Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 93 (2005) 571–576
Food Chemistry
www.elsevier.com/locate/foodchem

Effects of reducing agents on premature browning in ground beef

H.A. Sepe, C. Faustman *, S. Lee, J. Tang, S.P. Suman, K.S. Venkitanarayanan

Department of Animal Science, University of Connecticut, Storrs, CT 06269-4040, USA

Received 16 December 2003; received in revised form 16 April 2004; accepted 16 April 2004

Abstract

This study was performed to investigate the effects of food-grade reducing agents on counteracting premature browning (PMB) and to determine the relative heat stability of different redox states of bovine myoglobin (Mb). Sodium erythorbate (SE), erythorbic acid (EA), sodium ascorbate (SA), ascorbic acid (AA) and ascorbyl palmitate (AP) were added to ground beef (15% fat) at a concentration of 2.3 mM; patties were prepared and stored at 4 °C and at −18 °C. Surface redness (a^* values), lipid oxidation and total reducing activity (TRA) were measured on raw beef, and a^* values recorded from cooked internal surfaces. Bovine Mb was purified from biceps femoris muscle and heat capacity was measured using differential scanning calorimetry. All reducing agents decreased lipid oxidation and increased TRA relative to controls ($p < 0.05$). In general, SE and SA were more effective at maintaining red color in cooked ground beef patties than other reducing agents ($p < 0.05$). The temperature at peak heat capacity, T_m, of metmyoglobin appeared lower than that of oxymyoglobin and deoxymyoglobin. Reducing agents can maintain Mb in the reduced state, and their addition to ground beef may be one method of preventing PMB.

Keywords: Premature browning; Myoglobin; Color; Ground beef; Reducing agents

1. Introduction

It was discovered at Kansas State University that myoglobin (Mb) in ground beef patties could denature at a temperature lower than expected, turning the meat brown in color before pasteurization at the USDA recommended temperature of 71 °C was achieved (Hague et al., 1994; USDA, 1997; Bigner-George & Berry, 2000). Lavelle, Hunt, and Kropf (1995) presented evidence for 'premature browning' (PMB) in a study in which ground beef patties cooked to only 55 °C exhibited doneness as judged by internal color; at 55 °C, pink color is normally expected in ground beef. The USDA conducted a study to determine the frequency of ground beef patties appearing done when cooked to temperatures up to the recommended 71 °C (Lyon, Berry, Soderberg, & Clinch, 2000). As the internal temperature of patties increased, meat color became more brown, juice color less pink, and the texture more cooked in appearance. However, at no cooked internal temperature did 100% of the patties appear done when evaluated on those three criteria. This suggests that internal color and appearance are not necessarily acceptable measures of doneness in ground beef patties. PMB has been estimated to occur in 47% of ground beef patties cooked in the home (Killinger, Hunt, & Campbell, 1998), and this could result in the consumption of under-cooked ground beef.

PMB appears related to the redox state of Mb in raw ground beef, prior to cooking (Hague et al., 1994). There are two reduced ferrous forms of Mb, cherry red oxymyoglobin (OxyMb) and purple red deoxymyoglobin (DeoxyMb), and one oxidized ferric form, metmyoglobin (MetMb). Hague et al. (1994) reported that ground beef patties containing predominantly MetMb or OxyMb had PMB occur more readily than patties containing

* Corresponding author. Tel.: +1 860 486 2413; fax: +1 860 486 4375.
E-mail address: Cameron.Faustman@uconn.edu (C. Faustman).

0308-8146/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2004.04.045

predominantly DeoxyMB. This was attributed to a PhD thesis report that MetMb and OxyMb are denatured at lower temperatures than DeoxyMb (Machlik, 1965). Warren (1994) initially reported that ground beef containing predominantly MetMb – but not DeoxyMb – was predisposed to PMB. The effects of Mb redox state on the internal cooked color of ground beef were subsequently extended and confirmed by Hunt, Sorheim, and Slinde (1995). It was determined that when cooked to 55 °C, only patties containing predominantly DeoxyMb retained a red, undercooked appearance. Patties made of OxyMb and MetMb appeared brown and done enough to consume, despite a maximal internal temperature of only 55 °C (Hunt et al., 1995).

Food grade reducing agents are ingredients that can maintain Mb in a reduced, ferrous state (Shivas et al., 1984). If they are able to prolong DeoxyMb presence in raw ground beef, then it appears logical that the occurrence of PMB could be subsequently decreased or eliminated in the cooked meat. Phillips, Mancini, Sun, Lynch, and Faustman (2001) reported that the addition of erythorbic acid to ground beef patties at 0.04% and 0.06% (w/w) concentrations resulted in prolonged red color in patties cooked to internal endpoint temperatures of 60, 66, 71, or 77 °C from the raw state. However, this was the only reducing agent tested and no work was done to test the effects of freezing on the effectiveness of reducing agents added to ground beef. The objective of this study was to compare the effects of different reducing agents and storage conditions (i.e. fresh and frozen) on PMB in cooked ground beef patties, and to determine the heat stability of different forms of bovine Mb.

2. Materials and methods

2.1. Patty preparation

Six 4.54-kg tubes of coarse ground beef (15% fat) were obtained locally. The reducing agents, sodium erythorbate (SE), erythorbic acid (EA), sodium ascorbate (SA) and ascorbic acid (AA) were each dissolved in 50 ml of distilled water; ascorbyl palmitate (AP) was dissolved in 50 ml of 100% ethanol. These solutions were added to individual batches of beef to obtain a final concentration of 2.3 mM. Controls (CT) contained an equivalent volume of water or ethanol used to deliver the reducing agents. Ground beef was mixed and fine ground through a 4.8-mm plate using a Biro Meat Grinder (Model 346, Biro, Marblehead, OH). Ground beef patties (112 g each, 10.2 cm diameter × 1.2 cm thick) were formed, wrapped in oxygen-permeable PVC film (E-Z Wrap Crystal Clear PVC Wrapping Film, Koch Supplies Inc., Kansas City, MO) and stored at 4 °C for 48 h, or frozen at −18 °C for 14 days.

2.2. Cooking

Patties were cooked individually to internal endpoint temperatures of 65, 71, and 77 °C on a George Foreman Lean Mean Grilling Machine (Stalton Inc., Columbia, MO) heated at 170–180 °C. Patties were placed on the grill, seared lightly, and turned initially at 2 min, and then every min thereafter until the desired endpoint temperature was reached. The internal temperature of patties was monitored continuously with an Atkins probe thermometer (AccuTuff 34032, Gainesville, FL) inserted horizontally into the geometric center of the patty. For a given internal temperature endpoint, five patties within each treatment were cooked.

2.3. Color analysis

Immediately prior to cooking, patties were removed from storage and the surface color (a^* values) was measured with a Minolta Colorimeter (Model CR-200b, Osaka, Japan). After reaching the appropriate internal temperature, patties were cooled for 3 min at room temperature, sliced in half horizontally, and allowed to cool for an additional 3 min prior to color measurement.

2.4. Total reducing activity

Total reducing activity (TRA) of raw beef was measured following storage (Lee, Cassens, & Fennema, 1981). For duplicate samples, two grams of meat sample were blended with 10 ml of 25 mM Piperazine-*n*, *n*-bis 2-ethane-sulfonic acid (PIPES) buffer using a homogenizer (Polytron Model PT 10/35, Switzerland), and 5 ml of homogenate transferred to a 10 ml volumetric flask. Two ml of 5 mM potassium ferricyanide was added to the homogenate and chilled at 2 °C for 1 h with occasional stirring. Ammonium sulfamate (0.1 ml of 0.5%) and lead acetate (0.2 ml of 0.5 M) were added and the sample held at room temperature for 5 min. Trichloroacetic acid (20%; 25 ml) was added to denature protein and the solution brought to 10 ml total volume. After filtration (Whatman No. 42; Whatman International Ltd., Maidstone, England) the absorbance of samples was measured at 420 nm using a Shimadzu UV-2101PC spectrophotometer (Shimadzu Inc., Columbia, MD). A solution of 1 mM potassium ferricyanide was prepared for use as the standard. Total reducing activity was expressed as the difference in absorbance between the sample filtrate and the standard.

2.5. Lipid oxidation

Lipid oxidation of raw beef was measured after storage using the thiobarbituric acid assay as reported by Yin, Faustman, Riesen, and Williams (1993). Briefly, 5 g samples were mixed with trichloroacetic acid, homog-

enized and filtered. One ml of aqueous filtrate was mixed with 1 ml of aqueous thiobarbituric acid and incubated at 25 °C for 20 h. The absorbance of samples was measured spectrophotometrically at 532 nm and reported as thiobarbituric acid-reactive substances (TBARS).

2.6. Myoglobin preparation

Bovine Mb was purified according to Faustman and Phillips (2001). OxyMb was prepared by sodium hydrosulfite-mediated reduction of Mb (Brown & Mebine, 1969) and MetMb was prepared by oxidation with potassium ferricyanide. Dialysis against pH 5.6 citrate buffer was performed to remove excess potassium ferricyanide or sodium hydrosulfite. DeoxyMb was formed through the use of a Warburg flask. A 10 ml Mb sample was added to the flask and sodium hydrosulfite was placed in one arm of the flask. The sample was flushed for 10 min with nitrogen gas. The Mb sample and the sodium hydrosulfite were then mixed by inversion of the capped flask and the contents flushed for 15 min with nitrogen before it was carefully transferred to dialysis tubing to prevent exposure to oxygen. Nitrogen flushing of the dialysis buffer continued during removal of residual sodium hydrosulfite. The concentration of different redox forms of Mb was determined based on the absorbance at 525 nm with an absorbance extinction coefficient of 7.6 mM^{-1} cm^{-1} and adjusted to 2.5 mg/ml using 50 mM citrate, pH 5.6 (Faustman & Phillips, 2001).

2.7. Differential scanning calorimetry

Differential scanning calorimetry was performed using an MCS microcalorimeter (MicroCal Incorporated, Northhampton, MA). Cells were heated from 55 to 85 °C at a rate of 1.5 °C/min for OxyMb and MetMb samples. For DeoxyMb, cells were heated from 60 to 85 °C at a rate of 1.5 °C/min. DeoxyMb is highly susceptible to oxidation to MetMb, or readily oxygenated to OxyMb. In order to decrease this kind of interference a higher starting temperature was used for DeoxyMb. A lower initial temperature was used for MetMb and OxyMb to obtain a smooth and flat baseline. A higher initial temperature was used for DeoxyMb because of its greater relative instability and the associated need to expedite the analysis before sample denaturation. Heat capacity data were collected and the temperature at peak heat capacity, T_m, was determined.

2.8. Statistical analysis

The data from this experiment were analyzed using the general linear model (GLM) procedure of SAS (SAS, 1985). Differences among the means for a^* color values, lipid oxidation, and total reducing activity were detected at the 5% level using the least significant difference (LSD) test.

3. Results and discussion

In order to evenly distribute the reducing agents throughout the meat, 50 ml water was used for delivery of SE, EA, SA, and AA. For AP, a water-insoluble compound, 50 ml ethanol (100%) was used for delivery. Preliminary work with both water and ethanol controls revealed no significant solvent-dependent differences for color, lipid oxidation, or total reducing activity (results not shown). In addition, there was no effect of added reducing agent on meat pH (results not shown).

The effects of reducing agent addition on total reducing activity and lipid oxidation in ground beef patties are presented in Tables 1 and 2, respectively. Reducing agent addition increased total reducing activity over that of controls, and this effect persisted through refrigerated and frozen storage (Table 1; $p < 0.05$). SE, EA, SA, and AA demonstrated greater total reducing activity than AP, and all treatments were greater than CT for storage at 4 °C ($p < 0.05$). For frozen samples, all treatments had greater total reducing activity than CT ($p < 0.05$). Lipid oxidation that occurred in both the refrigerated and frozen patties was lower for all reducing agent treatments when compared to controls ($p < 0.05$; Table 2).

Table 1
Total reducing activity of beef patties after refrigerated (4 °C, 48 h) and frozen (−18 °C, 14 days) storage

	Refrigerated	Frozen
Control	0.396 ± 0.011^{c}	0.398 ± 0.035^{c}
Sodium erythorbate	0.537 ± 0.041^{a}	0.610 ± 0.013^{a}
Erythorbic acid	0.541 ± 0.013^{a}	0.560 ± 0.004^{b}
Sodium ascorbate	0.526 ± 0.028^{a}	0.597 ± 0.006^{ab}
Ascorbic acid	0.553 ± 0.021^{a}	0.595 ± 0.012^{ab}
Ascorbyl palmitate	0.457 ± 0.011^{b}	0.606 ± 0.038^{ab}

Results are expressed as means ± SD.
Within each storage condition values with different superscripts are significantly different ($p < 0.05$).

Table 2
Thiobarbituric acid-reactive substances (TBARS) of beef patties after refrigerated (4 °C, 48 h) and frozen (−18 °C, 14 days) storage

	Refrigerated	Frozen
Control	0.100 ± 0.006^{a}	0.055 ± 0.011^{a}
Sodium erythorbate	0.032 ± 0.002^{b}	0.028 ± 0.000^{b}
Erythorbic acid	0.032 ± 0.002^{b}	0.030 ± 0.002^{b}
Sodium ascorbate	0.028 ± 0.000^{b}	0.029 ± 0.001^{b}
Ascorbic acid	0.027 ± 0.001^{b}	0.029 ± 0.003^{b}
Ascorbyl palmitate	0.031 ± 0.003^{b}	0.030 ± 0.000^{b}

Results are expressed as means ± SD.
Within each storage condition values with different superscripts are significantly different ($p < 0.05$).

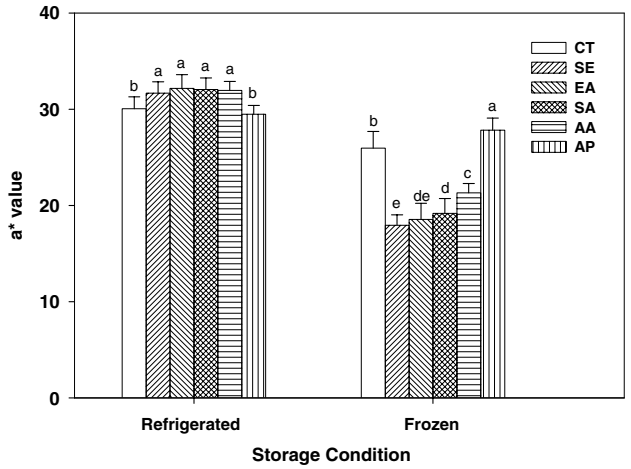

Fig. 1. Surface a^* values (redness) of beef patties after refrigerated (4 °C, 48 h) and frozen (−18 °C, 14 days) storage. Standard deviation bars are indicated; bars with different letters are different within each storage condition ($p < 0.05$). CT, control; SE, sodium erythorbate; EA, erythorbic acid; SA, sodium ascorbate; AA, ascorbic acid; AP, ascorbyl palmitate.

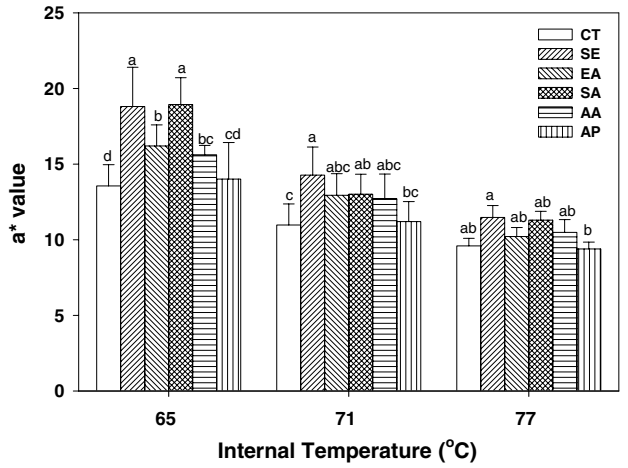

Fig. 2. Internal a^* values (redness) of beef patties stored at 4 °C for 48 h and cooked to different internal endpoint temperatures. Standard deviation bars are indicated; bars with different letters are different within each storage condition ($p < 0.05$). CT, control; SE, sodium erythorbate; EA, erythorbic acid; SA, sodium ascorbate; AA, ascorbic acid; AP, ascorbyl palmitate.

Refrigerated samples that contained SE, EA, SA, and AA had higher a^* values than control and AP treatments ($p < 0.05$). There were no differences between SE, EA, SA and AA, or between CT and AP ($p > 0.05$). In frozen samples, a^* values for patties containing SE, EA, SA and AA were lower than those for CT and AP ($p < 0.05$; Fig. 1) indicating that they were less red in color. However, this was not due to increased brown color in patties, but rather to increased purple color that we observed during the course of the experiment. The purplish color was attributed to increased levels of DeoxyMb caused by the reducing agent treatments. It is not clear why this phenomenon occurred in frozen but not fresh patties, especially given that the packaging was the same for meat in both storage conditions.

Ground beef patties were cooked to internal temperatures of 65, 71, or 77 °C. Refrigerated patties containing SE, EA, SA, and AA had higher internal a^* values compared with controls when cooked to 65 °C ($p < 0.05$; Fig. 2). Only patties containing SE or SA had higher internal a^* values when cooked to 71 °C ($p < 0.05$; Fig. 2). There was no difference for a^* values between treatment and control patties when they were cooked to 77 °C ($p > 0.05$).

Similar to results for patties stored at 4 °C, frozen patties containing SE, EA, and SA had higher internal a^* values compared to controls when cooked to 65 or 71 °C ($p < 0.05$; Fig. 3). However, for patties cooked to 77 °C, only SE and SA resulted in higher internal a^* values compared to controls ($p < 0.05$; Fig. 3). Ground beef patties containing SE always had the greatest internal a^* values after cooking ($p < 0.05$). It is possible that the observed purplish color and hypothesized increase in DeoxyMb in frozen patties containing SE, EA, SA, and AA resulted in cooked patties containing more OxyMb. This would account for the increase in cooked red color for these treatments.

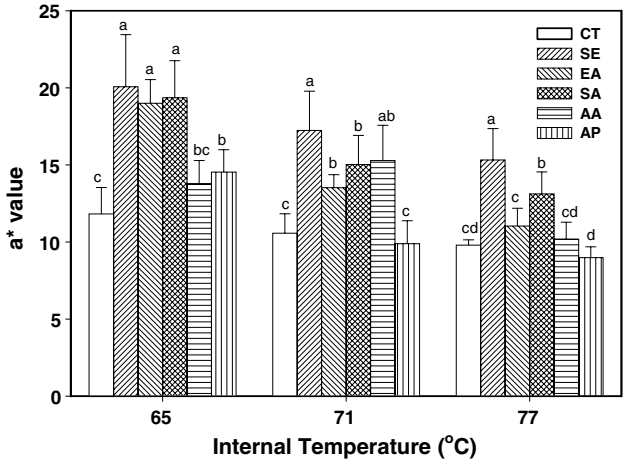

Fig. 3. Internal a^* values (redness) of beef patties stored at −18 °C for 14 days and cooked to different internal endpoint temperatures. Standard deviation bars are indicated; bars with different letters are different within each storage condition ($p < 0.05$). CT, control; SE, sodium erythorbate; EA, erythorbic acid; SA, sodium ascorbate; AA, ascorbic acid; AP, ascorbyl palmitate.

Minimal work has been performed to determine the T_m of Mb redox forms at pH 5.6. The T_m determined for redox forms of Mb at pH 5.6 did not vary to the extent that we anticipated. The measured T_m for MetMb was 73.3 °C; DeoxyMb, 73.7 °C; and OxyMb, 74.0 °C (Fig. 4). Our result would suggest that OxyMb is most heat stable, followed by DeoxyMb; MetMb would ap-

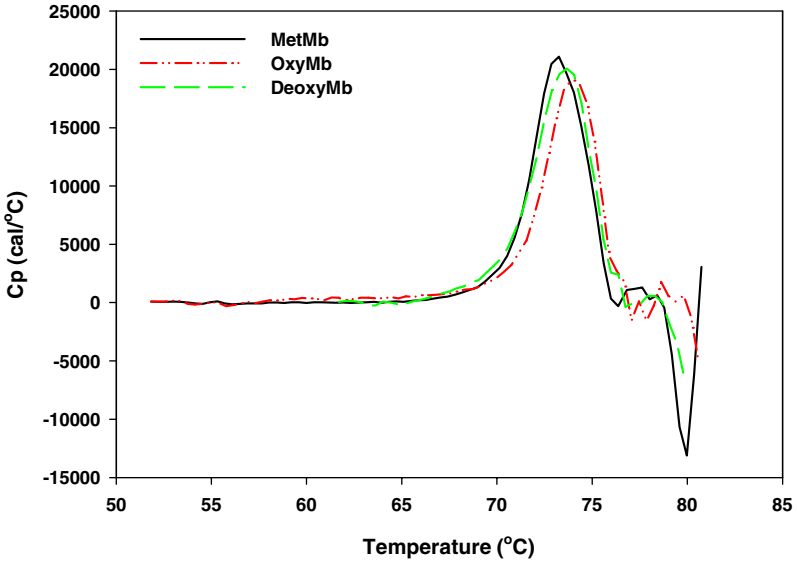

Fig. 4. Heat capacity (Cp) as measured by differential scanning calorimetry of bovine metmyoglobin (MetMb), oxymyoglobin (OxyMb), and deoxymyoglobin (DeoxyMb) at pH 5.6.

pear to be most easily denatured by heat, though sufficient replicates were not carried out to test statistical significance. This order in which ferrous redox forms of Mb denatured according to T_m was not consistent with the results of Machlik (1965). Machlik (1965) determined the resistance to denaturation by heat was greatest in DeoxyMb, followed by OxyMb, and was lowest for MetMb. Inconsistencies between this study and the work of Machlik (1965) could be due to procedural differences. For example, Machlik (1965) used acetate buffer rather than citrate buffer. Additionally, sodium hydrosulfite was used to facilitate heme protein reduction in both studies, but was not specifically indicated to have been removed prior to denaturation analysis in the Machlik (1965) study. Hunt et al. (1995) reported that patties containing DeoxyMb had greater a^* values at 55, 65, and 75 °C than patties comprised predominantly of OxyMb or MetMb, suggesting that patties with DeoxyMb are less likely to display PMB.

The relatively higher measured T_m of OxyMb and DeoxyMb suggest that these forms of Mb are more heat stable than MetMb. However, it is important to note that these measurements were necessarily made on purified proteins in buffer. The sarcoplasmic milieu within which myoglobin resides in postmortem muscle is very different from this chemical buffer environment, and this could be expected to affect the relative heat stabilities of the different redox forms of this heme protein. The studied reducing agents increased total reducing activity and decreased lipid oxidation in raw patties following storage. The addition of SE, EA, SA, and AA to ground beef maintained red surface color (a^* values and OxyMb presence) of refrigerated raw patties. The addition of reducing agents led to decreased a^* values but a more purple appearance in frozen raw patty surfaces. When these treated patties were cooked to the indicated temperatures, they stayed red longer, due to the relatively higher heat capacities of reduced forms of myoglobin.

Acknowledgements

This work was supported by beef and veal producers and importers through their $1-per-headcheckoff and was produced for the Cattleman's Beef Board and State Beef Councils by the National Cattlemen's Beef Association and by the Storrs Agricultural Experiment Station, University of Connecticut. We thank Dr. Davey Kalonia, School of Pharmacy, for his assistance with T_m measurement.

References

Bigner-George, M. E., & Berry, B. W. (2000). Thawing prior to cooking affects sensory, shear force, and cooking properties of beef patties. *Journal of Food Science, 65*, 2–8.

Brown, W. D., & Mebine, L. B. (1969). Autoxidation of oxymyoglobins. *Journal of Biological Chemistry, 244*, 6696–6701.

Faustman, C., & Phillips, A. L. (2001). Ch F3 Unit F3.3. Measurement of discoloration in fresh meat. In *Current protocols in food analytical chemistry*. New York: Wiley.

Hague, M. A., Warren, K. E., Hunt, M. C., Kropf, D. H., Kastner, C. L., Stroda, S. L., et al. (1994). Endpoint temperature, internal cooked color, and expressible juice color relationships in ground beef patties. *Journal of Food Science, 59*, 465–470.

Hunt, M. C., Sorheim, O. & Slinde, E. (1995). Effects of myoglobin form on internal cooked color development in ground beef. In *Proceedings 41st International Congress of Meat Science and Technology* (p. 394–395).

Killinger, K. M., Hunt, M. C. & Campbell, R. E. (1998). Incidence of premature browning in ground beef purchased retail. *Abstract in Proceedings 51st Reciprocal Meat Conference* (p. 185).

Lavelle, C. L., Hunt, M. C., & Kropf, D. H. (1995). Display life and internal color of ground beef from vitamin E-supplemented steers. *Journal of Food Science, 60*, 1175–1178, 1196.

Lee, M., Cassens, R. G., & Fennema, O. R. (1981). Effect of metal ions on residual nitrite. *Journal of Food Processing and Preservation, 5*, 191–205.

Lyon, B. G., Berry, B. W., Soderberg, D., & Clinch, N. (2000). Visual color and doneness indicators and the incidence of premature brown color in beef patties cooked to four end point temperatures. *Journal of Food Protection, 63*, 1389–1398.

Machlik, S.M. (1965). The effect of heat on bovine myoglobin derivatives in model systems and in beef semitendinosus muscles. PhD. Dissertation, Purdue University.

Phillips, A. L., Mancini, R., Sun, Q., Lynch, M. P., & Faustman, C. (2001). Effect of erythorbic acid on cooked color in ground beef. *Meat Science, 57*, 31–34.

SAS (1985). *SAS user guide: Statistics* (5th ed.) Cary, NC: SAS Institute.

Shivas, S. D., Kropf, D. H., Hunt, M. C., Kastner, C. L., Kendall, J. L. A., & Dayton, A. D. (1984). Effects of ascorbic acid on display life of ground beef. *Journal of Food Protection, 47*, 11–15.

USDA (1997). USDA advises consumers to use a meat thermometer when cooking hamburger. FSIS News and Information Bulletin. Washington, DC: FSIS, USDA.

Warren, K. E. (1994). Chemical properties of ground beef with normal and premature brown cooked color. PhD. Dissertation, Kansas State University.

Yin, M. C., Faustman, C., Riesen, J. W., & Williams, S. N. (1993). α-Tocopherol and ascorbate delay oxymyoglobin and phospholipid oxidation in vitro. *Journal of Food Science, 58*, 1273–1276.