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Review Article

Review: Lipid and myoglobin oxidations in muscle foods

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Abstract

Lipid oxidation and myoglobin oxidation in muscle foods occur in a concurrent manner and each process appears to enhance the other. During oxidation of oxymyoglobin, both superoxide anion and hydrogen peroxide are produced and further react with iron to produce hydroxyl radical. The hydroxyl radical has the ability to penetrate into the hydrophobic lipid region and hence facilitates lipid oxidation. The prooxidant effect of oxymyoglobin on lipid oxidation is concentration-dependent. At equimolar concentrations, oxymyoglobin shows higher prooxidative activity towards lipid than metmyoglobin. However, the catalytic activity of metmyoglobin is promoted by hydrogen peroxide. The reaction between hydrogen peroxide and metmyoglobin results in the formation of two active hypervalent myoglobin species, perferrylmyoglobin and ferrylmyoglobin, which are responsible for lipid oxidation. Additionally, lipid oxidation results in a wide range of aldehyde products, which are reported to induce the oxidation of oxymyoglobin. Metmyoglobin formation is generally greater in the presence of unsaturated aldehydes than their saturated counterparts of equivalent carbon chain length. In addition, increasing chain length of aldehydes, from hexenal through nonenal, results in the increased metmyoglobin formation. Moreover, aldehydes alter myoglobin redox stability by increasing oxymyoglobin oxidation, decreasing the metmyoglobin reduction via enzymatic process, and enhance the prooxidant activity of metmyoglobin. Therefore, the oxidation of both lipid and myoglobin directly affect the quality and acceptability of muscle foods and the lowering of such a phenomenon can enhance the shelf-life stability of those foods.

Key words: lipid, myoglobin, oxidation, muscle foods

1. Lipid oxidation in muscle foods

Lipid oxidation is one of the main factors limiting the quality and acceptability of meats and other muscle foods (Zamora and Hidago, 2001, Renerre, 2000, Morrissey *et al.*, 1998). Oxidation of lipids is accentuated in the immediate post-slaughter period, during handling, processing, storage and cooking. This process leads to discoloration, drip losses, off-odor and off-flavor development, texture defects and the production of potentially toxic compounds (Richards *et al.*, 2002; Morrissey *et al.*, 1998). Lipid oxidation is a chain reaction that consists of initiation, propagation, and termination reactions, and involves the production of free radicals (Nawar, 1996; Renerre, 2000; Figure 1). A three-step simplified free-radical scheme has been postulated as follows:

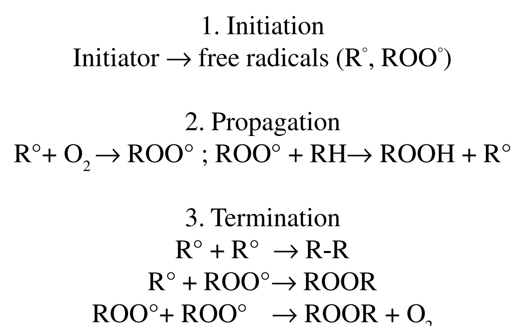

Figure 1. Mechanism of lipid oxidation (Nawar, 1996).

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This phenomenon can be influenced by both intrinsic and extrinsic factors such as the concentration of pro-oxidants, endogenous ferrous iron, myoglobin, enzymes, pH, temperature, ionic strength, oxygen consumption reaction and the fatty acid composition of the meat (Andreo *et al.*, 2003; Undeland, 2001; Harris and Tall, 1994; Renerre and Labas, 1978). Meats such as fish and poultry contain a high concentration of polyunsaturated fatty acids and are therefore more susceptible to oxidation (Pacheco-Aguilar *et al.*, 2000; Apgar and Hultin, 1982). Oxidation of lipids also occur during postmortem storage of muscle tissue. Lynch *et al.* (2001) demonstrated that lipid oxidation occurred progressively in stored ground beef at 4°C and produced a variety of aldehydes. Fatty fish such as sardine underwent rapid lipid oxidation during iced storage due to the high content of polyunsaturated fatty acids (Chaijan *et al.*, 2006; Pacheco-Aguilar *et al.*, 2000).

Furthermore, the concentration of ferrous iron and its ability be active in the lipid oxidation reaction will be a key factor causing differences among species and cuts of meat. In general, dark meats tend to have more reactive iron. Chaijan *et al.* (2004) reported that lipid and myoglobin contents were higher in dark muscle than in ordinary muscle of both sardine and mackerel. Saturation of red color in meat was directly related to myoglobin concentration (Faustman *et al.*, 1992). Other constituents of meat including enzymatic and non-enzymatic reducing systems can accelerate oxidation by converting iron from the inactive ferric form to the active ferrous state (Foegeding *et al.*, 1996).

As with most chemical reactions, lipid oxidation rates increase with increasing temperature and time (Hultin, 1992). Saeed and Howell (2002) reported that the rate of lipid oxidation in frozen Atlantic mackerel increased with increasing storage time and storage temperature. Furthermore, freezing can facilitate lipid oxidation, partly because of concentration effects (Foegeding *et al.*, 1996). Additionally, NaCl is able to catalyze lipid oxidation in muscle tissue (Nambudiry, 1980). Alternatively, the Na^+ may replace iron from a cellular complex via an ion exchange reaction (Kanner and Kinsella, 1983). The displaced iron may then participate in the initiation of lipid oxidation (Hultin, 1992). It is most likely that meat or meat products containing salt such as surimi and cured meat are susceptible to lipid oxidation. Lipid oxidation seems to be a distinct problem in surimi made from some dark-fleshed fish and particularly surimi from mammalian and avian muscle (Lanier, 2000).

2. Myoglobin oxidation in muscle foods

Myoglobin is a globular heme protein found in the muscle of meat-producing animals (Faustman and Phillips, 2001; Figure 2). It has been known to be a major contributor to the color of muscle, depending upon its redox state and concentration. Myoglobin concentration is affected by both genetics and environment (Giddings, 1974; Livingston and Brown, 1981; Faustman *et al.*, 1996).

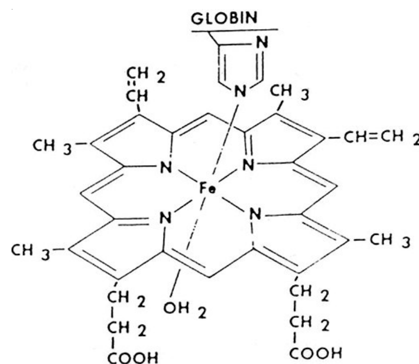
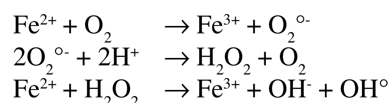


Figure 2. Chemical structure of myoglobin (Pearson and Young, 1989).

Myoglobin is made up of a single polypeptide chain, globin, consisting of 153 amino acids and a prosthetic heme group, an iron (II) protoporphyrin-IX complex (Hayashi *et al.*, 1998; Pegg and Shahidi, 1997). This heme group gives myoglobin and its derivatives their distinctive color (Dunn *et al.*, 1999; Pegg and Shahidi, 1997). The structure and chemistry of the iron atom have an impact on the reactions and color changes that myoglobin undergoes (Livingston and Brown, 1981). The oxidation of ferrous-oxymyoglobin (Fe^{2+}) to ferric-metmyoglobin (Fe^{3+}) is responsible for discoloration of meat during storage. Ferrous iron (Fe^{2+}) can react with molecular oxygen to produce superoxide anion ($O_2^{\circ-}$) with concomitant oxidation to ferric iron (Fe^{3+}). Hydrogen peroxide (H_2O_2), which may be produced by dismutation of $O_2^{\circ-}$, can react with Fe^{2+} to produce hydroxyl radical (OH°) (Hultin, 1992). This reaction termed the Fenton reaction is the principal mechanism for myoglobin oxidation. (Figure 3)



$$Fe^{2+} + O_2 \rightarrow Fe^{3+} + O_2^{\circ-}$$
$$2O_2^{\circ-} + 2H^+ \rightarrow H_2O_2 + O_2$$
$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^\circ$$

Figure 3. Reactive oxygen species generated by the Fenton reaction (Hultin, 1992).

In general, fish myoglobins are more readily oxidized than the mammalian counterpart (Haard, 1992). Discoloration of tuna meat during frozen storage is associated with the formation of metmyoglobin (Haard, 1992). Benjakul and Bauer (2001) suggested that the freeze-thaw process caused damage of cell and heme-proteins, resulting in the release of prooxidants. Haard (1992) also reported that fish myoglobins are at least 2.5 times more sensitive to autoxidation than mammalian myoglobins. Autoxidation of myoglobin becomes greater as temperature increased and pH decreased (Livingston *et al.*, 1981). Chaijan *et al.* (2007) demonstrated that sardine myoglobin was prone to oxidation and denaturation at temperature above 40°C and at very acidic or alkaline pHs as evidenced by the formation of metmyoglobin, the changes in tryptophan fluorescence intensity as well as the disappearance of Soret absorption.

Furthermore, the rate of myoglobin autoxidation was related to oxygen concentration (Brown and Mebine, 1969). Atmospheres enriched in carbon dioxide (CO_2) are effective in delaying spoilage of meat; however, one problem is that carbon dioxide can promote the oxidation of oxymyoglobin to metmyoglobin, thereby causing the discoloration (Haard, 1992).

3. Interrelationship between lipid and myoglobin oxidations in muscle foods

3.1 Myoglobin-initiated lipid oxidation

Heme pigments, especially myoglobin, catalyze the lipid oxidation in meat (Love, 1983; Han *et al*., 1994). Myoglobin and other heme compounds in red meats function as prooxidants in muscle tissue. It has been generally assumed that lipid oxidation in meat is nonenzymatic and myoglobin is the major catalyst of lipid oxidation (Love, 1983). Morey *et al*. (1973) found that H_2O_2 acting as an oxidizing agent caused changes in the oxidation state of the iron in myoglobin, and induced the formation of red-brown color. The interaction of H_2O_2 with metmyoglobin led very rapidly to generation of an active species, which could initiate lipid peroxidation (Chan *et al*., 1997; Kanner *et al*., 1987).

1) Role of deoxymyoglobin in lipid oxidation

The prooxidative activity of deoxymyoglobin in the biological system including muscle foods has been rarely reported (Baron and Andersen, 2002). Richards and Hultin (2000) suggested that deoxyhemoglobin was able to initiate lipid oxidation even at low lipid hydroperoxide concentrations. Deoxyhemoglobin was a potent catalyst of lipid oxidation in fish muscle (Richards *et al*., 2002).

2) Role of oxymyoglobin in lipid oxidation

Oxymyoglobin oxidation and lipid oxidation are coupled (Yin and Faustman, 1993). A high correlation between oxymyoglobin oxidation and lipid oxidation both in microsomes and liposomes was reported by Yin and Faustman (1993; 1994) and O'Grady *et al*. (2001). Lipid oxidation in fresh meat is influenced by the oxidation of oxymyoglobin since the oxymyoglobin oxidation results in production of two species known as the prooxidants, namely metmyoglobin and H_2O_2 (Chan *et al*., 1997). It has been proposed that $O_2^{\circ-}$ and H_2O_2 are produced during oxidation of oxymyoglobin to metmyoglobin (Gotoh and Shikama, 1976). $O_2^{\circ-}$ can further react with H_2O_2 and Fe^{3+} via the Fenton reaction to produce OH° and facilitate lipid oxidation (Chan *et al*., 1997). The prooxidative effect of oxymyoglobin towards lipid oxidation was concentration-dependent (Chan *et al*., 1997). Additionally, H_2O_2 can react with metmyoglobin to form a prooxidative ferrylmyoglobin radical (Decker *et al*., 1995). Kanner and Harel (1985) reported that H_2O_2-activated metmyoglobin caused the rapid oxidation of poultry skeletal muscle microsomes. Moreover, reactive oxygen species including superoxide, hydroperoxyl radical (HOO°), and H_2O_2, originated by the autoxidation of oxymyoglobin (Krüger-Ohlsen and Skibsted, 1997), can cause damage to muscle lipids via oxidation reaction (Skulachev, 1996; Hultin and Kelleher, 2000).

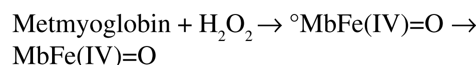
3) Role of metmyoglobin in lipid oxidation

Formation of metmyoglobin is highly correlated with lipid oxidation in muscle foods (Andersen and Skibsted, 1991). Baron *et al*. (1997) found that metmyoglobin is an effective prooxidant at acidic pH and in the presence of hydroperoxides. In contrast, at physiological pH and in the presence of lipids, metmyoglobin can undergo a rapid neutralization due to formation of the noncatalytic heme pigment. However, further denaturation of the heme proteins due to a high lipophilic environment may result in heme release or further exposure of the heme group to the surrounding lipids, thereby inducing lipid peroxidation (Baron and Andersen, 2002). Metmyoglobin acts as a prooxidant in raw fish more effectively than in raw turkey, chicken, pork, beef and lamb (Livingston *et al*., 1981).

In addition, the lipid to heme protein ratio is an important factor affecting the prooxidative activity of heme proteins (Kendrick and Watts, 1969). At lower linoleate/heme protein ratios, heme proteins become ineffective initiators of lipid oxidation (Nakamura and Nishida, 1971). The mechanism responsible for the inhibition of lipid oxidation at low linoleate/heme protein ratios has been proposed. The fatty acid anions bind reversibly to metmyoglobin, resulting in a spin transition, to yield the low-spin metmyoglobin derivative which is not prooxidative (Baron *et al*., 1998). At high linoleate-to-heme ratios, metmyoglobin immediately denatures and results in exposure or release of the heme group to the environment that instantly initiates hematin-induced lipid peroxidation in the system (Baron *et al*., 2002).

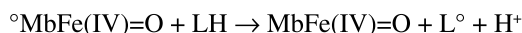
4) Role of ferrylmyoglobin in lipid oxidation

The reaction between H_2O_2 and metmyoglobin results in the formation of a red pigment, ferrylmyoglobin (Baron and Andersen, 2002). During this interaction, the production of free radicals occurs in the globin part of the heme protein. H_2O_2 activation of metmyoglobin is a necessary step in the conversion of metmyoglobin to a prooxidant (Kanner and Harel, 1985). Interaction between metmyoglobin and H_2O_2 is a complex mechanism, resulting in the generation of two distinct hypervalent myoglobin species, perferrylmyoglobin (°MbFe(IV)=O) and ferrylmyoglobin (MbFe(IV)=O) (Davies, 1990; 1991) as follows:



Metmyoglobin + H_2O_2 → °MbFe(IV)=O →
MbFe(IV)=O

Perferrylmyoglobin is a transient species with a very short half-life and autoreduces rapidly to the more stable ferrylmyoglobin (Baron and Andersen, 2002). Ferrylmyoglobin is a relatively stable species, which is slowly reduced back to metmyoglobin at physiological pH but with an increasing rate at decreasing pH due to an acid-catalyzed process (Mikkelsen and Skibsted, 1995). Perferrylmyoglobin can effectively transfer its radical to other proteins and subsequently induces lipid oxidation (Baron and Andersen, 2002). However, the ability of perferrylmyoglobin to initiate lipid oxidation by abstracting a hydrogen atom from fatty acids (LH) was suggested by Kanner and Harel (1985) as shown in the following reaction:



$$^{\circ}MbFe(IV){=}O + LH \rightarrow MbFe(IV){=}O + L^{\circ} + H^{+}$$

Ferrylmyoglobin is responsible for the oxidation of a variety of substrates (Baron and Andersen, 2002). Under conditions similar to those found in muscle foods, ferrylmyoglobin is able to initiate lipid oxidation (Hogg *et al*., 1994). However, under the conditions found in fresh meat (pH 5.5-5.8), ferrylmyoglobin autoreduces rapidly to metmyoglobin. Nevertheless, under physiological conditions (pH 7.4), ferrylmyoglobin is a strong prooxidant, which is able to abstract a hydrogen atom from fatty acids with subsequent stereospecific addition of oxygen (Rao *et al*., 1994). The prooxidative activity of ferrylmyoglobin is independent of pH and of lipid concentration (Baron *et al*., 2002). Therefore, ferrylmyoglobin is expected to be an effective prooxidant under the conditions found in muscle food, as well as under physiological conditions. However, ferrylmyoglobin formation in muscle tissues is determined by hydrogen peroxide and lipid hydroperoxide production. Its potential to oxidize lipids depends on the concentration of reducing agents and their compartmentalization in the muscle cells (Baron and Andersen, 2002).

3.2 Interaction between lipid oxidation products and myoglobin

Lipid oxidation generates a wide range of secondary aldehyde products, which are predominantly *n*-alkanals, *trans*-2-alkenals, 4-hydroxy-*trans*-2-alkenals, and malondialdehyde (Lynch and Faustman, 2000). Lynch *et al*. (2001) demonstrated that propional, pentenal, hexanal, and 4-hydroxynonenal (4-HNE) were the primary aldehydes formed during lipid oxidation in stored ground beef at 4°C (Table 1). The aldehyde products are more stable than free radical species and readily diffuse into the cellular media, where they may exert toxicological effects by reacting with critical biomolecules *in vivo* (Esterbauer *et al*., 1991).

Aldehydes produced during lipid oxidation can form adducts with proteins and this may have an impact on protein stability and functionality as well as the color stability of meat. Aldehyde products can alter myoglobin stability (Lynch and Faustman, 2000). Covalent modification of equine, bovine, porcine and tuna myoglobin by 4-hydroxynonenal (4-HNE) has been demonstrated (Faustman *et al*., 1999; Phillips *et al*., 2001a, b; Lee *et al*., 2003a, b).

Table 1. Concentration (mM) of the principal aldehydes formed during aerobically storage of ground beef for 9 days at 4°C (Lynch *et al*., 2001)

Aldehyde	Time			
	Day 0	Day 3	Day 6	Day 9
Pentenal	0.86	0.71	1.19	11.81
Hexenal	0.28	0.71	1.12	4.04
Hexanal	2.61	8.75	4.18	17.18
Heptenal	0.00	1.72	1.28	3.57
4-HNE*	0.23	3.36	3.58	10.94
Propional	0.49	17.18	20.84	31.32

***4-HNE: 4-hydroxynonenal**

Lynch and Faustman (2000) also determined the effect of aldehyde lipid oxidation products on oxymyoglobin oxidation, metmyoglobin reduction and the catalytic activity of metmyoglobin as a lipid prooxidant *in vitro*. Metmyoglobin formation was greater in the presence of α,β-unsaturated aldehydes than their saturated counterparts of equivalent carbon chain length (Faustman *et al*., 1999; Figure 4).

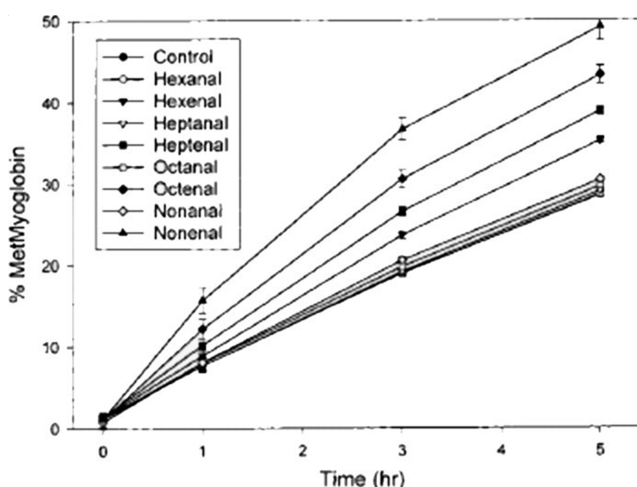


Figure 4. The potential basis for enhancement of oxymyoglobin oxidation by aldehyde lipid oxidation products (Faustman *et al*., 1999).

The covalent attachment of aldehydes to oxymyoglobin rendered oxymyoglobin more susceptible to oxidation (Faustman *et al*., 1998). Alderton *et al*. (2003) studied the effect of 4-HNE on bovine metmyoglobin formation. Increased metmyoglobin formation was found in the presence of 4-HNE. Similar results were observed by Faustman *et al*. (1999) during the incubation of 4-HNE with equine oxymyoglobin and by Lee *et al*. (2003a, b) with porcine and tuna oxymyoglobin. The covalent binding of α,

β-unsaturated aldehydes to oxymyoglobin at key amino acid residues may subsequently lead to alter tertiary structure of the protein and increasessusceptibility to oxidation. This would result in a loss of physiological activity and the brown discoloration in fresh meat (Alderton *et al*., 2003).

Alderton *et al*. (2003) and Lee *et al*. (2003a) demonstrated that 4-HNE covalently attached to bovine and porcine myoglobin, respectively. The LC-MS spectra revealed the covalent binding of up to three molecules of 4-HNE to bovine myoglobin (Figure 5A) and a di-adduct formed under the reaction of 4-HNE with porcine myoglobin (Figure 5B).

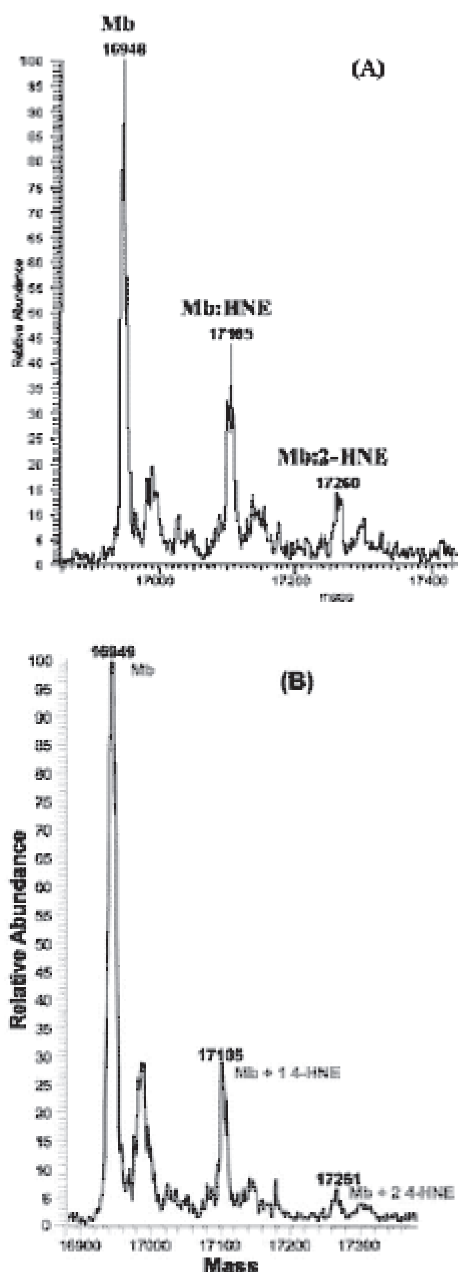


Figure 5. LC-MS spectra of bovine (A) and porcine (B) myoglobin (Mb) following reaction with 4-HNE at pH 7.4 for 120 min at 37°C (modified from Alderton *et al*., 2003 and Lee *et al*., 2003a).

4. Conclusion

Lipid oxidation and myoglobin oxidation in meat are coupled and both reactions appear capable of influencing each other. The oxidation of oxymyoglobin results in the production of metmyoglobin and H_2O_2 necessary to induce lipid oxidation. On the other hand, aldehyde lipid oxidation products alter myoglobin redox stability, resulting in the promoted oxidation of oxymyoglobin and the formation of adduct with myoglobin through covalent modification. Thus, studies of the relationship between lipid oxidation and myoglobin oxidation processes in muscle food are important in understanding reactions and mechanisms that may affect the quality and acceptability and could be useful in minimizing lipid oxidation of meats and meat products during handling, processing and storage.

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