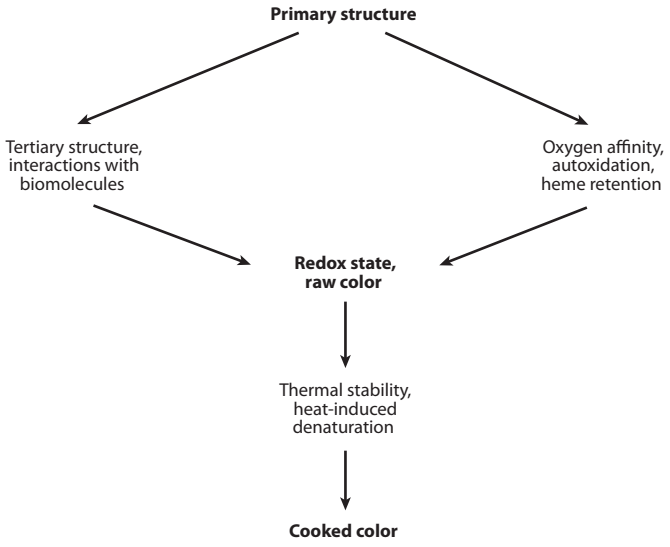

Figure 3

Mechanisms through which myoglobin primary structure influences meat color.

and Abov19Tbuf in helix A) leads to destabilization of the buffalo Mb and subsequent rapid meat discoloration.

Mbs from elephants (African and Asian species) have a glutamine in place of the usual distal histidine at position 64 (Dene et al. 1980), the latter of which is conserved in many other mammalian species. Investigations by Romero-Herrera et al. (1981) summarized that the glutamine substitution was responsible for the low rate of autoxidation of elephant Mb and conferred evolutionary advantage on the wild animal. Further investigations confirmed that glutamine substitution at distal histidine was responsible for the absence of proton-catalyzed autoxidation in elephant (Tada et al. 1998) and shark (Suzuki 1987) Mbs.

Oxygen Affinity

The redox state of Mb is governed by the oxygen affinity of the heme protein. Furthermore, the interactions between heme iron and oxygen are influenced by the accessibility to and size of the heme pocket. The influence of primary structure on the size of the heme pocket is well documented (Brantley et al. 1993). Substituting position 29 (leucine) in sperm whale Mb with phenylalanine resulted in a tenfold increase in oxygen affinity, with a concomitant decrease in oxygen dissociation (Carver et al. 1992). Marcinek et al. (2001) investigated the relationship between oxygen affinity and the amino acid sequence of fish Mbs and concluded that low oxygen affinities of mackerel Mb and bonito Mb are the results of multiple amino acid substitutions in nonhelical regions. The relationship between primary structure and oxygen affinity in Mb from four fish species inhabiting different ambient temperatures was investigated (Madden et al. 2004); significant variation in oxygen affinity was observed, which was attributed to the dissimilarities in amino acid sequence. Similarly, Stewart et al. (2004) reported that beluga whale Mb had the largest dissociation constant for molecular oxygen binding among vertebrate Mbs, possibly because of key amino acid substitutions in positions 66 and 67 near the distal histidine.

Heme Retention

Mb redox state and its ability to bind ligands are dependent on the efficiency of the protein to retain its heme prosthetic group, a biochemical property governed by the primary structure (Yang & Phillips 1996). Disassociation of heme from Mb results in meat color deterioration (Richards et al. 2005). The heme loss from sperm whale Mb mutants was assessed as a function of primary structure (Grunwald & Richards 2006a,b). These authors observed that single amino acid substitutions at specific locations resulted in variation (in a scale of up to 1,000-fold) in heme affinity. Mb variant V68T exhibited greater heme affinity than native Mb, whereas the H97A mutant demonstrated lower affinity than wild type because substitution of histidine 97 with small-sized alanine residue enabled easy access of water molecules to the heme pocket and subsequently weakened the coordination of heme by proximal histidine. Noticeably, these investigations confirmed that even a single residue substitution in Mb can alter heme retention ability and meat color stability.

Thermal Stability

The biophysical properties of Mb change with exposure to heat and high temperature, and these changes ultimately influence Mb denaturation and the development of cooked meat color. The thermal stability, or the resistance against heat-induced denaturation, of Mb can influence cooked-color biochemistry (King & Whyte 2006). The relationship between primary structure and thermal stability was investigated in Mb from closely related tuna species (Ueki & Ochiai 2004). Although the primary structure of bigeye tuna Mb shares more than 95% similarity with its yellowfin tuna and bluefin tuna counterparts, differential scanning calorimetry revealed that the thermal stability of bigeye tuna Mb is the lowest among the three species. These authors concluded that the differences in tertiary structures, as a result of variation in primary structure, were responsible for the observed differences in thermal stability. Similar results were reported in bullet tuna Mb in related studies (Ueki et al. 2005, Ueki & Ochiai 2005).

Thermal stability differences observed in fish Mbs, despite high sequence similarity, were explained on the bases of the presence or absence of structurally important amino acid residues. The effect of amino acid replacement on thermal stability was investigated in five mutants (P13A, I21M, V57I, A62G, and I21M/V57I) and the wild-type bigeye tuna Mb (Ueki & Ochiai 2006). The stabilities of V57I and I21M/V57I mutants were greater than that of the wild type, and this observation suggests that the structural stability of Mb is tuned up by the substitutions of a few amino acid residues located in the α-helical segments forming the hydrophobic heme pocket. Substitutions of key amino acid residue(s) influence the tertiary structure of Mb and are sufficient to impact thermal stability.

INTERACTIONS BETWEEN MYOGLOBIN AND BIOMOLECULES

In sarcoplasm, Mb continuously interacts with other water-soluble biomolecules. Such interactions are necessary to fulfill its function in live muscles and can also modulate the protein's functionality in meats. Exposure to prooxidants can destabilize Mb, whereas antioxidants can improve Mb stability. This principle contributes to certain color phenomena observed in meats.

Lipid oxidation generates reactive secondary products such as aldehydes, which compromise color stability by adducting at the histidine residues in Mb. Supranutritional supplementation of vitamin E increases color and lipid stability of beef (Faustman et al. 1989, Lynch et al. 1999) and lamb (Guidera et al. 1997, Strohecker et al. 1997). Although lipid oxidation was reduced significantly in vitamin E–supplemented pork, a color-stabilizing effect was not readily observable

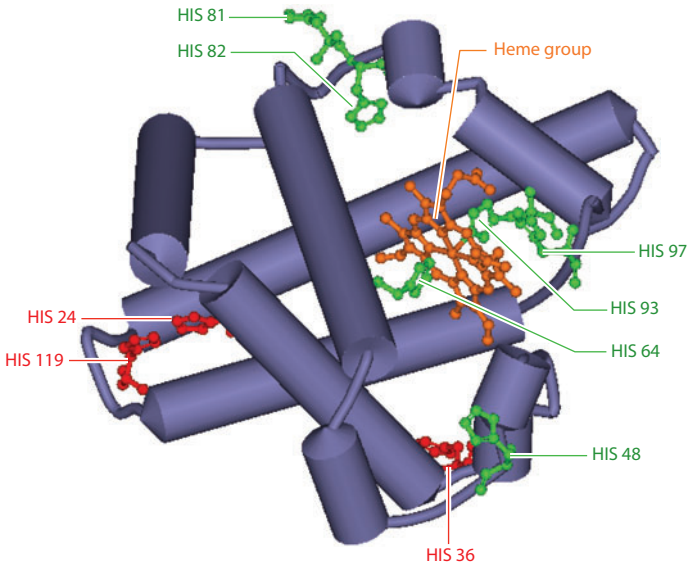

Figure 4

The three-dimensional structure of pork myoglobin showing the three histidines adducted by reactive aldehydes (*red*). Unadducted histidines (*green*) and the heme group (*orange*) are also indicated. (Adapted with permission from Suman et al. 2007.)

(Houben et al. 1998, Phillips et al. 2001a), indicating that the effect of vitamin E on meat color is not universal. Vitamin E protects highly oxidizable polyunsaturated fatty acids from oxidation by reactive oxygen species and free radicals (Buttriss & Diplock 1988). The antioxidant effects of vitamin E minimize the generation of reactive aldehydes, which attack Mb molecules. Pork generally has a greater proportion of unsaturated fatty acids than beef. Pork lipids, being more unsaturated, would undergo lipid oxidation more readily and produce more secondary products than beef lipids. In turn, this is expected to result in a reduction in color stability in pork, which would be prevented by vitamin E. However, findings that pork did not demonstrate the improved color stability from vitamin E supplementation indicated that interactions between Mb and lipid oxidation products are different in pork and beef. Pork Mb contains nine histidines, whereas beef Mb contains 13 histidines (**Figure 2**); fewer histidines in pork Mb than in beef Mb contribute, in part, to the lower susceptibility of pork Mb to the redox destabilizing effect of lipid oxidation than that of beef Mb. Use of mass spectrometry and proteomics revealed that beef Mb is more susceptible to lipid oxidation–induced oxidation than pork Mb (Suman et al. 2007); the reactive lipid oxidation product hydroxynonenal adducted seven histidines in beef Mb, but only three histidines were alkylated in pork Mb (**Figure 4**). Preferential aldehyde adduction at the proximal histidine (position 93), observed exclusively in beef Mb in this study, explained why lipid oxidation–induced Mb oxidation appears more extensive in beef than in pork and why the color-stabilizing effect of vitamin E is observed only in beef. Furthermore, recent studies (Yin et al. 2011) suggested that the susceptibility of mammalian and avian Mbs to lipid oxidation is directly proportional to the number of histidines. The aforementioned studies on lipid oxidation–induced meat discoloration were undertaken using OxyMb, the only cherry-red redox form that was relevant to the US meat industry until 2004. With the approval of CO MAP in red-meat retailing, COMb also became relevant to US meat merchandising, and the effect of lipid oxidation on COMb stability was also examined. Although COMb has been known as a more stable redox form than OxyMb in

meat, mass spectrometric investigations indicated that COMb undergoes lipid oxidation–induced oxidation in a manner similar to that of OxyMb (Joseph et al. 2009, 2010a).

Components of sarcoplasmic proteome, often known as soluble muscle proteins, interact with Mb and can influence color stability (Renerre et al. 1996). Joseph et al. (2012) reported that the relative abundance of the sarcoplasmic proteins is variable in beef muscles and contributes to muscle-specific color attributes. Color-stable beef muscle (Longissimus lumborum) possesses higher levels of soluble antioxidant proteins than does color-labile muscle (Psoas major), and this is reflected in the low rate of Mb oxidation and superior color stability observed in Longissimus lumborum (McKenna et al. 2005, Seyfert et al. 2007).

The primary structure of Mb from the Emperor penguin, known for its deep-sea diving ability in Antarctica, is different from human and sperm whale Mb (Tamburrini et al. 1999). The oxygen-binding capacity of penguin Mb was greater than human Mb and is not influenced by lactate, a heterotropic modulator of oxygen affinity (Giardina et al. 1996) in sperm whale and horse Mbs. These studies concluded that the primary structure of Emperor penguin Mb influenced its oxygen-binding capacity and mechanistic interactions with lactate, and thus provided species-specific advantage to the bird in its natural habitat. In the present-day meat industry, lactate is exploited as a color stabilizer in fresh beef products, as it promotes formation of ferrous forms of Mb (Kim et al. 2006, Mancini et al. 2009). A direct covalent interaction between lactate and Mb (via covalent binding) proposed by Giardina et al. (1996) as the underlying mechanism modulating oxygen affinity was not evident in the mass spectrometry–based investigations (Mancini et al. 2010b). The improved color stability observed in lactate-enhanced meats has been attributed to the indirect mechanism of regenerating NADH through the lactate dehydrogenase enzyme system, which favors mitochondria-mediated MetMb reduction and subsequent improvement in color stability (Ramanathan et al. 2010).

COLOR DEFECTS IN FRESH MEATS

Brown color formation is universally considered as a major indicator of quality deterioration in fresh meats, and the biochemical basis of brown discoloration in fresh meats has been extensively reviewed (Faustman & Cassens 1990, Mancini & Hunt 2005). In addition to surface browning, some specific color defects have been observed in meats, influencing consumer attitude and sales.

Discoloration of bone marrow is a major problem in bone-in cuts (Mancini et al. 2004). The red color of bone marrow is due to hemoglobin; being an erythropoietic organ, bone marrow is rich in hemoglobin. In addition, bone marrow has abundant lipids, which readily undergo oxidation upon exposure to air. Fabrication causes disruption of erythrocytes in bone marrow, exposing the hemoglobin to a prooxidant environment that leads to bone discoloration. The use of antioxidants such as ascorbic acid can prevent this defect (Grobbel et al. 2006, Mancini et al. 2004).

Iridescence is the shiny rainbow–, peacock feather–, or credit card hologram–like appearance in raw as well as cooked meats (Swatland 2012). This is a physical phenomenon attributed to the optical refraction and diffraction that results from the striated structure of skeletal muscles. Although iridescence is physical in nature and is not primarily caused by pigments, it is often misunderstood as the presence of spoilage and undesirable chemicals (Mancini 2007).

COLOR DEFECTS IN COOKED MEATS

At the point of consumption, many consumers consider the color of cooked meat as a reliable indicator of safety and doneness. Dull-brown interiors are considered a hallmark of a well-done product, whereas pink appearance is related to uncooked meats (King & Whyte 2006).

Unfortunately, the denaturation temperature of Mb is dependent on the redox state, and therefore cooked color may not always be an indicator of safety. The resistance to heat-induced denaturation is in this order: DeoxyMb > OxyMb > MetMb (Machlik 1965, Sepe et al. 2005).

Premature browning is the condition in cooked ground beef wherein Mb denaturation happens at a temperature lower than 71°C, which is the US Department of Agriculture–recommended temperature to destroy *Escherichia coli* O157:H7. Therefore, relying solely on cooked color can lead to consumption of undercooked beef and food-borne infections (Bigner-George & Berry 2000). It has been reported that approximately 50% of ground beef retailed in the United States is susceptible to premature browning (Killinger et al. 2000). Ground beef containing predominantly MetMb readily undergoes premature browning compared with ground beef containing mainly ferrous forms (Hunt et al. 1999). By modulating the redox state of Mb in raw meat, several exogenous and endogenous factors are known to govern the incidence of premature browning, including muscle source (Suman et al. 2004), storage (Suman et al. 2005), antioxidants (Phillips et al. 2001a, Sepe et al. 2005, Suman et al. 2011, Mancini et al. 2011), and packaging (John et al. 2005, Suman et al. 2005, Mancini et al. 2010a, 2011).

Pink color defect is a quality problem in fully cooked uncured turkey products and affects 1% to 2% of the turkeys harvested in the United States. The uncooked pink appearance leads to consumer rejection of otherwise well-done products (Holownia et al. 2003). Although food safety is not a concern, this defect is responsible for sales losses to the turkey industry because of the condemnation of microbiologically safe products. Incomplete denaturation of Mb (Girard et al. 1990, Trout 1989) and the interactions between Mb and combustion products (Cornforth et al. 1998, Nam & Ahn 2002) have been offered as the major reasons for pinking. Furthermore, recent studies (Joseph et al. 2010b) indicated that the inherent greater thermal stability of turkey Mb, in comparison with its beef counterpart, is a major factor contributing to the occurrence of pinking.

STRATEGIES TO IMPROVE FRESH MEAT COLOR

The antioxidant mechanisms within the skeletal muscles become dysfunctional once the muscle-to-meat conversion is complete (Huff-Lonergan et al. 2010). Meat discoloration is inevitable during storage and retail, and therefore strategies to prolong the color stability contribute significantly to profitability. Common strategies used for this purpose fall under two major categories: packaging and antioxidants. Packaging is purely a post-harvest approach, whereas antioxidants can be either post- or pre-harvest applications.

MAP is the enclosure of meat products in gas-barrier materials in which the gaseous environment has been changed. Approximately two-thirds of the fresh meat in the United States is marketed in case-ready MAP systems (McMillin 2008). The common MAP systems used for fresh meats are high oxygen (80% oxygen and 20% carbon dioxide) and low CO (0.4% CO, with the balance nitrogen and carbon dioxide). The ligands, oxygen and CO, in the gas mixture bind with Mb to provide the desirable cherry-red color. When used at 55% to 80% in high-oxygen MAP, oxygen helps maintain the consumer-desirable color of meat for a longer time than in aerobic packaging. The presence of high levels of oxygen (50% or more) in MAP saturates the meat surface with OxyMb and thus improves color shelf life (Jakobsen & Bertelsen 2000, Jayasingh et al. 2002). Fresh red meats retailed in high-oxygen MAP retain acceptable red color for up to 14 days of display, compared with less than seven days for aerobically packaged counterparts.

Compared with high-oxygen packaging, the use of CO in MAP is relatively new in the US meat industry (Cornforth & Hunt 2008). The toxic aspect of CO has limited its level to less than 1%, which is sufficient to maintain a stable cherry-red color. The Norwegian meat industry successfully used CO MAP between 1985 and 2004, and in the United States the CO MAP was approved for

retail in 2004. Although the appearances of fresh meats in high-oxygen and CO MAP systems are similar, those in CO MAP have longer color shelf life (21 days) than their counterparts in high-oxygen MAP (14 days). Several studies have documented that CO MAP significantly improves the color stability of beef as well as pork (Sorheim et al. 1999, Hunt et al. 2004, Wilkinson et al. 2006, Mancini et al. 2009). However, there is a concern among the consumers that CO MAP may mask spoilage because the stable cherry-red color can last beyond the microbiological shelf life of red meats (Cornforth & Hunt 2008). The color-stabilizing effect of MAP systems on meat is muscle dependent (Mancini et al. 2009); steaks from beef Longissimus (color-stable) muscle demonstrated an increase in color stability under high-oxygen MAP, whereas steaks from Psoas (color-labile) muscle exhibited improved color stability in CO MAP, suggesting the necessity to develop muscle-specific MAP strategies to improve meat color stability.

In meat production and the supply chain, antioxidants can be applied either pre-harvest or post-harvest to minimize lipid oxidation–induced color deterioration (Faustman et al. 2010). Vitamin E can be used in the finishing diet of cattle to increase color stability of beef (Faustman et al. 1989) and lamb (Strohecker et al. 1997). In addition, feeding food animals with plant extracts rich in antioxidant compounds also can increase meat color stability. O'Grady et al. (2006) reported that feeding tea catechin and rosemary extract to beef animals improved color stability of refrigerated patties. Furthermore, supplementing plant extracts that contain polyphenols in the diets of culled cows improved steak color stability (Gobert et al. 2010).

Synthetic and natural antioxidants can be employed in meat systems to improve color in post-harvest applications. With the potential health concerns associated with synthetic antioxidants, there is an increased interest in the use of natural antioxidants in fresh meat. A variety of natural antioxidants, including grape-seed extract (Kulkarni et al. 2011), chitosan (Georgantelis et al. 2007), rosemary (Sanchez-Escalante et al. 2001), and olive-leaf extract (Hayes et al. 2010) improve meat color stability. Noticeably, the effects of antioxidants can be packaging dependent. For instance, Suman et al. (2010a) observed that chitosan improved the surface redness of ground beef patties in CO MAP but exerted no effect on patties in high-oxygen MAP.

Injection enhancement of whole-muscle beef cuts with solutions that contain nonmeat ingredients is widely employed to improve color quality. In this respect, natural metabolites from carbohydrates in postmortem muscles, such as lactate, succinate, and pyruvate, have been extensively investigated in the past decade. Previous studies described lactate as a color-stabilizer minimizing surface discoloration (Kim et al. 2006, Knock et al. 2006). Further investigations suggested that the influence of lactate on meat color stability is packaging dependent. Beef steaks enhanced with lactate demonstrated greater redness in high-oxygen MAP than nonenhanced steaks, but this effect was not observable in CO MAP (Mancini et al. 2009). Similar observations were documented in ground beef incorporated with 2.5% lactate (Suman et al. 2010b). Recent investigations documented that succinate (Mancini et al. 2011) and pyruvate (Ramanathan et al. 2011) also improve the color stability of retail beef.

STRATEGIES TO IMPROVE COOKED MEAT COLOR

Controlling color defects in cooked meats can contribute to increased consumer confidence and improved food safety. Strategies to minimize premature browning have focused on increasing the relative proportion of heat-stable redox forms (DeoxyMb and COMb) in the fresh ground beef. Studies have reported that use of CO MAP (John et al. 2005, Suman et al. 2011, Mancini et al. 2010a) and vacuum packaging (Suman et al. 2011, Mancini et al. 2010a, 2011) minimizes premature browning. The same outcome can be achieved through antioxidant technology; the use of erythorbate (Phillips et al. 2001b, Suman et al. 2005), ascorbate (Sepe et al. 2005), lactate

(Suman et al. 2009b), succinate (Mancini et al. 2011), and chitosan (Suman et al. 2011) minimizes premature browning by increasing the percentage of ferrous Mb forms in beef and by inhibiting lipid oxidation. The results of the aforementioned studies indicate that antioxidants can improve beef safety by preventing premature browning. Nevertheless, antioxidants are not allowed in fresh ground beef marketed in the United States.

Minimizing pink color defect in turkey products can be accomplished by the incorporation of a variety of nonmeat ingredients. Citric acid at the 0.3% level inhibits the pink defect (Kieffer et al. 2000). Milk-derived products, such as nonfat dried milk (Schwarz et al. 1999) and whey proteins (Sammel & Claus 2003, Sammel et al. 2007), are known to minimize pinking. In addition, certain antioxidants have demonstrated an ability to decrease pinking in turkey. Sodium citrate, in combination with sodium tripolyphosphate and calcium chloride, inhibits pinking (Sammel et al. 2006). Although these strategies are effective in minimizing pinking, the molecular mechanisms through which the antioxidants and milk compounds exert their effects are yet to be completely understood.

CONCLUSIONS

The complex matrix of postmortem skeletal muscle is biochemically active during retail and storage. Postmortem biochemical processes influence the color of muscle foods, which is a major trait governing consumer perception of wholesomeness. Meat color is dictated by the mechanistic interactions of Mb with a multitude of exogenous and endogenous factors. Improving color stability and prolonging color shelf life are critical to consumer acceptance of fresh meat and contribute to the profitability of the industry. Significant advances have been achieved in characterizing the underlying molecular mechanisms of meat color and in engineering strategies to improve color stability of retail fresh meats. Although certain strategies are universal, the success of others depends on muscle source, meat species, and packaging systems.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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Contents

Errata

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