# STRATEGIES FOR IMPROVING FRESH MEAT COLOR

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## INTRODUCTION

The visual appearance of any food product is an important sensory property by which consumers judge quality (Cassens et al. 1987). This is especially true in fresh meat cuts where surface discoloration may be interpreted as an indication of unwholesomeness.

The color of meat is primarily due to the heme-containing protein, myoglobin. The heme group contains a central iron atom which can form six bonds. Four of these bonds anchor the iron into the heme protoporphyrin molecule while the fifth bond attaches the heme to the apoprotein. The chemical group which is bound at the sixth site and the redox state of the heme iron determine meat color (Fig. 1). Deoxymyoglobin lacks a sixth

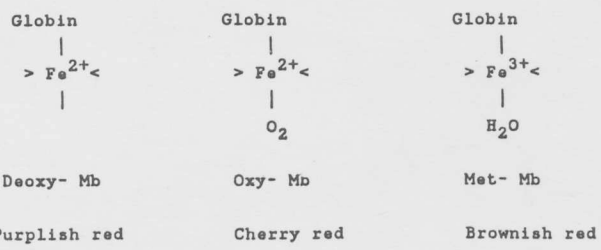


**Figure 1. The color of meat and the associated forms of myoglobin.**

ligand and is responsible for the purplish-red appearance of freshly-cut meat surfaces. If the meat is exposed to air, oxygen will bind as the sixth ligand and form oxymyoglobin, the pigment responsible for typical cherry-red, "fresh beef" color. Over time, oxymyoglobin will oxidize to undesirable brownish metmyoglobin which has $H_2O$ bound at the sixth position. The redox state of the heme iron in both deoxy- and oxy- myoglobin is +2 while in metmyoglobin it is +3.

There are two basic strategies for maintaining the desirable oxymyoglobin form in fresh meat. The first of these is to prevent/delay oxidation and can be accomplished by a variety of methods including cold temperature storage, proper sanitation, selective use of lighting, control of $pO_2$, and use of antioxidants. The major research effort into meat color has concentrated on this approach.

The second approach, and one which has received less research focus, is the enhancement of reduction of metmyoglobin to deoxy- and/or oxymyoglobin. From a practical standpoint, the rationale is to favor reduction of metmyoglobin as it is formed. The rate of discoloration is related to both oxidation and reduction; in theory oxidative processes should become dominant as reductive processes decrease due to exhaustion of the reductant pool. The biochemical basis of this reductant reserve is not understood. Ideally, it would be best to minimize oxidation and maximize reduction of the myoglobin pigment for optimum meat color shelf-life.

The purpose of our work has been to investigate methods for effecting both of these strategies in order to develop procedures for the stabilization of fresh meat color.

## MATERIALS AND METHODS

Materials and methods used are varied and extensive and are described elsewhere (Faustman et al., 1988; Faustman et al., 1989 a, b, c).

## RESULTS/DISCUSSION

Enzymatic reduction of metmyoglobin (*in vitro*) was investigated using a partially purified metmyoglobin reductase from bovine cardiac muscle and either partially purified cytochrome $b_5$ or potassium ferrocyanide as reaction mediators. NADH was an absolute requirement for pigment reduction in these assays.

The results for assays with metmyoglobin reductase preparation are presented in Table 1. Initial rates of reduction were significantly greater ($P<0.001$) at 37.5°C versus 22°C for all pH/mediator conditions. For each of the two mediators,

metmyoglobin reduction was greatest at pH 6.3 with a decrease in initial velocities occurring at pH 7.0 and 7.3. This trend occurred at both 22°C and 37.5°C.

*Table 1—Initial velocities for metmyoglobin reduction at two temperatures*

| pH | Mediator | Initial Velocity (nmol/min) 22°C | 37.5°C |
|---|---|---|---|
| 6.3 | Cyt $b_5$ | 17.4[a] | 33.2[a] |
| | Ferrocyanide | 11.3[b] | 20.7[b] |
| 7.0 | Cyt $b_5$ | 9.9[bc] | 17.4[c] |
| | Ferrocyanide | 8.6[bc] | 14.7[d] |
| 7.3 | Cyt $b_5$ | 8.6[bc] | 13.7[d] |
| | Ferrocyanide | 7.6[c] | 14.3[d] |

[a-d]Mean values in the same column with different superscripts differ significantly ($P<0.05$).

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*Table 2—Initial velocities of metmyoglobin reduction at various assay conditions (pH 7.0)*

| MetMb reductase | Assay Condition Cyt $b_5$ | FKeCN | NADH | Initial Velocity (nmol/min) 22°C | 37.5°C |
|---|---|---|---|---|---|
| + | + | − | + | 9.9[a] | 17.4[a] |
| + | − | + | + | 8.6[a] | 14.7[b] |
| − | + | − | + | 10.2[a] | 20.2[c] |
| − | − | + | + | 0.0[b] | 0.0[d] |
| − | − | − | + | 0.6[b] | 0.3[d] |

[a-d]Mean values in the same column with different superscripts differ significantly ($P<0.05$).

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Surprisingly, the cytochrome $b_5$ preparation reduced metmyoglobin in the absence of the metmyoglobin reductase preparation. Results for these assays run at pH 7.0 are shown in Table 2. Potassium ferrocyanide did not demonstrate a similar ability to reduce metmyoglobin in the absence of reductase preparation. It appears that the crude cytochrome $b_5$ preparation from liver contains components which interact with cytochrome $b_5$ to promote metmyoglobin reduction. Non-enzymatic reduction by NADH at both temperatures was minimal and not significant ($P<0.05$).

When the mixtures were allowed to stand at 22°C, it was observed that assays with reductase and potassium ferrocyanide re-oxidized more quickly than those with reductase and cytochrome $b_5$ preparation or cytochrome $b_5$ preparation alone. Spectral evidence for the observed re-oxidation behaviors are given in Figs. 1 and 2. Both assay types reached a maximum of reduced pigment at 0.5 h. However, spectral behavior of the reductase/ferrocyanide assay demonstrated a rapid return to the oxidized, met-state at 2 h (Fig. 1) while the assay with cytochrome $b_5$ preparation alone

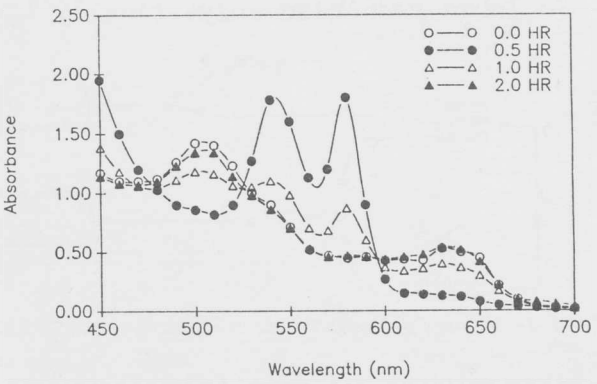


*Fig. 1—Absorbance spectrum of the cardiac metmyoglobin reductase assay with potassium ferrocyanide at 22°C displaying the time course of reduction and subsequent oxidation.*

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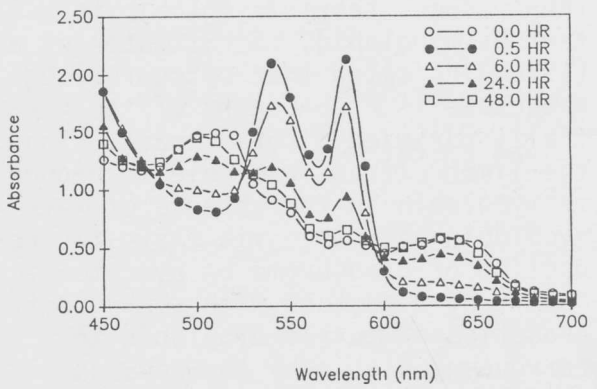


*Fig. 2—Absorbance spectrum of the assay with cytochrome $b_5$ preparation alone (i.e. without cardiac reductase) at 22°C displaying the time course of reduction and subsequent oxidation.*

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required 48 h to return to the time 0 spectra (Fig. 2). The cardiac reductase extract with cytochrome $b_5$ preparation as mediator showed a similar time course/spectrum relation to that in Fig. 2.

A schematic diagram of the metmyoglobin reduction pathways observed in these experiments is shown in Fig. 3. NADH is the ultimate source of reducing equivalents for all pathways. Potassium ferrocyanide is believed to facilitate metmyoglobin reduction in a manner similar to its role in methemoglobin reduction (Hegesh and Avron, 1967, Hagler et al., 1979). The ferrocyanide is not a simple electron carrier but binds to the heme protein in such a way as to make the heme

group accessible to the metmyoglobin reductase. Livingston et al. (1985) have demonstrated that purified metmyoglobin reductase enzymatically

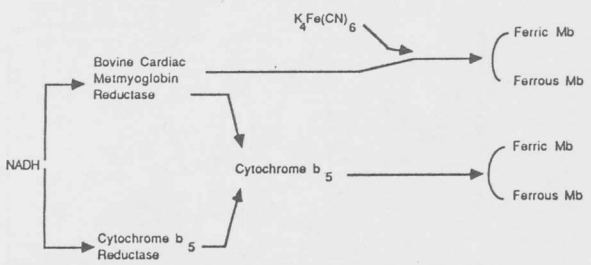


*Fig. 3—Proposed pathways for observed metmyoglobin reduction.*

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reduces cytochrome $b_5$, and subsequently, the reduced cytochrome $b_5$ then reduces ferric myoglobin to ferrous myoglobin. Strittmater et al. (1978) indicated that cytochrome $b_5$ reductase is a component of the partially purified liver extract. Thus, the liver extract was able to reduce metmyoglobin in the absence of metmyoglobin reductase via enzymatic reduction of cytochrome $b_5$ by cytochrome $b_5$ reductase with subsequent reduction of ferric myoglobin to ferrous myoglobin by the reduced cytochrome $b_5$. It may be possible that our bovine liver/cytochrome $b_5$ preparation contained metmyoglobin reductase. Work on the mechanism of reduction by Livingston et al. (1985) resulted in cardiac metmyoglobin reductase being classified as an NADH: cytochrome $b_5$ reductase. This classification also applies to liver microsomal cytochrome $b_5$ reductase, and so these two proteins perform similar functions albeit in different anatomical locations. Further work is necessary to define the exact proteins responsible for reduction by the liver preparation.

Vitamin E is a well characterized, lipid-soluble antioxidant located in cell membranes of biological systems. Holstein steer diets were supplemented with 370 I.U. $\alpha$-tocopheryl acetate/head/day for ca. 300 days; the color and lipid stability of meat subsequently obtained from these animals was compared with that of meat from non-supplemented, control animals.

Data describing differences in sirloin steak color stability between the two treatment group means utilizing Hunter 'a' value and chroma are represented in Figure 4A and 4B respectively. For both objective color measurements, values for sirloin

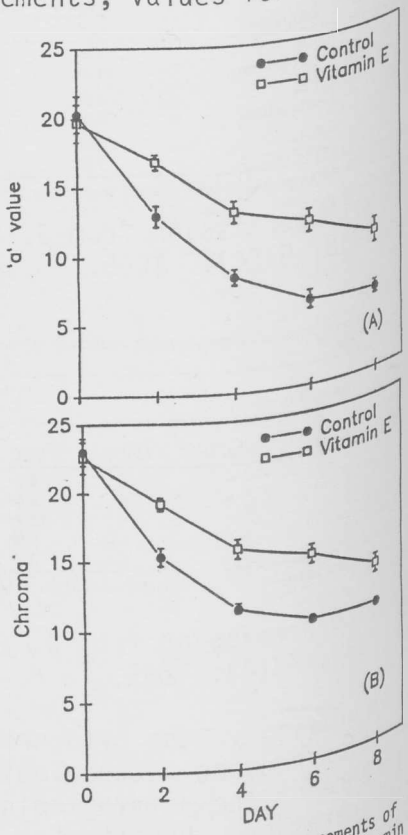


Fig. 4- Objective color measurements of sirloin steaks, from control and vitamin E-supplemented Holstein steers, during storage at 4°C. (A) 'a' value (redness) and (B) chroma (intensity). n=17 for each treatment group; std error bars are indicated. Taken with permission from J. Food Sci. 1989. In press.

steaks from control and vitamin E-supplemented Holstein steers were no different at day 0 ($P<0.05$). Following day 0, both groups tended to decrease in the two color parameter values during refrigerated storage. However, steaks from the vitamin E-supplemented animals were more resistant to this color change. The differences between the two treatment groups for both 'a' value and chroma were highly significant ($P<0.01$) at days 2, 4, 6, and 8 of 4°C storage. The chroma values for the control Holstein beef are similar to those of Taylor and MacDougall (1971) for beef stored in air. Mean L and 'b' values (data not shown) were greater ($P<0.05$) for the vitamin E-supplemented group

at days 0 and 2, and days 2, 4 and 6 of storage respectively.

The α-tocopherol, lipid, and moisture of the ground sirloin from control and vitamin E-supplemented Holstein steers are listed in Table 3. Tissue α-tocopherol levels of the supplemented animals were more than 2-fold greater than non-supplemented steers ($P<0.05$). There was no difference ($P>0.05$) between the 2 groups for lipid or moisture.

Data for metmyoglobin accumulation in ground sirloin patties is presented in Fig. 5. For d 0 and 2 ($P<0.05$), and d 2 and 4 ($P<0.10$), metmyoglobin was greater for control animals than for those supplemented with vitamin E. Fresh, ground sirloin from Holstein steers supplemented with vitamin E was also more resistant to lipid oxidation (Fig. 6). At d 2, 4 and 6 of 4°C storage, accumulation of TBA-reactive substances was greater in control steers ($P<0.05$). Additionally, TBA values for ground sirloin patties stored at -18°C were lower ($P<0.05$) following 1.5 and 3 mo storage for meat from supplemented versus control animals (Table 4).

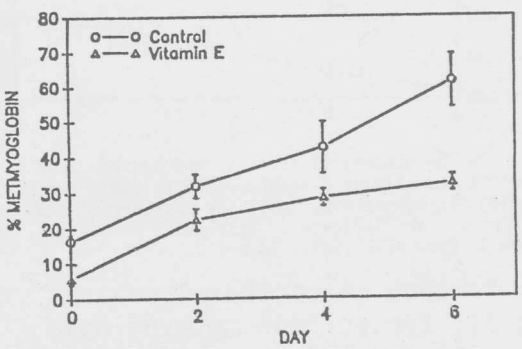


Fig. 5- Metmyoglobin accumulation during storage at 4°C for fresh ground sirloin patties from control and vitamin E-supplemented Holstein steers. n=11 for each group; std error bars are indicated. Taken with permission from J. Food Sci. 1989. In press.

Table 3. Alpha-tocopherol, moisture and ether-extractable lipid for ground sirloin muscle from control and vitamin E-supplemented Holstein steers.

| Treatment | α-tocopherol (mg/100g tissue) | % lipid | % moisture |
|---|---|---|---|
| Control | 0.16[a] (±0.07) | 5.9 (±3.1) | 76.1 (±2.4) |
| Vitamin E-Supplemented | 0.44[b] (±0.09) | 5.7 (±1.8) | 76.5 (±1.4) |

Mean values (± S.D.) in the same column with different superscripts differ ($P<0.05$). N=11 for each treatment group.
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Table 4. Mean TBA values for ground sirloin patties under frozen storage (-18°C), from control and vitamin E-supplemented Holstein steers.

| Treatment | Storage period (months) 0 | 1.5 | 3.0 |
|---|---|---|---|
| Control | 0.11 (±0.08) | 1.19[a] (±0.37) | 0.48[a] (±0.14) |
| Vitamin E-supplemented | 0.13 (±0.05) | 0.19[b] (±0.07) | 0.26[b] (±0.07) |

Mean values (± S.D.) in the same column with different superscripts differ ($P<0.05$). N=11 for each treatment group.
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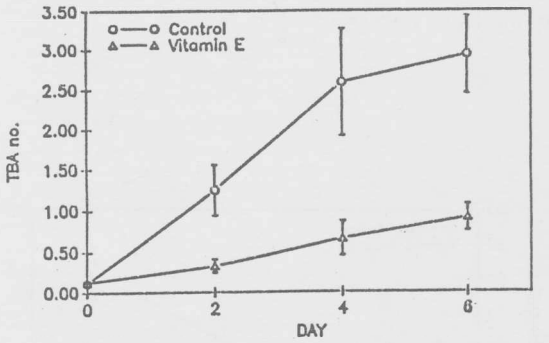


Fig. 6- TBA numbers of fresh ground sirloin from control and vitamin E-supplemented Holstein steers during storage at 4°C. n=11 for each group; std error bars are indicated. Taken with permission from J. Food Sci. 1989. In press.

Data on α-tocopherol content (Fig 7), the percent metmyoglobin (Fig. 7A) and TBA no. (Fig. 7B) of ground sirloin at 6 d storage are presented in Fig. 7. The correlation coefficients for control animals were -0.76 and -0.78 for Fig. 7A and 7B, respectively, and were significant ($P<0.05$). The correlation coefficients for vitamin E-supplemented animals were 0.16 and 0.14 for Fig. 7A and 7B, respectively, and were not significant ($P>0.05$). Alpha-tocopherol concentrations in excess of ca. 0.3 mg per 100 g meat did not appear to yield any added benefit for reducing metmyoglobin accumulation or TBA no. in ground sirloin. However, concentrations below ca. 0.3 mg α-tocopherol per 100 g meat were not sufficient to achieve minimal pigment and lipid oxidation. Thus, the data indicated that dietary vitamin E supplementation should be maintained at a level to obtain ca. 0.3 mg per 100 g meat.

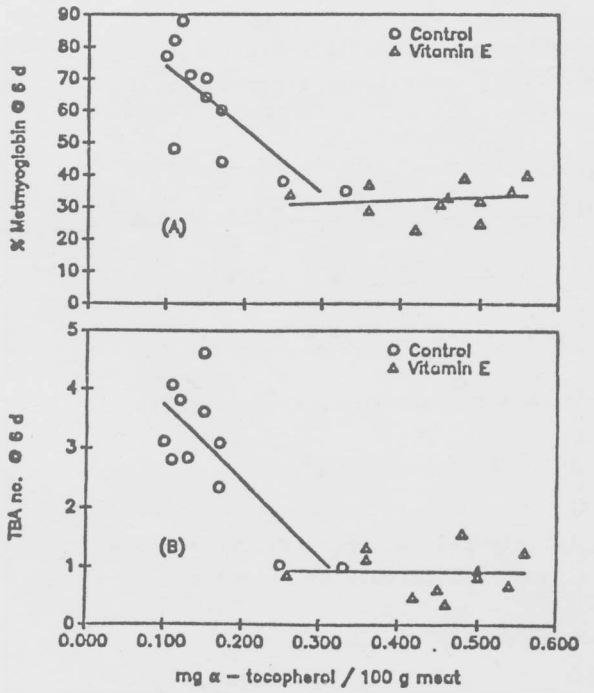


Fig. 7- Relationship between α-tocopherol content and, (A) percent metmyoglobin at 6 days and (B) TBA no. at 6 days in ground sirloin. First-order regression lines are indicated. Taken with permission from J. Food Sci. 1989. In press.

Our results indicate that vitamin E-supplementation of Holstein steers stabilizes the redness and color intensity of sirloin steaks and also delays pigment and lipid oxidation of ground beef obtained from these animals. Vitamin E quenches radicals derived from lipid oxidation and this is likely the mechanism by which pigment oxidation is delayed. This could be accomplished directly, indirectly via sparing of pigment reducing mechanisms, or both. Consistent research results for supporting the exogenous addition of vitamin E to meat products are lacking. Our results indicate a real advantage for supplementing Holstein steers with vitamin E to obtain ground beef with a greater shelf-life. We believe that this advantage is due to incorporation of vitamin E into the cell membranes where it has its physiological antioxidant effect. Addition of exogenous tocopherol to meat products does not ensure that vitamin E is properly positioned to perform its antioxidant role.

Ground beef homogenates inoculated with fluorescent *Pseudomonas* and/or *Brochothrix thermosphacta* and held at 4°C, were assayed for bacterial growth, pH, and metmyoglobin content during 10 d of storage. Time to reach maximum metmyoglobin was ca. 5 d for *Pseudomonas* and ca. 8 d for *B. thermosphacta*. Both types of bacteria demonstrated metmyoglobin reduction at approximately $10^8$ CFU/g. Frequency of sampling time was a critical factor in being able to accurately document peak oxidation and subsequent reduction. Data for the fluorescent *Pseudomonas* are

presented in Figure 8. Metmyoglobin reduction again occurred, and began at ca. 5.5 days. If homogenates

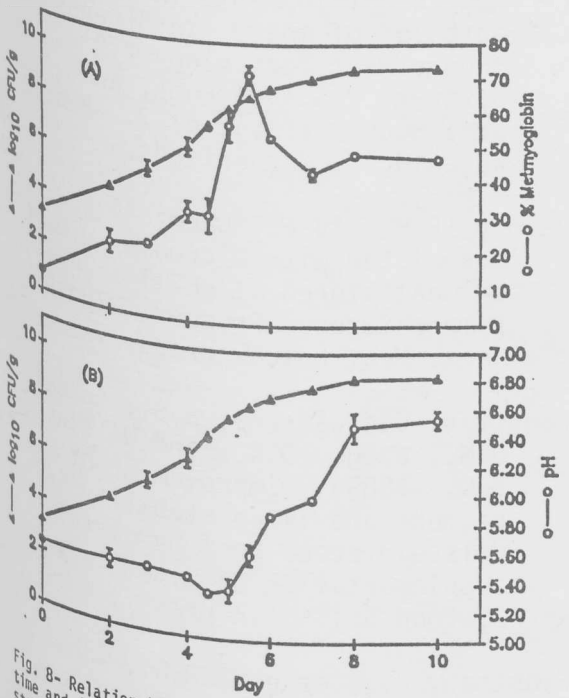


Fig. 8- Relationship between Pseudomonas population over time and (A) percent metmyoglobin, and (B) pH, during storage at 4°C.

had been assayed on alternate days (i.e. days 4 & 6), the recorded color reversion would have been less dramatic. Interestingly, at 5.5 days, the population of Pseudomonas was approximately $10^8$ CFU/g and the pH had begun to increase.

Filtered, cell-free supernatant from a fluorescent Pseudomonas culture grown in trypticase soy broth was effective in reducing metmyoglobin in an *in vitro* assay at 22°C (Fig. 9A). For purposes of comparison, spectral scans of metmyoglobin, oxymyoglobin, and deoxymyoglobin are presented in Fig. 9B. The spectral scans in Fig. 9A reveal that metmyoglobin was reduced and that assay #2 (90% filtrate) was more effective than assay #1 (50% filtrate) at effecting the reduction over the 3 hr time period. Control assays did not reduce metmyoglobin, indicating that TSB possessed no reductive capacity of its own. It is well established that the 3 forms of myoglobin are isobestic at 525 nm (Hunt, 1980) and this is demonstrated in Fig. 9B. Reaction of metmyoglobin with culture filtrate appeared to shift the isobestic point to ca. 515 nm. We do not have an explanation for this phenomenon.

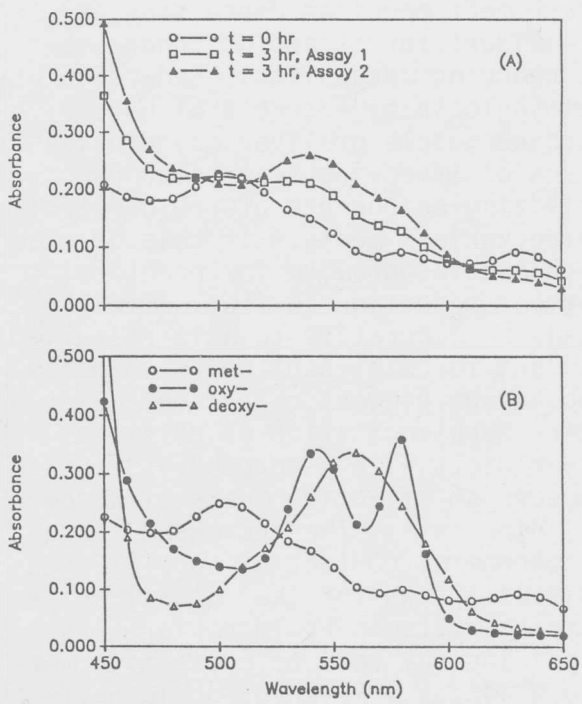


Fig. 9- Absorbance spectra for (A) 3 hr assays of metmyoglobin reduction, and (B) metmyoglobin, oxymyoglobin and deoxymyoglobin. Assays contained 0.025mM metmyoglobin in addition to 50% (#1) or 90% (#2) Pseudomonas culture filtrate.

It has generally been assumed that the desirability of color and the bacterial population of meat are inversely related. Our results support this observation in a meat homogenate system but only for low bacterial populations (ca. $10^4$ CFU/g). At very high populations (ca. $10^8$ CFU/g) of psychrotrophic bacteria, however, reduction of metmyoglobin may occur. For fluorescent pseudomonads, the reduction occurs concomitantly with a pH increase and the latter is likely a result of deamination of protein substrate by bacteria (Dainty, 1986). The mechanism by which this reduction occurs is unknown. Butler et al. (1953) attributed metmyoglobin reduction on beef steak surfaces to oxygen consumption by high numbers of bacteria; they maintained that this would be sufficient to provide reducing conditions. However, in our study, cell-free culture supernatant was capable of reducing metmyoglobin in vitro and this would counter the

latter argument.

## SUMMARY

Improving color stability of fresh meat requires depression of prooxidant forces and/or enhancement of reducing mechanisms. Reduction of metmyoglobin by extracts of bovine cardiac muscle or liver may provide a means of improving meat color by utilizing components of low commercial value variety meats. If this or other technologies provide for practical pigment reduction in meat, it will still be imperative to have an antioxidant force present so as to delay subsequent pigment oxidation. Dietary supplementation of Holstein steer diets with α-tocopheryl acetate appears an effective means of accomplishing this. The increased tissue α-tocopherol content of supplemented animals allows for the natural antioxidant, vitamin E, to perform its physiological role of protecting membrane lipids. Psychrotrophic bacteria were demonstrated to be prooxidant in low population numbers (ca. $10^4$ CFU/g) and proreductive at very high populations (ca. $10^8$ CFU/g). The mechanism by which metmyoglobin reduction occurred is unknown but the participation of viable intact cells can be eliminated. Results indicate that a threshold population of bacteria must be attained before culture filtrate may reduce metmyoglobin. The responsible agents are either bacterial metabolites elaborated during growth, or intracellular components freed during cell lysis. It is critical that bacterial quality be reported whenever new innovations for improving meat color are employed. Additionally, the relationship between meat discoloration and bacterial quality is complex and meat color is not necessarily a reliable indicator of bacterial load.

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