FACTORS AFFECTING BEEF LUMBAR VERTEBRAE DISCOLORATION

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Background

Marrow discoloration is a factor that influences the appearance of bone-in meat products. This is particularly true in beef steaks containing lumbar vertebrae. However, to date, little research has focused on beef bone marrow discoloration, regardless of its affect on product display life.

Bone discoloration appears to be promoted by high-oxygen modified atmosphere packaging (MAP), which likely favors hemoglobin oxidation within exposed bone marrow (Lanari *et al.* 1995; Sorheim *et al.* 1999). In contrast, removing oxygen from modified atmosphere packages should encourage the deoxygenated form of hemoglobin; therefore, stabilizing vertebrae marrow color during storage and display. In addition, low levels of carbon monoxide in MAP should maintain a bright-red marrow color due to the formation of carboxyhemoglobin. Sorheim *et al.* (1999) speculated that carbon monoxide and oxygen exclusion might limit bone blackening. Although packaging technology has the potential to influence bone color, no published reports have evaluated the effects of package atmosphere on bone discoloration.

The oxidative state of hemoglobin released from red blood cells on the surface of cut bones likely determines marrow color (Lanari *et al.* 1995; Gill 1996). Thus, antioxidants that limit heme-protein oxidation within marrow should prove useful for preserving vertebrae surface color. Although ascorbic acid's ability to reduce methemoglobin in purified solutions has been reported, no published work has assessed the effects of ascorbic acid on bone marrow. The ability of ascorbic acid to delay myoglobin oxidation has made it a useful tool for minimizing surface discoloration in muscle (Lee *et al.* 1999; Shivas *et al.* 1984).

Objectives

Because bone discoloration is (1) an undesirable result of prolonged storage and (2) a process for which there is no established preventative measure (Warren *et al.* 1992; Gill 1996), our objectives were to:

1.) Investigate ultra low-oxygen and carbon monoxide modified atmosphere packaging (CO MAP) as a means of limiting lumbar vertebrae discoloration during storage.
2.) Investigate the ability of ascorbic acid, ascorbate-6-palmitate, and sodium erythorbate to minimize beef lumbar vertebrae discoloration during display.

Materials and methods

Experiment 1

Ten beef short loins (6 days postmortem) were cut perpendicular to the vertebral column into 2.54 cm-thick steaks. All lean, fat, and ribs were removed, yielding 2.54 cm-thick sections of only lumbar vertebrae. From each loin, 4 vertebrae were packaged separately in either ultra low-oxygen (80% N_2 and 20% CO_2) or carbon monoxide MAP (0.4% CO, 30% CO_2, and 69.6%N_2). One vertebra from each loin was packaged in high-oxygen (80%O_2 and 20% CO_2). Before packaging, initial instrumental color on the surface of each vertebrae section was evaluated (CIE L*a*b* and reflectance from 400 to 700nm; Illuminant A, 0.64 cm aperture, 10° observer). Packages were stored at 4°C.

From each loin, 1 vertebra per packaging treatment was evaluated after 1 day in storage. Because of the rapid discoloration resulting from high-oxygen MAP, vertebrae packaged in 80%O_2 were no longer evaluated. Instrumental color variables at 2, 4, and 6 weeks after packaging were then used to evaluate atmosphere-induced stability during storage (low-oxygen and 0.4% carbon monoxide packaging). The experimental design was a completely randomized block with repeated measures. Vertebral columns served as blocks (n = 10) to which packaging was assigned to vertebrae sections within each loin. Time of color evaluation was a

repeated measure assigned to units within a column. Data for all experiments was analyzed using the mixed procedure of SAS. Significance represents probabilities < 0.05.

Experiment 2:
Eight vertebral columns containing lumbar vertebrae were obtained at 6 days postmortem. Each was cut perpendicular to the length of the column into 1.91 cm-thick sections. From each column, two vertebral sections (n = 2 per treatment per column) with at least 2.54-cm² of freshly exposed lumbar marrow were randomly assigned to 1 of 7 treatments: Treatment 1 = No topical antioxidant; Treatment 2 = Distilled water; Treatment 3 = 1.5% Ascorbic acid; Treatment 4 = 2.5% Ascorbic acid; Treatment 5 = Ethanol; Treatment 6 = 1.5% Ascorbate-6-Palmitate; and Treatment 7 = 2.5% Ascorbate-6-Palmitate. Ascorbic acid and ascorbate-6-palmitate were made on a wt/wt basis using distilled water and ethanol, respectively. Vertebrae were topically treated with 1 ml of an assigned treatment, which thoroughly covered the entire fresh cut vertebrae.

To minimize package-to-package variation, 7 vertebrae sections from each column (1 per treatment) were packaged together in 80% O_2 and 20% CO_2. Packages were displayed at 1°C for 5 days in open-top display cases under continuous fluorescent light (1614 lux; 3000 K).

The color of marrow from each vertebrae section was evaluated initially and on days 1, 3, and 5 of display by 8 trained visual-color panelists using a scale of 1 = Bright reddish-pink to red (typical fresh cut bone), 2 = Dull pinkish-red, 3 = Slightly grayish-pink or grayish-red, 4 = Grayish-pink or grayish-red, 5 = Moderately gray, 6 = All gray or grayish-black, 7 = Black discoloration (0.5 point intervals). Instrumental color (CIE L*a*b*; Illuminant A, 0.64 cm aperture, 10° observer) was evaluated at 0, 1, and 5 days of display. Initial color (day 0) was evaluated prior to topical antioxidant treatment and packaging. The experimental design and analysis were similar to experiemnt 1.

Experiment 3
Ten bone-in short loins containing *longissimus* muscle and lumbar vertebrae (6 days postmortem) were fabricated into 1.9 cm-thick steaks. From each loin, 6 steaks with at least 2.54-cm² of freshly exposed lumbar marrow were assigned to one of the following treatments: Treatment 1 = Untreated control (0% Erythorbate); Treatment 2 = 0.05% Erythorbate; Treatment 3 = 0.1% Erythorbate; Treatment 4 = 0.5% Erythorbate; Treatment 5 = 1.0% Erythorbate; and Treatment 6 = 1.5% Erythorbate. All treatments were made on a wt/wt basis using distilled water. Experiment 2 indicated that there was little advantage to using 2.5% ascorbic acid; thus, 1.5% erythorbate was selected as the maximum concentration. Five milliliters of an assigned treatment was applied to the entire fresh cut surface of each steak, including the bone surface. To maximize distribution, treatments were spread over the entire steak surface using sterile cell spreaders.

Before treatment, the initial color (0 hours) on the fresh cut surface of lumbar vertebrae and *longissimus* muscles was measured instrumentally (Illuminant A, 1.27 cm aperture, 10° observer). Following treatment, steaks were packaged in 80% O_2 (20% CO_2) and displayed for 24 hours at 1ºC before instrumental color again was evaluated (24 hours post packaging) on both the *longissimus* muscle and the porous bone marrow of lumbar vertebrae for each steak. The experimental design and analysis were similar to experiment 1.

Results and discussion

Experiment 1
Initial vertebrae color prior to packaging was bright-red, typical of freshly-cut, oxygenated marrow (Table 1). High-oxygen packaging resulted in a rapid and significant loss of both redness ($\Delta a^* = 7.7$) and red color intensity (Δchroma = 6.8) within 24 hours after packaging. In addition, high-oxygen MAP decreased reflectance at 630 nm and increased reflectance between 540 and 580 nm, both of which indicate the conversion of oxy- to met- pigments. Thus, discoloration on the surface of lumbar vertebrae was likely due to methemoglobin. Our results support those with pork (Lanari *et al.* 1995), indicating that high-oxygen is detrimental to beef bone marrow color stability. Our reflectance data supports the role of hemoglobin's oxidative state (redox stability) in bone darkening (Lanari *et al.* 1995; Gill, 1996).

The benefits of 0.4% CO in MAP were immediately noted, and this packaging system increased surface redness within as little as 24 hours of storage (Table 1). In addition, vertebrae packaged in 0.4% CO MAP

remained bright-red for up to 6 weeks while those packaged in ultra low-oxygen MAP discolored after 2 weeks of storage. Sorheim *et al.* (1999) hypothesized that low levels of CO might limit bone discoloration. Our results support this, suggesting that formation of carboxyhemoglobin maintained a bright-red marrow color on the surface of lumbar vertebrae for up to 6 weeks in storage at 4°C. We agree that bone discoloration is increased by storage time (Warren *et al.* 1992; Gill 1996), but also recognize that beef vertebrae stored in high-oxygen MAP will darken within as little as 24 hours after packaging.

Experiment 2

Initial vertebrae color prior to treatment was bright-red. Untreated vertebrae and vertebrae treated with water or ethanol significantly discolored within 1 day after packaging in high-oxygen, resulting in no significant difference between negative and positive controls (Table 2). Applying ascorbic acid to vertebrae marrow significantly limited the rapid discoloration observed in control samples. Concentration of antioxidant had no significant affect on discoloration (1.5% was comparable to 2.5%). Overall, surface color during the 5-day display was preserved by topical antioxidant treatment in the following order: ascorbic acid > ascorbate-6-palmitate > controls; $P < 0.05$). During the 5-day display, ascorbic acid treated marrow had the least surface discoloration, the ascorbate-6-palmitate treatments were intermediate, and applying only water or ethanol had no benefits compared to untreated samples.

Experiment 3

Prior to display, all vertebrae were bright-red with no surface discoloration. Sodium erythorbate at 0.5, 1.0, or 1.5% prevented vertebrae discoloration within the first 24 hours of high-oxygen MAP display whereas untreated vertebrae and vertebrae treated with 0.05 or 0.1% erythorbate significantly discolored (Table 2). The *longissimus* from all treatments was bright-red with no signs of discoloration; therefore, erythorbate had no detrimental affects on muscle color (data not shown).

When bone-in beef steaks containing lumbar vertebrae were packaged in high-oxygen MAP, the marrow discolored rapidly while the *longissimus* muscle remained bright-red early in display. In experiments 2 and 3, both ascorbic acid and its isomer (erythorbate) proved useful for minimizing lumbar vertebrae discoloration. The reducing activity of ascorbic acid plays a role in muscle color stability via metmyoglobin reduction (Lee *et al.* 1999). We speculate that ascorbic acid maximized color stability on the surface of lumbar vertebrae by reducing methemoglobin. However, it is also possible that ascorbic acid limited methemoglobin accumulation by protecting hemoglobin from prooxidants derived from lipid oxidation.

Conclusions

We conclude that the detrimental effects of storage on beef lumbar vertebrae can be minimized through package atmosphere. Removal of oxygen from packages will limit vertebrae marrow discoloration during storage (2 weeks at 4 °C). To maximize the stability of hemoglobin on the surface of bone, combining 0.4% carbon monoxide with oxygen exclusion from modified atmosphere packages will maintain bright-red lumbar vertebrae for up to 6 weeks after packaging.

Although storage tends to promote discoloration, we conclude that high-oxygen MAP will induce rapid bone marrow discoloration. However, this rapid color deterioration on the surface of lumbar vertebrae can be prevented by ascorbic acid and sodium erythorbate. To maximize color stability through a 5-day display period at 1ºC, 2.5% ascorbic acid may be required. While ascorbate-6-palmitate minimizes vertebrae discoloration compared to untreated bone-in product, it does not perform as well as its water-soluble counterparts, ascorbic acid and sodium erythorbate.

References

Gill, C.O. 1996. Extending the storage life of raw chilled meats. Meat Sci. 43:S99-S109.

Lanari, M.C., Schaefer, D.M., and Scheller, K.K. 1995. Dietary vitamin E supplementation and discoloration of pork bone and muscle following modified atmosphere packaging. Meat Sci. 41:237-250.

Lee, B.J., Hendricks, D.G., and Cornforth, D.P. 1999. A comparison of carnosine and ascorbic acid on color and lipid stability in a ground beef pattie model system. Meat Sci. 51:245-253.

Shivas, S.D., Kropf, D.H., Hunt, M.C., Kastner, C.L., Kendall, J.L.A., and Dayton, A.D. 1984. Effects of ascorbic acid on display life of ground beef. J. Food Prot. 47:11-15.

Sorheim, O., Nissen, H., and Nesbakken, T. 1999. The storage life of beef and pork packaged in an atmosphere with low carbon monoxide and high carbon dioxide. Meat Sci. 52:157-164.

Warren, K.E., Hunt, M.C., Marksberry, C.L., Sorheim, O., Kropf, D.H., Johnson, D.E., and Windisch, M.J. 1992. Modified-atmosphere packaging of bone-in pork loins. J. Muscle Foods 3:283-300.

Table 1: Effects of packaging atmosphere[a] and storage time at 4°C on the instrumental color of marrow from beef lumbar vertebrae

Variable	Package atmosphere	Storage time				
		Initial color	Day 1	Week 2	Week 4	Week 6
a*	High-O_2	25.7c	18.0d	---[b]	---[b]	---[b]
	Ultra-low O_2	25.7c	24.9c	24.4c	17.6d	18.5d
	CO MAP	25.7c	32.1e	34.4f	31.0de	29.3d
Chroma	High-O_2	31.9c	25.1d			
	Ultra-low O_2	31.9d	30.2d	29.5d	22.5c	23.8c
	CO MAP	31.9c	38.9de	41.1e	36.9d	34.9d

[a]High-O_2 = 80%O_2 & 20% CO_2; Ultra low = 80% N_2 & 20% CO_2; CO = 0.4% CO, 30% CO_2, & 69.6%N_2.
[b]Vertebrae packaged in high-oxygen were bright red prior to packaging and moderately gray within 24 hours of storage. Thus, these vertebrae significantly discolored and were no longer evaluated.
[cdef]Least square means within a row with a different letter differ ($P < 0.05$).

Table 2: Effects of ascorbic acid and ascorbate-6-palmitate on the visual[a] color and a* values of beef lumbar vertebrae packaged in 80% O_2 and displayed at 1°C

Visual color[a]	Controls			Ascorbic acid		Ascorbate-6-palmitate	
Display (days)	Untreated	Water	Ethanol	1.5%	2.5%	1.5%	2.5%
0[b]	Prior to treatment, all vertebrae were bright-red (typical fresh cut bone)						
1	5.4c	5.4c	5.9c	1.8e	2.0e	4.2d	4.6d
3	5.8c	5.8c	6.2c	2.9e	3.0e	4.4d	4.7d
5	6.0c	6.0c	6.4c	4.2e	4.2e	5.1d	5.5d
a* values	Prior to treatment[b], all vertebrae had an average a* value of 30.8						
1	14.0c	14.0c	16.3c	33.2e	32.7e	25.4d	23.6d
5	15.7c	15.7c	14.8c	19.4e	18.3e	20.9d	22.8d

[a]Color scale of 1 = Typical fresh cut bone, 2 = Dull pinkish-red, 3 = Slightly grayish-pink or grayish-red, 4 = Grayish-pink or grayish-red, 5 = Moderately gray, 6 = All gray or grayish-black, 7 = Black discoloration.
[b]Day 0 initial color for all vertebrae prior to antioxidant treatment had an average color score of 1.0.
[cde]Least square means within a row with a different letter differ ($P < 0.05$).

Table 3: Effects of sodium erythorbate on the a* values of beef lumbar vertebrae packaged in 80% O_2 and displayed for 24 hours at 1°C

Display	Sodium erythorbate					
	Control[a]	0.05%	0.1%	0.5%	1.0%	1.5%
Initial[b]	Prior to treatment, all vertebrae were bright-red with an average a* value of 24.5					
24 hours	17.9c	17.1c	16.4c	26.9d	32.7de	32.5de

[a]Control samples recieved no topical antioxidant treatment (untreated control samples).
[b]Initial color was measured on all vertebrae prior to antioxidant treatment.
[cde]Least square means within a row with a different letter differ ($P < 0.05$).