Strategies for Increasing Oxidative Stability of (Fresh) Meat Color

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Introduction

Fresh meat color is determined by the oxidation status of myoglobin. Several reviews of myoglobin chemistry and meat color stability, including cured and/or cooked meat color, have been published (Livingston and Brown, 1981; Giddings, 1977; Faustman and Cassens, 1990a; Renerre, 1990; Cornforth, 1994). The purpose of this presentation is to emphasize recent research findings which impact the oxidative stability of myoglobin in fresh meat. Specific attention is given to metmyoglobin reduction and antioxidant approaches for minimizing oxymyoglobin oxidation.

Maintenance of oxymyoglobin and thus a desirable appearance in fresh meat has significant economic impact. Liu et al. (1995) noted that reduction of discoloration discounts at the retail level could result in added value to the U.S. beef industry in excess of $700 million per year. In addition to this sensory aspect, the redox chemistry of myoglobin is critical for considerations of lipid oxidation and appears to play an important role in the extent to which internal color predicts cooking doneness in ground beef (Hunt et al., 1995).

Heme-Containing Proteins in Muscle-Based Food Products

Muscle-based food products are derived from the skeletal muscle of livestock and fish species following slaughter/harvest. Historically, there has been a differentiation between red (dark) meat and white meat. The basis for this difference is the concentration of heme proteins found in particular muscles. Of specific interest are the heme proteins hemoglobin, myoglobin and cytochromes. These compounds are responsible for oxygen-binding and storage (hemoglobin and myoglobin) and for electron transport during respiration (cytochromes) in the living animal. Skeletal muscles which obtain energy primarily by aerobic means contain relatively high concentrations of heme proteins when compared to those which depend primarily on anaerobic glycolysis.

Of the heme proteins found in post-mortem skeletal muscle, myoglobin is the most important for considerations of meat color. The majority of hemoglobin found in the living animal is lost during slaughter as a result of exsanguination. Some blood is retained in meat (Fleming et al., 1960) and Warriss and Rhodes (1977) estimated the average residual blood content of butcher's meat to be 0.3%. Han et al. (1994) recently published a procedure for determining the concentrations of hemoglobin and myoglobin in meat. While visible blood spots are considered undesirable in meat (Lyon et al., 1986), hemoglobin can provide a source of pigmentation in processed meat products. Both blood and spleen which contain high concentrations of hemoglobin have been utilized for this purpose (Bittel et al., 1981; Mielnik and Slinde, 1983; Oellingrath and Slinde, 1985). The use of blood at high concentration may result in excessive pigmentation and an unacceptable dark product (Caldironi and Ockerman, 1982).

Cytochromes are present in low concentrations in skeletal muscle and not considered to be of primary importance to meat color (Ledward, 1984). Pikul et al. (1986) investigated the cytochrome c content of several different species of poultry. As a percent of total heme pigments, cytochrome c ranged from 2.33% to 2.58% in breast meat and 2.14% to 4.23% in thigh meat. In cardiac muscle, cytochromes contribute a substantial portion of the pigment present (Drabkin, 1950); heart muscle has been utilized to improve the color of bologna (Lozano and Cassens, 1984). Cytochrome c concentrations may also affect darkness of breast muscle color in turkeys subjected to pre-slaughter stress (Ngoka and Froning, 1982). The relative contributions of these heme proteins in a given muscle food may become more discernible in the near future as Boyle et al. (1994) recently published a procedure for quantifying the reduced and oxidized forms of hemoglobin and cytochromes in biological samples.

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Structure

Heme proteins contain a globin moiety or amino acid sequence and heme. Myoglobin (MW ca. 17,000) contains a single polypeptide chain and heme group. Hemoglobin (MW ca. 64,000) can be essentially considered a tetramer of myoglobin.

The heme structure within myoglobin contains an iron atom bound within a protoporphyrin ring. Protoporphyrin is a cyclic compound formed from the joining of four pyrolle rings (porphobilinogen) by methene bridges (Kalyanasundaram, 1992). The binding of iron within the planar protoporphyrin yields the metalloporphyrin, protoporphyrin IX, known commonly as 'heme.' In general, metalloporphyrins are highly colored as a result of their cyclic conjugated tetrapyrolle structure (Kalyanasundaram, 1992). The solubility of myoglobin and hemoglobin in aqueous solution is quite high while that of free heme is very low. Methods used to quantitate heme within meat products will typically employ protein denaturing conditions with subsequent extraction of heme into organic solvents (Hornsey, 1956).

Heme iron may exist in a ferrous (+2) or ferric (+3) state. The use of the prefixes ferro- and ferri- to describe heme structures indicates the redox state of the central iron. Six coordination sites exist on iron. Four of these anchor it to the four pyrolle rings of heme. The fifth site, which exists at right angles to the other four, serves to attach the heme to the amino acid sequence of the protein. In myoglobin (and hemoglobin), the sixth coordination site which is directly opposite to the fifth typically binds oxygen in the normal physiological functioning of the molecule. However, a variety of chemical compounds may bind to the heme iron of myoglobin and yield substantially different spectra and perceived color.

The heme moiety of cytochrome c is covalently bound to the globin portion of the protein via thioether linkages between cysteine residues and the heme molecule. The 5th and 6th ligands of cytochrome c are linked to the apoprotein - thus the 6th position is not available to bind ligands as in hemoglobin and myoglobin (Lemberg and Barrett, 1973).

Myoglobin Concentration

Myoglobins have been characterized in a wide variety of animal species (Drabkin, 1950; Rickansrud and Henrickson, 1967; Hapner et al., 1968; Satterlee and Zachariah, 1972) and used as the basis for identifying species origin in meat products (Janssen et al., 1990). In general, a high myoglobin concentration results in more red color within skeletal muscle. Myoglobin concentration in a given muscle is influenced by both genetics (Enfield, 1968; Boccard et al., 1979) and environment (Thomas and Judge, 1970)

The effects of animal species, age and muscle type on myoglobin concentration have been summarized by Lawrie (1985). Myoglobin concentration within a given muscle increases with animal age. Species- and muscle-specific differences also exist and in part, reflect the need for oxygen storage and delivery. Red myofibers contain more myoglobin than white myofibers (Cassens, 1977) and within a given carcass, the concentration of myoglobin will differ among muscles based on their myofiber composition. The breast muscle of domestic poultry is typically very white and extremely low in myoglobin, while thigh muscles are more red and contain higher concentrations of myoglobin. The *longissimus* muscle of the pig contains a substantially lower concentration of myoglobin than that of the lamb or cow/steer (Lawrie, 1985). The blue whale, which makes long dives and thus requires large stores of oxygen, contains greater concentrations of myoglobin than terrestrial animal species.

The control of myoglobin concentration in meat has been critical to the veal industry. The husbandry of 'special-fed' veal calves is targeted in part to obtain a muscle food product of relatively low myoglobin concentration without anemia; dietary iron is controlled carefully (Bremner et al., 1976; McFarlane et al., 1988). Veal calves raised on iron-supplemented milk diets display higher myoglobin concentrations than their non-supplemented pen mates (MacDougall et al., 1973). Grain-fed calves generally receive more dietary iron and yield meat which is more red in color than that from special-fed calves (Buege, 1989). The addition of an iron chelator appears to assist in development of low pigmented muscle in grain-fed veal calves (Pommier et al., 1992).

Myoglobin Chemistry and Perceived Color in Fresh Meat

Heme iron in myoglobin generally exists in a ferrous (+2) or ferric state (+3). A ferryl (+4) form may result from hydrogen peroxide activation of the ferrous/ferric states, but this is short-lived and is more important for considerations of heme protein catalysis of lipid oxidation than meat color (Kanner, 1994).

The ferrous forms of myoglobin include deoxymyoglobin and oxymyoglobin. Deoxymyoglobin heme iron lacks a ligand at the sixth coordination site (Dickerson and Geis, 1983). The color of deoxymyoglobin is purplish-red and is typically viewed when the deep interior of a muscle is exposed during meat cutting, or when freshly-cut meat is vacuum-packaged. When meat is exposed to the atmosphere, oxygen binds to heme iron and forms oxymyoglobin, the cherry-red pigment in beef. The process of myoglobin oxygenation is referred to as blooming and appears to differ in rate and extent among different species (Millar et al., 1994).

Ferric myoglobin results from heme iron oxidation in either of the ferrous myoglobins. Partial or complete loss of tertiary structure results in an increased rate of oxymyoglobin oxidation (Sugawara et al., 1995).

Occasionally as a result of bacterial contamination, green discolorations may form. These have been attributed to the presence of sulfmyoglobin. Berzofsky et al. (1972) performed studies which demonstrated attachment of radioactive sulfur (^{35}S) to the protoporphyrin ring of myoglobin.

When extracted into organic solvent, the sulfheme group was unstable. The authors hypothesized that ferryl species were likely necessary for sulfur incorporation.

Bacterial contamination may also cause formation of red myoglobin pigments, the structure of which is not always known (Faustman et al., 1990; Arihara et al., 1993). In experiments concerned with dietary intake of moldy feed by turkeys, Wu et al. (1994) found that redness of breast muscle increased with intake of some fusarial cultures; the authors did not elucidate a mechanism.

Stability of Myoglobin

At the retail level, fresh meat is generally displayed in oxygen-permeable film and oxymyoglobin is the pigment of concern. Deoxymyoglobin is typically associated with fresh meats which are vacuum-packaged in oxygen-impermeable film. O'Keefe and Hood (1982) maintained that deoxymyoglobin is less stable than oxymyoglobin; the presence of oxygen stabilizes myoglobin (Brunori, 1995). Practical considerations of meat color stability must therefore be concerned with the maintenance of myoglobin in the oxy- form. At any time point during the storage and/or display of fresh meat, a measurable quantity of metmyoglobin can be detected. Though oxymyoglobin is readily oxidized to metmyo-globin, a process which is thermodynamically favored (Shikama, 1985), reduction of metmyoglobin to ferrous myoglobin may also occur. As conversion to metmyoglobin occurs, some ferric pigment will be reduced to the ferrous form and in an aerobic environment, oxymyoglobin will result. Once reducing equivalents in the meat are exhausted, complete metmyoglobin formation will occur (Ledward, 1984). Thus, the color observed in meat represents the relative concentrations of ferrous and ferric pigments present at any given time.

Metmyoglobin Reduction

Metmyoglobin reduction in meat has been reported (Ledward, 1970; O'Keefe and Hood, 1982; Ledward, 1985; Echevarne et al., 1990; Faustman and Cassens, 1990b; Reddy and Carpenter, 1991) although the mechanism by which it may occur has only recently received significant attention. A specific enzymatic pathway for this reaction was first proposed in cardiac muscle by Hagler et al. (1979). The authors demonstrated that the enzyme metmyoglobin reductase required reduced nicotinamide adenine dinucleotide (NADH) and an appropriate mediator for activity. A subsequent investigation by Livingston et al. (1985) suggested that cytochrome b_5 was the likely mediator in skeletal muscle; earlier work revealed that cytochrome b_5 was the mediator for the closely related enzyme, methemoglobin reductase (Kuma and Inomata, 1972). Metmyoglobin reductase has been identified as an NADH-cytochrome b_5 reductase in both bovine cardiac (Livingston et al., 1985) and skeletal muscle (Arihara et al., 1989a). It appears that metmyoglobin is reduced non-enzymatically by reduced cytochrome b_5. Cytochrome b_5 can be regenerated from its oxidized form to an active reductant by cytochrome b_5 reductase which utilizes reducing equivalents from NADH.

Work by Arihara et al. (1989b, 1990) has contributed substantially to an understanding of cytochrome b_5 activity in muscle. Arihara et al. (1989b) utilized electrophoretic immunoblotting to demonstrate the presence of cytochrome b_5 in bovine skeletal muscle. Subsequent work (Arihara et al., 1990) led to the discovery that an outer membrane (OM) cytochrome b was present in rat skeletal muscle which was distinct from cytochrome b_5. The OM cytochrome b was also capable of reducing metmyoglobin under appropriate conditions. Recently, Arihara et al. (1995) demonstrated that cytochrome b_5 was localized to sarcoplasmic reticulum, while OM cytochrome b was found predominantly in the mitochondria. Cytochrome b_5 reductase was found in both sarcoplasmic reticulum and mitochondrial fractions. The authors proposed that cytochrome b_5 reductase would utilize NADH to enzymatically reduce cytochrome b_5 in sarcoplasmic reticulum, or cytochrome b on the outer membrane of mitochondria; either of these reduced cytochromes could then non-enzymatically reduce metmyoglobin.

Investigations concerned with the potential practical implications of enzymatic metmyoglobin reduction for improving meat color stability have been reported. Faustman et al. (1988) demonstrated that a crude liver extract was capable of effecting metmyoglobin reduction in the presence of NADH. They attributed this to the presence of cytochrome b_5 and cytochrome b_5 reductase in the extract. Application of a similar liver extract with NADH to meat did not improve color stability (Mikkelsen and Skibsted, 1992). Reddy and Carpenter (1991) adapted the assay procedure originally proposed by Hagler et al. (1979) to measure metmyoglobin reductase activity in beef. They found that this activity followed the order tensor *faciae latae > longissimus dorsi > gluteus medius > diaphragma medialis > semimembranosus = psoas major*. In subsequent work, Madhavi and Carpenter (1993) found that *psoas* steaks had greater metmyoglobin accumulation, lower metmyoglobin reductase activity and greater oxygen consumption activity than *longissimus* steaks.

Interestingly, meat with high levels of bacterial contamination may display red coloration (Butler et al., 1953; Robach and Costilow, 1961). This appears to coincide with an increase in meat pH (Faustman et al., 1990). Arihara et al. recently (1994) demonstrated that some strains of enterococci can reduce metmyoglobin to oxymyoglobin *in vitro*.

Oxymyoglobin Oxidation - pO_2 and Modified-Atmosphere Packaging

Myoglobin stability is poor at low partial oxygen pressures. This has been demonstrated in meat (Ledward, 1970) and *in vitro* (George and Stratmann, 1952). Muscles with high oxygen consumption rates favor a relatively low pO_2 environment; metmyoglobin formation is favored where low pO_2 conditions exist.

Poor color stability has been attributed to high oxygen consumption rates in muscles such as the *psoas* (O'Keefe and Hood, 1982) and *diaphragma medialis* (Renerre and Labas, 1987). In addition, the concentration of nicotinamide adenine dinucleotide (NAD), which appears directly proportional to oxygen consumption rate in post-mortem muscle, has been shown to be higher in lamb than in beef or pork (Atkinson and Follett, 1973). Atkinson and Follett (1973) attributed a higher oxygen consumption rate for the relative instability of color in lamb when compared to its red meat counterparts. Faustman and Cassens (1991) reported that Holstein beef, which had a higher NAD concentration than beef from crossbred animals, was less color stable.

The packaging environment can impact significantly partial oxygen pressure and have profound effects on the color stability of fresh meat (Renerre and Labadie, 1993). Packaging with high oxygen partial pressure can extend fresh meat color shelf-life (Daun et al., 1971; Taylor and MacDougall, 1973); vacuum-packaged meats retain the purplish color of deoxymyoglobin. Some approaches have utilized anoxic atmospheres in master packs for shipment to retail; meat cuts are subsequently displayed in traditional oxygen-permeable packaging. Failure to completely remove oxygen (to <1%) can result in prooxidative conditions associated with low pO_2. Allen (1991) reported on the use of oxygen-scavenging sachets for enhancing color shelf-life of retail lamb cuts which were stored in modified atmospheres (MAP) of 50%CO_2:50%N_2. Lamb chops were stored in MAP for up to 4 weeks and then placed on trays and overwrapped with oxygen-permeable film. Those retail cuts which were stored in MAP with oxygen-scavengers demonstrated significantly greater retail color shelf-life than those which were not exposed to oxygen-scavengers. The effect has been recently demonstrated in beef (Allen et al., 1996) and in turkey meat packaged in 100% CO_2 atmospheres with oxygen scavengers (Sante et al., 1994).

Oxymyoglobin Oxidation and Lipid Oxidation

The formation of metmyoglobin from oxymyoglobin appears related to lipid oxidation and to be dependent on antioxidant status (Yin et al., 1993). It may be that the process of lipid oxidation yields products which are prooxidative towards oxymyoglobin. Renerre et al. (1992) demonstrated that the autoxidation rate of oxymyoglobin extracted from *psoas* (color labile) and *longissimus* (color stable) muscles stored for only 2 hours post-mortem was not different. However, at 192 hours post-mortem, oxymyoglobin obtained from *psoas* oxidized more rapidly than oxymyoglobin from *longissimus*. Foucat et al. (1994) repeated this work utilizing ^{1}H-NMR. Their results supported the concept that oxidative processes occurring during extended storage of the two muscles led to greater oxidative susceptibility of myoglobin after extraction from *psoas* than from *longissimus*.

Water-soluble antioxidants added to meat appear efficacious in protecting myoglobin against oxidation. Ante-mortem infusion of ascorbic acid to cattle has been effective at increasing the color stability of some beef cuts subsequently obtained from treated animals (Hood, 1975; Schaefer et al., 1994). The addition of ascorbic acid to fresh beef (Greene et al., 1971; Harbers et al., 1981; Shivas et al., 1984; Mitsumoto et al., 1991ab) enhances color stability.

The application of the dipeptide, carnosine, has been shown to delay meat discoloration in salted ground pork (Decker and Crum, 1991). A direct protective effect of carnosine on oxymyoglobin does not appear likely (Decker et al., 1995). Rather, the pigment stabilizing effect of carnosine in salted pork appears to be indirect; carnosine effectively delays the production of lipid oxidation products which may enhance oxymyoglobin oxidation.

Oxymyoglobin stability is greater in meat which contains higher concentrations of α-tocopherol. Several reviews have been published on dietary supplementation of vitamin E for improving beef color stability (Faustman, 1993; Schaefer et al., 1994; Liu et al., 1995). Improved color stability has been observed in fresh (Faustman et al., 1989; Arnold et al., 1993; Lavelle et al., 1995; Sherbeck et al., 1995; Chan et al., 1996; Liu et al., 1996) and frozen (Lanari et al., 1993) beef, fresh pork (Asghar et al., 1989), lamb (Wulf et al., 1995; Guidera et al., 1995) and turkey (Sante and Lacourt, 1994).

It is important to note that the relative color stability of different muscles (O'Keefe and Hood, 1982) is not changed as a result of vitamin E supplementation. Chan et al. (1996) recently demonstrated that the color stability of beef *psoas* < *gluteus medius* < *longissimus lumborum* within control or vitamin E-supplemented treatments. Interestingly, within each treatment, *psoas* contained the highest concentration of α-tocopherol. Thus, while vitamin E has profound effects on oxymyoglobin stability, there remain other factors equally or more critical to meat color stability. This was supported further by Chan et al. (1996) who isolated microsomal fractions from each of the three muscle types and combined them with oxymyoglobin; pigment stability was equivalent amongst the three different muscle microsome treatments.

The protection of water-soluble myoglobin against oxidation by a lipid-soluble antioxidant is interesting. Liposome and microsome models have been used to investigate this effect and results are consonant with those obtained in meat (Anton et al., 1991, 1993; Yin et al., 1993; Yin and Faustman, 1993, 1994; Chan et al., 1996). The incorporation of α-tocopherol into myoglobin:liposomes (Yin et al., 1993), or the incubation of oxymyoglobin in the presence of microsomes prepared from tissues of vitamin E-supplemented livestock (Yin and Faustman, 1994; Chan et al., 1996) enhanced oxymyoglobin stability over that of controls. Schaefer et al. (1995) proposed a model by which oxymyoglobin stability is improved in the presence of high concentrations of α-tocopherol. In brief, α-tocopherol slows the process of lipid oxidation directly via radical quenching. This delays production of lipid oxidation breakdown products (eg peroxides) which can accelerate oxymyoglobin oxidation. Thus, the protection of oxymyoglobin by α-tocopherol appears to be indi-

rect. Mitsumoto et al. (1993) provided evidence that endogenous incorporation of α-tocopherol within meat results in greater color stability than when α-tocopherol is added exogenously. Further work is needed to determine if a direct interaction between α-tocopherol and oxymyoglobin exists and whether or not α-tocopherol improves meat color stability by enhancing metmyoglobin reduction.

Summary

Many factors affect oxymyoglobin stability in fresh meat. Temperature is potentially the most important environmental variable and is easily controlled through existing technology. In order to continue progress towards stabilizing fresh meat color, new approaches which emphasize nutritional and/or genetic strategies will be necessary. In addition, further investigations in packaging and control of packaging atmosphere are required. The supplementation of vitamin E to cattle to dramatically improve color stability of meat subsequently obtained from these animals is just one example of a successful nutritional strategy. Optimization of metmyoglobin reduction will require a better understanding of meat biochemistry. Improved fresh meat color stability will continue to be needed in order to better serve export markets and to provide improved shelf stability in domestic markets where distances between food production and food consumption continue to increase.

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