SYMPOSIUM: METAL-CATALYZED LIPID OXIDATION

presented at the ISF-AOCS World Congress, Chicago, Illinois

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Oxidations Involving the Heme Complex in Raw Meat[1]

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Abstract

In raw meat systems one is concerned with both lipid and pigment oxidations. Heme pigments catalyze oxidation of tissue lipids causing a stale or rancid odor and flavor. Free radicals from lipid oxidation can oxidize and decompose the red ferrous hemes. This results in the brown colored meat commonly rejected by the consumer. This paper reports three approaches taken to study means of reducing these nonmicrobial oxidative changes: (1) enzymatic reduction of metmyoglobin to maintain ferrous pigments; (2) inhibition of lipid and pigment oxidation and decomposition by means of an antioxidant and reducing agent; (3) the use of model systems to study the kinetics of lipid and heme oxidations. While a biological metmyoglobin reducing system has been found to exist in post mortem muscles, its practical significance in relation to retention of meat color and odor is not fully understood. Anaerobic conditions are usually necessary to achieve complete reduction. The common phenolic antioxidants, butylated hydroxyanisole (BHA) and propyl gallate, along with ascorbic acid will protect meat color and odor for up to eight days according to both chemical analyses and sensory evaluations. Such meat can be packaged aerobically. Surface pigments will remain as the bright red oxymyoglobin familiar to the consumer rather than the purple reduced myoglobin of anaerobically packaged meat.

The peroxide-heme ratio may be an important factor in determining rate of oxidation of both pigment and lipid. Maintenance of a high proportion of heme to lipid appears to prevent catalysis of lipid oxidation. The importance of this in relation to actual storage life of meat has not been explored.

[1] One of 28 papers presented at the Symposium, "Metal-Catalyzed Lipid Oxidation," ISF-AOCS World Congress, Chicago, September 1970.

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In raw meat systems one is concerned with both lipid and pigment oxidations. Heme pigments catalyze oxidation of the tissue lipids causing a stale or rancid odor. The brown ferric pigments are believed to be the more active catalysts (1,2). Free radicals from lipid oxidation can, in turn, oxidize and decompose the red ferrous pigments (3). This results in the brown colored meat commonly rejected by the consumer. These nonmicrobial oxidative changes result in economic loss to the retailer and pose a limitation on the use of central packaging by the meat packer. This paper reports three approaches which have been taken to study means of retarding these reactions: (1) investigations on the enzymatic reduction of metmyoglobin (MetMb) to maintain ferrous pigments and prevent lipid oxidation catalysis; (2) addition of an antioxidant and a reducing agent to the meat to inhibit lipid and pigment oxidations; (3) use of model systems to study the kinetics of lipid and heme oxidations.

The rationale behind the first approach is based on the idea that the amount of MetMb (brown ferric pigment) that is formed in a raw meat sample during storage is a result of both autoxidation of the ferrous pigment and enzymatic reduction of the ferric pigment. Walters and Taylor (4) first demonstrated

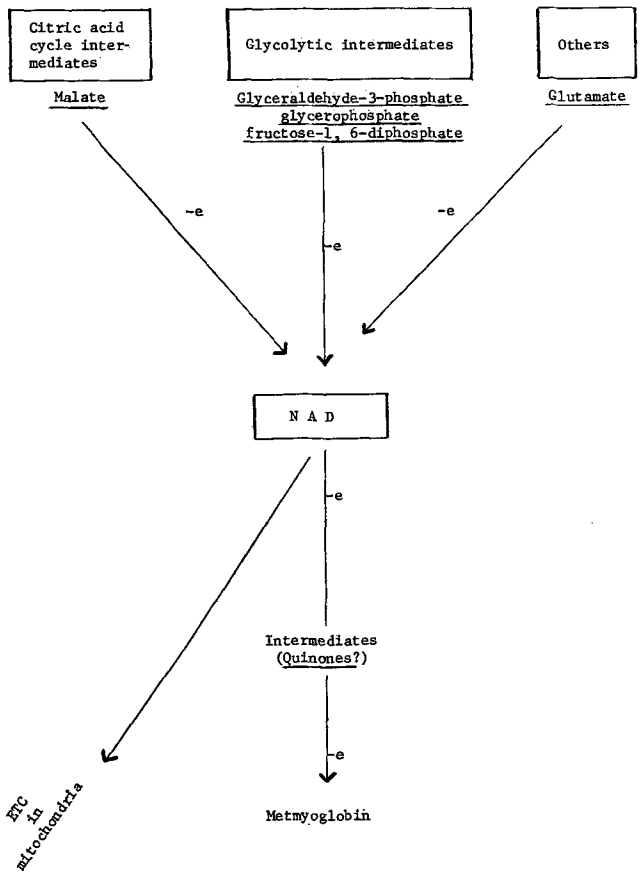

FIG. 1. Hypothetical scheme for the role of substrates and intermediates in MetMb reduction in meat. *Underscored* = compounds tested which demonstrated activity (8).

that reduction of MetMb was enzymatic in nature. This was further substantiated by Stewart et al. (5). The latter workers employed reflectance spectrophotometry to study MetMb reduction in raw ground beef. This method measures the absorption spectra of pigments in an intact meat sample. Results are expressed in terms of percentage MetMb (6).

Watts et al. (7) demonstrated that this reduction is mediated through nicotinamide adenine dinucleotide (NAD) and that anaerobic conditions are necessary to assure complete reduction. To determine the significance of this reaction in relation to retention of meat color, Saleh and Watts (8) attempted to locate the specific compounds responsible for reduction of MetMb. They measured the reducing activity produced by a number of substrates and intermediates which might be expected to act through NAD to reduce MetMb. This was done by adding the various compounds plus NAD to ground beef which had been treated with $K_3Fe(CN)_6$, to oxidize the pigments to 100% MetMb and measuring per cent of reduction (decrease in per cent MetMb) within a given time limit. Figure 1 presents a schematic drawing of pathways which could be expected to function in the nonliving animal along with the various compounds tested which demonstrated reducing activity. It can be seen that a number of compounds were found to be capable of supplying electrons to NAD and MetMb. The study did not single out one specific enzyme system responsible for reduction. Therefore the term "MetMb reducing activity" (MRA) was adopted to represent this general type of reduction.

As to the practical significance of these findings:

TABLE I

Effect of Additives on Pigment and Lipid Oxidation in Refrigerated Raw Ground Beef Stored Eight Days (10)

Variable	Metmyoglobin, %	Color score[a]	Total pigment loss, %	TBA[b] number
Control	77	....	20.2	8.9
BHA[b]	38	3.9	13.6	0.3
AH_2[b]	28	4.4	14.3	2.7
BHA + AH_2	16	5.3[c]	7.3	0.3

[a] Score of 6 = very good color, 1 = very badly discolored.
[b] Abbreviations: BHA = butylated hydroxyanisole; AH_2 = ascorbic acid; TBA = thiobarbituric acid.
[c] Significant >BHA.

the amount of MRA has been found to vary greatly between carcasses and between muscles from the same carcass. While this is undoubtedly due to the varying amounts of the above compounds present, without knowledge of the specific system it would not be practical at this point to recommend any one compound as an additive. Of all the compounds tested only glutamate is inexpensive enough to consider as an additive. The amount of NAD present in meat may vary. With insufficient NAD, reduction will also be insufficient. The cost of NAD would prohibit its use as an additive. Meat packaged anaerobically is the purple color of reduced Mb rather than the familiar bright red of oxymyoglobin (MbO_2). Without reeducation of the consumer, this meat may be rejected also.

By employing the second approach, that of adding an antioxidant and a reducing agent, the meat can be wrapped in an oxygen permeable film allowing the pigment to oxygenate to the familiar bright red color. The common phenolic antioxidants, butylated hydroxyanisole (BHA) and propyl gallate (PG) have been found to retard lipid oxidation in raw ground beef (9). This treatment also retarded MetMb formation to a certain extent. When ascorbic acid (AH_2) was added along with BHA or PG, MetMb formation was inhibited to an even greater extent. This meat usually remained an acceptable color for up to eight days or longer (10). Table I presents typical data from this study. Additives were used in accepted amounts for similar type products, i.e., BHA, PG 0.01% AH_2 0.05% (11). Percentage of MetMb was measured by reflectance spectrophotometry according to Stewart et al. (6); total pigment by aqueous extraction, conversion to cyano pigments and absorbance read at 540 $m\mu$ (12); lipid oxidation by the 2-thiobarbituric acid (TBA) test for malonaldehyde (mg MA/1000 g meat (13)); color score by a trained panel. Control samples were usually not presented to the panel after storage to avoid bias from the extreme discoloration of these samples. The important points exemplified by this table are: (1) inhibiting lipid oxidation also inhibited MetMb formation to a great extent (>50% over control); (2) AH_2 as a reducing agent was able to retard MetMb formation but was not able to completely inhibit lipid oxidation as measured by the TBA test; (3) the combination of additives gave greater protection to both pigments and lipids as measured by per cent MetMb, TBA number and sensory color scores, than did either additive alone. Odor scores on both raw and cooked samples (not presented) also showed that rancid odor was inhibited; (4) total pigment loss (per cent decrease from initial amount present) was largely dependent upon amount of ferric heme. A high correlation was obtained between per cent MetMb and total pigment loss. This suggests that heme destruction proceeds through breakdown of the ferric

heme moiety.

From a practical standpoint the combination of antioxidant and reductant offered noticeable protection to stored raw meat as measured chemically and organoleptically. Meat samples treated with these additives were also presented to an untrained consumer-type panel for color judgment. Panel members were simply asked which samples they would purchase and which they would reject. Samples had been stored eight days at the times of testing. From 75–100% of the samples containing 30–40% MetMb were rated as "purchase" while 50–100% of the panel rated samples with >60% MetMb as "reject." The samples rated "purchase" were almost always the samples containing both antioxidant and AH_2. "Reject" samples contained either additive alone or no additive.

In all samples tested throughout this study there was seldom a sample, regardless of treatment, that contained no MetMb. These samples were packaged as thin hamburger patties to allow maximum exposure to oxygen. Zimmerman and Snyder (14) have shown that high oxygen tensions, although important for maintenance of MbO_2, are also destructive to MRA. AH_2 as a reductant probably exerts its effect by being preferentially oxidized rather than by functioning as a part of an oxidative chain as in the case of natural MRA. More MRA may have enabled the additive treated samples to remain at 0% MetMb. Sufficient MRA may make AH_2 unnecessary. In the study of Saleh and Watts (8) glutamate was actually found to be most effective when tested under aerobic conditions and without the addition of NAD. However when this meat was stored under aerobic conditions, even though MRA remained high, the amount of MetMb in the samples was not significantly lower than untreated controls and actual meat color was not improved.

The use of model systems is considered by many to be the more informative approach to gaining an understanding of the actual mechanisms responsible for these oxidative changes. Kendrick and Watts (15) employed model systems to study the relation of type and amount of heme catalyst to rate of lipid oxidation. An oxygen analyzer was used to measure oxygen uptake of emulsions of pure fatty acids and pure heme compounds. They observed, as had others previously, that smaller amounts of heme compounds were catalytic to lipid oxidation whereas larger amounts were inhibitory. While rapid destruction of heme occurred with catalytic proportions of heme to lipid, a stable red pigment was formed with inhibitory ratios of these compounds. Heme degradation products in various amounts were then added to linoleate, and oxidation rate was measured. These were found to act as antioxidants as well. These workers concluded that stable heme peroxide complexes or stable heme radicals, or both, may form during early stages of oxidation. Therefore oxidation of the ferric pigment must proceed beyond the initial stages before it can function as a lipid oxidation catalyst. Thus a larger amount of heme would keep the peroxide-heme ratio low enough to prevent pro-oxidant activity and therefore act as an antioxidant. As lipid peroxides build up and the peroxide-heme ratio is overbalanced, rapid destruction of both pigments and lipids takes place.

Speculations on the relation of these findings to the results obtained from the more applied studies discussed earlier would be premature. Conclusions that can be made at this point are: (1) a biological MetMb reducing system exists in post mortem muscles. The specific enzyme(s) and substrate(s) is not known, but a number of compounds that increase reducing activity have been determined. The practical significance of this activity in relation to preservation of meat color and flavor is not fully recognized at this point. Studies relating amount of MRA to actual storage life of meat samples are needed; (2) the addition of a phenolic antioxidant and ascorbic acid to raw meat helps to maintain an acceptable color and odor in these samples for up to eight days. Studies relating the effect of MRA on the effectiveness of these additives may result in a means to provide maximum protection against adverse chemical changes in stored raw meat; (3) a knowledge of the degree of heme degradation, the amount of lipid oxidation products and the relative ratios of heme-lipid present in meat samples at various stages during storage may provide further insight into the mechanisms responsible for color and flavor changes in raw meat.

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[Received January 25, 1971]