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Review Article

Metmyoglobin reducing activity and meat color: A review

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Abstract

Meat color is a crucial factor that determines customer purchasing decisions. Metmyoglobin reducing activity is essential in the preservation of meat quality since it directly influences color stability. Metmyoglobin, an oxidized form of myoglobin found in meat, is converted to its reduced, oxygen-binding state, oxymyoglobin, in this process. A number of both internal and external factors closely control the activity of metmyoglobin reduction. Intrinsic considerations include the pH of the meat, which affects the redox potential and, as a result, the effectiveness of metmyoglobin reduction. The presence of other reducing agents, such as enzymatic systems, also aids in the reduction process. Extrinsic factors such as storage conditions and temperature have an effect on metmyoglobin production, with oxygen exposure, light, and long storage durations all contributing to undesirable discoloration. Furthermore, differences in metmyoglobin reducing activity are detected across meat types and animal breeds, emphasizing the need to address meat sources in quality management. This review aims to define the current understanding of factors that influence metmyoglobin reduction in meat, with special emphasis on recent advancements in this field.

Introduction

One of the main factors influencing consumers' decisions to buy is the color of the meat. The main protein found in the sarcoplasm, myoglobin, is what gives meat its color. Myoglobin's globular structure comprises 153 amino acids that are made up of 8 α-helices and a heme prosthetic group located in the core of the protein. Three distinct redox forms of myoglobin can be found in fresh meat: deoxymyoglobin (DMb), also known as reduced myoglobin, oxymyoglobin (OMb), and metmyoglobin (MMb) (AMSA, 2012). With the iron state of Fe2+, Mb has a purple-red color; OMb has a bright red color; and MMb has oxidized myoglobin brown color with Fe3+. MMb is formed, and the flesh becomes discolored as a result of the oxidation of both OMb and DMb (Faustman and Cassens, 1990). Meat has the innate ability to prevent MMb from accumulating or forming. It occurs through a process known as metmyoglobin-reducing activity (MRA) (Ledward, 1985). Various pre- and post-harvest factors can influence MRA.

Beef color is determined by the processes that regulate the interconversion of myoglobin redox states. This reversible reaction is dependent on oxygen current, active enzymes, and decreasing chemicals in the muscle (Figure 1).

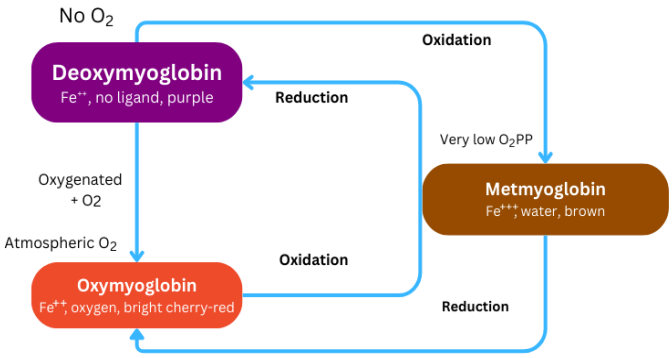

Figure 1. Interconversion of myoglobin redox states.

This review paper undertakes a comprehensive examination of metmyoglobin reducing activity, seeking to illuminate the multifaceted landscape of the factors. Recent and previous findings have been listed in Table 1, Table 2, Table 3, Table 4, Table 5, and Table 6, with their results/conclusions.

Temperature and MMb reducing activity

Table 1. Summary of research evaluating temperature affecting the MMb reducing activity

Publication	Results/conclusions
Hutchins et al., 1967; Stewart et al., 1965; Zimmerman and Snyder, 1969	MMb reduction is accelerated with increased temperature in situ
Shimizu and Matsuura, 1971	Purified MMb reductase from tuna muscle performs best at 25℃.
Hagler et al., 1979	At 37℃, bovine cardiac enzyme activity was at its peak.
Echevarne et al., 1990	The greatest MMb reduction activity of bovine skeletal muscle homogenates was at 37.5 C, with activity near zero at ≤10℃ and ≥50℃.
Mikkelsen and Skibsted (1992)	MMb reducing activity in beef liver increased with increasing temperature up to 30 ℃.
Mikkelsen et al. (1999)	Temperatures between 15 and 30℃ (pH 6.95) increased the MMb-reducing activity in porcine LD muscle.
Bekhit et al. (2001)	Lamb LD muscle extracts' MMb-reducing activity was unaffected by temperatures up to 25℃, whereas enzymatic activity dropped at temperatures above 30℃.
Reddy and Carpenter (1991)	Temperature impacts were minimal at pH 5.3, while temperature increases from 4 to 30℃ tripled MMb reducing activity from bovine LD homogenate at pH 6.4 and 7.0.
Mikkelsen et al. (1999)	While storage at -80℃ for one week had no effect on the activity of MMb reductase in porcine LD tissue, muscle extracts lost 14 percent of their initial activity after just two weeks at -80℃.
Pong et al. (2000b)	Significant MMb-reducing activity was observed in LD muscle after overnight incubation at 25℃ as opposed to 10℃. This activity was eliminated when the meat was incubated at 37℃.
Chiou et al. (2001)	MMb reductase activity gradually decreased in tuna muscle following extended storage; after 60 days, samples kept at 4 and 20℃ retained 25% and 33% of their initial activity, respectively.
Li et al. (2017)	The impact of controlled freezing point storage (CFPS, -0.8℃) on lamb meat color stability over a 10-day period compared to standard storage at 4℃. Compared to the 4℃ control, CFPS storage resulted in continuously higher color values, increased oxymyoglobin content, and decreased metmyoglobin content, indicating greater color stability.
Thiansilakul et al. (2011)	Myoglobin isolated from the dark muscle of the Eastern small tuna displayed distinct color and absorption spectra based on its forms, OxyMb and MMb. Myoglobin may be destabilized as a result of pH and heating. At pH 3 and temperatures above 60℃, tuna myoglobin was prone to oxidation and denaturation. MMb was shown to be more stable than OxyMb.
Sen et al. (2014)	Mutton chops appeared less red as the endpoint cooking temperature climbed and myoglobin denaturation increased.
Mungure et al. (2016)	The rigor temperature can be adjusted to speed up the glycolysis process in hot-boned beef SM muscle without jeopardizing aspects of the meat's quality qualities, like tenderness. Tenderness, color, and cooking loss are all factors to consider.

pH and MMb reducing activity

The activity of metmyoglobin reducing activity increases as pH rises (Ledward, 1970; Stewart et al., 1965). The optimal pH for MMb reducing activity appears to be dependent on the source of reducing activity (purified preparation vs. extract) and assay conditions. Taylor and Hochstein (1978) reported that methylene blue and NADH were used with pure bovine cardiac MMb reductase, and the optimal pH was 7.0. In contrast, Hagler et al. (1979) reported that pure bovine cardiac MMb reductase activity was highest at pH 6.5 in the pH range of 5.7-7.3. Lanier et al. (1978) reported that when samples were under nitrogen for 1 hour, MMb reduction in ground beef, beef slurries, and extracts increased with rising pH (5.6- 7.0). The action reached its peak at pH 6.2-6.6. When exposed to carbon monoxide for 1 hour. Partially purified MMb reductase from beef heart muscle with added cytochrome b5 and NADH demonstrated higher reducing activity at pH 6.3 than at pH 7.0 or 7.3 (Faustman et al., 1988).

Table 2. Summary of research evaluating pH affecting the MMb reducing activity

Publications	Results/ Conclusions
Echevarne et al. (1990)	MMb reducing activity was highest in a bovine muscle preparation at pH 7.3 with methylene blue and NADH.
Reddy and Carpenter (1991)	The optimal reducing activity for bovine LD muscle preparation was about pH 6.4.
Pong et al. (2000a)	Metmyoglobin (Met-Mb) reductase was isolated to electrophoretic homogeneity from blue-fin tuna conventional muscle using ammonium sulfate fractionation, ion exchange, and organomercurial agarose affinity chromatography. The best pH for met-Mb reduction was 7.3.
Bekhit et al. (2001)	MMb-reducing activity in ovine muscle was greatest at pH 7.4. However, measurements between pH 5.5 and 7.5 revealed no significant effect on reducing activity in this species.
Mikkelsen and Skibsted (1992)	The reduction process was enzymatic and pH-dependent. At pH 6.21, the decrease of MMb in the beef liver extract was notably greater in contrast to pH 6.96.
Hutchison et al. (2010)	MRA was highest at pH 7.4 and reduced when pH decreased to 5.7, a normal fresh meat pH. The MRA of the three lactate cations varied, with CaL being the highest at pH 7.4 and KL being the highest at pH 5.7. The effects of lactate concentration were greatest between pH 6.4 and 7.4 and least at pH 5.7. This

investigation suggests that lactate is beneficial for MRA at all pH levels, including postmortem muscle.

Storage time and MMb reducing activity

While the methodology and test conditions used to determine MMb-reducing activity can explain some of the disparities in findings among researchers, the debate concerning the impact of storage time on MMb-reducing action is not primarily due to the methodology used. Stewart et al. (1965) reported that six days of cold storage of complete beef rib-eye had a minor impact on decreasing activity. In contrast, Lanari and Cassens (1991) found that over 7 days of storage, there was no significant change in MMb decreasing activity by purified beef mitochondria/mediated reduction by methylene blue.

On the other hand, using MMbRA assay, Madhavi and Carpenter (1993) and Zhu and Brewer (1998) found that during storage, muscle MMb-reducing activity decreased, whereas others have found an increase (Bekhit et al., 2001; Echevarne et al., 1990; Feldhusen et al., 1995).

Table 3. Summary of research evaluating storage time affecting the MMb reducing activity

Publications	Results/Conclusions
Pong et al. (2000b)	After three days of cold storage, tuna muscle MMb reductase activity was reduced to less than 20% of its original level.
Liu et al. (2015)	The results showed that after 7 days of storage, MRA was considerably higher ($p< 0.05$) with all patties than on the first day.
Gao et al. (2014)	During storage, there was a decrease in MRA across all muscles. From day 1 to day 7, muscle in group A showed considerably higher MRA than muscle in groups C and E. A significant ($P<0.05$) difference was also noticed between groups B and D from day 1 to day 5.
Jeong et al. (2009)	MRA of Hanwoo muscles reduced considerably ($P <0.05$) with increasing storage time and regardless of muscle type. MRA value fell slightly ($P <0.05$) during the first three days of cold storage but significantly ($P <0.05$) on the fifth and seventh days of cold storage for all three muscles.
Mancini and Ramanathan (2014)	Changes in mitochondrial activity caused by aging can have an impact on color intensity and stability. In particular, decreased mitochondrial oxygen consumption coupled with prolonged aging durations would increase the intensity of the first hue. This gain in initial color development, however, will most certainly be offset by the lower color stability caused by the unfavorable effects of storage time on mitochondria-mediated metmyoglobin reduction.
Vieira et al. (2009)	The results indicate that frozen storage under vacuum for up to 3 months in Morucha × Charolais cattle resulted in changes in color parameters and lipid oxidation despite the quality being good in all cases.
King et al. (2011)	Metmyoglobin reducing activity was substantially associated to color stability when assessed at the start of the display, and this association was marginally strengthened by the end of the presentation, demonstrating that color stability is influenced by fluctuation in both starting levels and reducing ability maintenance.
English et al. (2016)	For the first MMb development (indicating MRA), there was a significant interaction between muscle type and aging period ($P <0.001$). Across all age times, dark-cutting steaks exhibited higher MRA (reduced early MMb formation; $P<0.001$) than normal pH steaks. D 0 aging time demonstrated higher MRA than other age periods for normal pH and dark-cutting beef. There was no significant ($P = 0.14$) difference in MRA for dark-cutting between d 42 and 62 and beef with a normal pH.
Gao et al. (2013)	MRA decreased in three different muscle groups during storage. During storage, the MRA of ST dropped constantly ($P<0.05$). MRA of LD, on the other hand, increased from day 2 to day 3. An increase in ST MRA was also noticed from day 3 to day 4. Following the ranking LD>ST>PM, the MRA of three separate muscles significantly differed over most of the storage periods ($P<0.05$).

Oxygen and MMb reducing activity

There is disagreement on how oxygen affects MMb-reducing activity. The decrease in metmyoglobin was an anaerobic occurrence proposed by Watts et al. (1966). Subsequent studies discovered that, in contrast to aerobic circumstances, MMb reduction was higher in anaerobic environments (Al-Shaibani et al., 1977; O'Keeffe and Hood, 1982; Shimizu and Matsuura, 1968). Zimmerman and Snyder (1969) found that MRA was very responsive to oxygen. Ledward (1970) observed that while autoxidation peaked at low p O_2 (1-4 torr), MRA peaked in anoxia and claimed that at low oxygen partial pressures, the rate of myoglobin autoxidation was higher than the rate of reduction. Later, Ledward (1985) reported that the MMb reductase did not require oxygen. However, Echevarne et al. (1990) reported no appreciable distinctions between anaerobic and aerobic MMb-reducing activities for four distinct beef muscle fractions. Hagler et al. (1979) also agreed earlier that under both anaerobic and aerobic circumstances, the quantified rates of metmyoglobin reduction using pure enzymes were the same.

Lanier et al. (1978) reported a decrease in metmyoglobin in beef extracts, slurries, and ground beef stored in CO–air, nitrogen, or air mixes. They discovered that both anaerobic and aerobic circumstances resulted in a notable decrease in MMb. Mikkelsen et al. (1999) reported that while MMb reduction mechanisms were less impacted by oxygen pressure, the oxygen-depleting enzyme system in meat surfaces was pressure-sensitive.

Table 4. Summary of research evaluating Oxygen affecting the MMb reducing activity

Publications	Results/Conclusions
Mikkelsen and Skibsted (1992)	It is not possible to explain the observed quick oxygen exhaustion and suggested alternative electron-transfer reactions only by the rise in the MMb reduction rate and subsequent oxygenation.
Sammel et al. (2002)	Investigators looked at the correlations between six assays for metmyoglobin (MMb) decreasing ability to color stability and the chemical distinctions between beef semimembranosus (SM) muscle after 5 or 14 days of storage. Regardless of the time after death, the ISM exhibited lower ($p < 0.05$) color stability than the OSM, and both muscle sections showed greater color stability after 5 days as opposed to 14 days. The assay that showed the strongest correlation with visual color ratings ($r = -0.58$) and MMb accumulation ($r = -0.61$) in the SM was aerobic decreasing ability. The ISM consumed less oxygen than the OSM, which is partially explained by lower NAD concentrations and oxygen consumption rates ($p < 0.05$).

Ions and Chemicals and MMb reducing activity

After papain therapy, MRA rose, and this was explained by the cell network's proteolysis, which may have released the enzyme and boosted its capacity for activity (Hutchins et al., 1967). Metmyoglobin reduction was prevented by amytal, a barbiturate that prevents pyridine nucleotide oxidation and reduction (Watts et al., 1966). This discovery suggested that nucleotides play a part in the reduction reaction; rotenone and antimycin A were found to prevent MMb reduction completely (Watts et al., 1966). elevated oxidation of pigment in samples treated with rotenone and amytal reported by Govindarajan et al. (1977).

Zimmerman and Snyder (1969) reported that MRA was suppressed in the samples by the addition of malonic acid, which blocks the use of oxygen by blocking succinic dehydrogenase. The authors came to the conclusion that malonic acid reduced the amount of oxygen used and raised the concentration in meat, both of which reduced MRA. These inhibitors function at various stages of the mitochondrial electron transport chain. Thus, it makes sense to propose that the MMb decrease may be facilitated by enzymes other than NADH cytochrome b5 reductase.

It was discovered that MRA was partially inhibited by dicoumarol, an anticoagulant and DT diaphorase inhibitor Saleh and Watts (1968). They also reported that Metmyoglobin reduction was sped up when NAD and NADH were added to minced meat; MRA was not improved by NADP addition alone. The authors came to the conclusion that the rate at which metmyoglobin reduces is determined by the pace at which pyridine nucleotide reduces.

(Kendrick and Watts, 1969) reported that Nicotinamide delayed the loss of MRA in meat but not nicotinic acid, probably because it shielded NAD from nucleosidase (NAD glycohydrolase, E. C. 3.2.2.5). Additionally, they examined substrates connected to the NAD-dehydrogenase system (α-glycerophosphate, malate, and glutamate) and intermediates of the glycolytic pathway (glyceral-dehyde-3-phosphate and fructose-1, 6-diphosphate) in meat and discovered that these substances improved MMb reduction. The scientists found that the rate of NAD reduction regulated the total rate of metmyoglobin decrease. Their results suggested that these chemicals may be used to deliver electrons directly to NAD and subsequently indirectly to MMb.

It has been reported that pure NADH-cytochrome b5 reductase is totally inhibited by flavin analogs (acrinol and proflavine) at concentrations of 1 mM (Yubisui and Takeshita, 1980).

Stewart et al. (1965) reported that in beef muscle, 5% salt chloride totally suppressed the enzymatic lowering activity. Al-Shaibani et al. (1977) reported that the activity in tuna meat was 17% lower with 5% NaCl. Al-Shaibani et al. (1977) reported that Cl^- inhibited bluefin tuna's pure metmyoglobin reductase and was activated by Mn^{2+}, Fe^{3+}, and I^-.

Tamura et al. (1988) studied the impacts of bivalent cations on NADPH- and NADH-mediated cytochrome b5 and c reductases. The results showed that $MgCl_2$ or $CaCl_2$ strongly inhibited NADPH-cytochrome b5 reductase (requiring concentrations of 8 and 18 mM, respectively) while $CaCl_2$ or $MgCl_2$ activated NADPH-cytochrome c reductase (5 mM $CaCl_2$ produced a 24-fold increase in activity compared to the control, and the magnitude of activation by $CaCl_2$ was 2-3 times higher than $MgCl_2$).

It was discovered that EDTA increased MMb enzymatic reduction rate by a factor of 2 (Mikkelsen and Skibsted, 1992), whereas, chelator treatment improved activity in porcine longissimus dorsi extracts by 6% (Mikkelsen et al., 1999).

Arihara et al. (1996) examined the connection between rat myocyte MMb decrease and the glycolytic pathway. They discovered that whereas malonic acid, an inhibitor of the citric acid cycle, did not reduce MMb reduction, 2-deoxy-D-glucose, an inhibitor of the glycolytic pathway, did. These findings showed that the enzymatic decrease of MMb could involve the glycolytic pathway.

Chiou et al. (2001) examined how different reductants, including glutathione, cysteine, dithiothreitol (DTT), and β-mercaptoethanol (β-Me), affected the activity of MMb reductase in tuna muscle during storage at 4℃. They discovered that during storage, MMb reductase activity was maintained by 0.1 mM β-Me or DTT, 0.5 mM glutathione, and 5 mM cysteine. They proposed that the oxidation of the enzyme's sulfhydryl groups may be the cause of the activity deficits that are commonly seen during storage. Hutchison et al. (2010) reported that after adding CaL, NaL, or KL, lactate oxidation at postmortem muscle pH produces reducing equivalents, which should improve MRA and color stability.

Availability of nucleotides and MMb reducing activity

Watts et al. (1966) showed that increasing NAD in beef results in increased MMb-reducing activity. The lowering of MMb is not the only significant biochemical activity that the nucleotides may play a role in; they may also play a role in other critical biochemical processes that may affect the overall stability of fresh meat color. For example, Atkinson et al. (1969) found that during post-mortem storage, there was a sharp decline in both the rate of meat oxygen consumption and the concentration of

total NAD (oxidized and reduced). They also said that adding NADH resulted in an increase in oxygen consumption. In another study, Atkinson and Follett (1973) found that lower NAD levels were associated with lower muscular absorption of oxygen in lamb, pig, and beef muscles. No conclusions were drawn about NAD content despite the fact that they showed a logarithmic relationship between oxygen absorption and color stability in semimembranosus muscles from the three species. Compared to color-stable muscles like LD, color-unstable muscles like the *diaphragma medialis* oxidize NADH at a greater rate Echevarne et al. (1990). Therefore, some writers believe that the rate-limiting factor is NADH availability rather than the total quantity of MMb-reducing enzyme available (Echevarne et al., 1990). Considering the relationship between O_2 intake and fading of color, Atkinson and Follett (1973) recommended that red meat color be successfully preserved by any therapy that reduces O_2 absorption and/or sustains endogenous NAD levels. For example, Niacin (nicotinamide), for instance, is useful in preserving the color of meat (Naruse et al., 1998) due to the fact that it inhibits NAD nucleosidase (Schwimmer, 1981). Reduced reducing activity was seen when NAD glycohydrolase, also referred to as NAD nucleosidase (EC. 3.2.2.5), was added to crude hemolysate preparations or purified methemoglobin reductase enzyme (Scarrà et al., 1974).

Several researchers are interested in the possibility that NADH regeneration could aid in MMb decrease. However, there is not much experimental evidence to support this theory. Giddings and Hultin (1974) suggested that reversing electron transit may offer a way to regenerate NADH. Electron transport from succinate or cytochrome c to NAD^+ can regenerate NADH, and this process can be reversed when the energy potential is greater than the equilibrium potential, which is defined as the point at which there is no net movement of electrons in either direction (Pfaff and Klingenberg, 1968). They also indicated that reversed electron transfer from succinate has the potential to reach exterior acceptors in addition to reducing intramitochondrial NAD^+. This is especially true for sub-mitochondrial particles, which are devoid of a functional permeability barrier. Giddings and Hultin (1974) proposed that the whole electron transport chain would be involved in the reversal under anaerobic settings, whereas just a partial involvement was indicated in aerobic situations.

It is possible for several cytoplasmic dehydrogenases to produce NADH when given the right substrates (Bodwell et al., 1965). Pong et al. (2000b) reported that NADPH levels began at higher levels than NADH and only reduced during storage; in contrast, elevated NADH levels were present in tuna muscle during the first three days of storage, followed by a sharp decline. Their hypothesis was that the observed alterations in NADH and NADPH were due to NADPH recycling and NADH regeneration, which took place exclusively within the initial 72 hours of storage via specific enzymatic systems. Additionally, they showed that the cells' redox pool would be maintained by NAD (P)H recycling if there was a steady supply of electron donors (by either NADH or NADPH).

Table 5. Summary of research evaluating Nucleotides affecting the MMb reducing activity

Publications	Results/Conclusions
Sammel et al. (2002)	In semimembranosus muscle, NAD and NADH concentrations varied depending on the location (internal vs. exterior). The concentrations of NAD, NADH, and OCR were all higher in the muscle's outer layer. The location-dependent variations were ascribed to variations in relative chilling, meaning that the outer part would cool more quickly and would not cause rapid metabolite depletion. During vacuum storage, NADH concentrations dropped. In beef semimembranosus, neither hot boning nor ES changed the concentrations of NAD and NADH.
Ramanathan et al. (2010)	When lactate-LDH-NAD was combined with isolated mitochondria, the result was an increase in oxygen consumption and a decrease in metmyoglobin when compared to either control (lactate-free) or added lactate mitochondria at pH 5.6 and 7.4 ($p < 0.05$). Metmyoglobin reduction and oxygen consumption were both reduced ($p < 0.05$) when mitochondrial and LDH inhibitors were added to lactate-LDH-NAD. For both enzymatic (NADH-dependent metmyoglobin reductase) and non-enzymatic (via the electron transport chain) metmyoglobin reduction, NADH, which is produced from lactate-LDH-NAD, can be utilized.
Kennett et al. (2005)	In erythrocytes from humans, cattle, dogs, horses, grey kangaroos, pigs, and sheep, the impact of extracellular NADH on the rate of decrease of nitrite-induced methemoglobin was examined. The rate of methemoglobin reduction in erythrocytes from humans, dogs, pigs, and kangaroos was shown to be enhanced by extracellular NADH. However, it was found to have virtually little effect on erythrocytes from sheep, cattle, or horses.
Kim et al. (2009)	Different rates of NADH replenishment via various LDH isozymes control the variation in color stability of physiologically distinct muscles.
Rodríguez et al. (2011)	Through the interplay of the lactate-NAD^+-enzyme coupling, the addition of L-lactate can cause a decrease in metmyoglobin.
Ramanathan and Mancini (2010)	Pyruvate can lower metmyoglobin and improve mitochondria's capacity to take in oxygen.
Elroy et al. (2015)	Materials like quinone, cytochrome c, or methylene blue can transport electrons from NADH to metmyoglobin during non-enzymatic metmyoglobin reduction.

Lipid peroxidation and MMb reducing activity

Hutchins et al. (1967) reported that metmyoglobin and lipid oxidation have a strong positive correlation, while metmyoglobin and MRA have a significant negative correlation. Although they hypothesized that lipid antioxidants would have an impact on MRA, they did not report any direct correlation between lipid peroxidation and MRA. Greene (1969) reported that Antioxidants added to fresh red meat delayed the formation of metmyoglobin and prevented lipid oxidation. In aerobic packing, they found that samples with high MRA enzymatic activity browned more quickly than less active samples and those enzymatically active samples treated with antioxidants browned more slowly than similarly treated enzymatically less active samples. According to their theory, antioxidants shielded enzyme-based MMb reducing systems from lipid oxidation products, thereby preserving the red color of fresh meat. Giddings and Hultin (1974) argued that metabolically active meat would have a higher degree of lipid oxidation-related hemo pigment oxidation and is more vulnerable to oxygen reaching exposed surfaces. Given that lipid

peroxidation (which raises the MMb%) and MMb reduction (which lowers the MMb%) are two of the several factors that affect the net quantity of metmyoglobin generated in meat, it stands to reason that the presence of antioxidants could provide protection against the products of lipid peroxidation. Interestingly, Yang and Cederbaum (1995) reported that the enzymatic MMb reduction system (cytochrome b5 reductase and cytochrome b5) has been linked to a catalytic involvement in lipid oxidation. For example, it has been demonstrated that the lipid oxidation rate is increased when microsomes containing pure cytochrome b5 are added. In the presence of NADH, partially purified cytochrome b5 reductase and cytochrome b5 from beef liver enhanced lipid peroxidation in frozen beef patties (Mikkelsen and Skibsted, 1992).

An earlier study by Hirokata et al. (1978) reported that because antibodies against NADH-cytochrome b5 and cytochrome b5 reductase prevented NADH-dependent lipid peroxidation, electrons from NADH were provided to the lipid peroxidation reaction via these proteins. In a beef system, it was discovered that potassium ferrocyanide, a chemical mediator utilized in MMb reduction tests, accelerated lipid peroxidation Hansen et al. (1996). They reported that ferrocyanide could induce MMb reduction through a ferricyanide reductase enzyme, which would then oxidize ferrous Mb to MMb through both oxidative and reductive chemical pathways. These would not occur in the same time order. This might be comparable to the cytochrome b5 activity documented by Yang and Cederbaum (1995).

O'Grady et al. (2001) reported that for every 1 lg/g tissue increase in muscle vitamin E, there was an 8.5% rise in MMb reductase activity. Contrary to their findings, they hypothesized that either a feedback mechanism existed whereby low vitamin E levels caused MMb reductase levels to rise or that vitamin E partially produced its color-stabilizing impact by increasing MMb reductase activity. Lynch et al. (1999) reported that in vitro MMb reduction mediated by cytochrome b5 was inhibited by the presence of aldehydic lipid products. This may indicate that vitamin E does not directly shield MMb reductase nevertheless, by shielding a cofactor (like cytochrome b5) that is required to lower activity suggested by O'Grady et al. (2001).

Table 6. Summary of research evaluating lipid peroxidation affecting the MMb reducing activity

Publications	Results/Conclusions
Lynch and Faustman (2000)	It has been discovered that lipid oxidation, in particular the existence of aldehyde lipid oxidation products, greatly affects metmyoglobin's reducing activity (MMb)
Egelandsdal et al. (2010)	A summary After thorough washing, the lipid and protein composition of cod muscle was examined for 47 hours at pH 5.6 and 2°C with and without horse metmyoglobin (MMb). MMb markedly elevated the production of TBARS and lipid peroxides. The addition of MMb slowed the enzymatic release of soluble proteins and protein fragments, such as collagen fragments, and decreased the solubility of proteins, such as myosin heavy chain and its fragments.

Exercise and diet and MMb reducing activity

Exercise had no effect on NADH-erythrocyte MetHb reductase, whereas rats that underwent the most intense treadmill exercise showed a 25% increase in NADH-MMb reductase activity in the soleus muscle alone Hagler et al. (1980). While diet had no effect on NADH-erythrocyte MetHb reductase, it did produce a 35% reduction in the muscle's NADH-MMb reductase activity. Diets lacking in iron caused a 35% decrease in Hb content, and skeletal samples had 20–37% less Mb than control samples. However, no significant change was seen in the heart Hagler et al. (1981). A recent study by Gao et al. (2014) found that daily grazing over an extended period of time combined with a diet high in herbs helped to sustain reduced oxygen consumption, increased metmyoglobin-reducing activity, and decreased metmyoglobin buildup.

Conclusions

It has been observed that a wide variety of enzymatic and non-enzymatic systems can reduce MMb. The presence of NADH and an electron transfer mediator is required for MRA. Intrinsic and extrinsic factors like pH, temperature, oxygen, storage time, ions and chemicals, availability of nucleotides, lipid peroxidation, exercise, and diet were involved in MRA and meat color stability. Future research can be on the application of artificial intelligence and machine learning to forecast color-stable muscles and/or employed in the meat processing production line to enhance the value and reduce waste of nutritionally dense meat.

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