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# Effects of polyvinyl chloride overwrap film, high-oxygen modified atmosphere packaging, or ultra-low-oxygen modified atmosphere packaging on bone marrow discoloration in beef humerus, rib, thoracic vertebra, and scapula<sup>1</sup>

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**ABSTRACT:** Meat retailers have reported bone marrow discoloration to be a problem, especially in modified atmosphere packages (MAP). Therefore, it is important to determine the prevalence and cause(s) of bone marrow discoloration in different beef bones and packaging systems. Thirty-six beef humeri, ribs, scapulas, and thoracic vertebrae from USDA Select and Choice carcasses were obtained from a commercial abattoir, cut into 2.54-cm-thick sections at 4 d postmortem, and packaged into 1 of 3 systems: 1) polyvinyl chloride film (PVC) overwrap; 2) high-oxygen (80% O<sub>2</sub>, 20% CO<sub>2</sub>) MAP; and 3) ultra-low-oxygen (70% N<sub>2</sub>, 30% CO<sub>2</sub>) MAP. Instrumental reflectance and visual color scores were taken on d 0, 2, and 4, and on d 0 to 4 of display, respectively. Bone marrow was extracted from humeri, ribs, and thoracic vertebrae for analysis but not from scapulas. Ribs, scapulas, and thoracic vertebrae packaged in PVC and high-oxygen MAP developed undesirable gray or black discoloration. In ultra-low-oxygen MAP, mean visual color scores were acceptable throughout the entire display period. Discoloration (darkening) was more extensive for ribs, scapulas, and thoracic vertebrae than for humeri, especially for bones packaged in PVC and high-oxygen MAP. Humeri had

lower ( $P < 0.05$ ) a\* values (larger positive a\* values indicate a redder color) than the other bones. The a\* values for ribs, scapulas, and thoracic vertebrae decreased ( $P < 0.05$ ) over time. Chroma showed that bone marrow discolored during display, but graying was dramatically less for all bones packaged in ultra-low-oxygen MAP and for humeri in PVC and high-oxygen MAP. Humeri marrow had lower ( $P < 0.05$ ) 2-thiobarbituric acid reactive substances (TBARS) than did ribs and thoracic vertebrae marrow. Ultra-low-oxygen MAP resulted in the least amount of change in TBARS from d 0 to 4, whereas thoracic vertebrae marrow had greater ( $P < 0.05$ ) TBARS values at d 4 of display than at d 0 in PVC and high-oxygen MAP. Humeri marrow had dramatically less total Fe and hemoglobin than did that of ribs and thoracic vertebrae for all packaging systems. Myoglobin was undetectable in humeri marrow. The much larger amounts of Fe and hemoglobin in ribs and thoracic vertebrae likely contribute to marrow discoloration. Bone marrow discoloration was distinct in ribs, scapulas, and thoracic vertebrae packaged in PVC or high-oxygen MAP. Bones packaged in ultra-low-oxygen MAP had minimal discoloration.

**Key words:** beef, bone marrow, color, modified atmosphere packaging, simulated retail display

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## INTRODUCTION

Occurrence of the “black bone” condition in modified atmosphere packaged (MAP), bone-in, beef retail cuts

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has been reported by meat retailers. Consumers may perceive bone discoloration as unwholesome, and it may affect their overall perception of a fresh meat product (Gill, 1996). Bone marrow discoloration has been reported in high-oxygen MAP beef and pork and also in cuts packaged in polyvinyl chloride film (PVC). As more and more meat is being sold as case-ready, it is important to find cause(s) for and prevention(s) of this problem.

Gill (1996) suggested that bone blackening occurred when bone is cut and hemoglobin is released to the surface, where it will accumulate when the red blood cells are disrupted. Over time and through exposure to air, hemoglobin on the surface of the bone turns from red to brown to black (Gill, 1996). Other possible causes

of bone marrow discoloration include greater total pigments, more hemoglobin, and more Fe when compared with muscle (Field et al., 1980). Furthermore, lipid content in bovine bone marrow differs among bones and among their locations. Miller et al. (1982) analyzed the lipid content of bovine bone marrow from cervical vertebrae, lumbar vertebrae, and femurs, and found that bone marrow from cervical vertebrae contained the least lipid, whereas marrow from femurs had the most. In addition, Miller et al. (1982) stated that bone marrow resembled adipose tissue more than it resembled muscle or liver tissue. Thus, lipid oxidation may also be a factor in the development of bone marrow discoloration.

Lanari et al. (1995) found that supplementing pigs with vitamin E (198 and 207 mg/kg) for 105 d increased  $a^*$  values of lumbar vertebrae over nonsupplemented pigs during 5 d of display. Mancini et al. (2004) demonstrated that beef lumbar vertebrae discolored within 24 h when packaged in high-oxygen MAP, primarily due to the oxidation of hemoglobin. Therefore, the objectives of this experiment were to determine the prevalence of bone marrow discoloration in different packaging systems in beef humeri, ribs, thoracic vertebrae, and scapulas and to determine factors that may cause bone marrow discoloration.

## MATERIALS AND METHODS

### *Samples and Packaging*

Thirty-six beef humeri, ribs, scapulas, and thoracic vertebrae from 2 replications of 18 different USDA Select and Choice carcasses were obtained from a commercial abattoir and cut into 2.54-cm-thick sections at 4 d postmortem by using a band saw (model 3334, Biro, Marblehead, OH). Cross-sections of the humeri (shaft), ribs, and thoracic vertebrae (medial to lateral through the main body of the vertebrae), and cross-sections perpendicular to the spinous process of scapulas were cut on a band saw. The only meat remaining on the bones was the small amounts left from commercial boning procedures.

Bones were packaged into 1 of 3 package types: 1) PVC overwrap; 2) high-oxygen (80% O<sub>2</sub>, 20% CO<sub>2</sub>; certified, Linweld Gases, Lincoln, NE) MAP; and 3) ultra-low-oxygen (70% N<sub>2</sub>, 30% CO<sub>2</sub>; certified, Linweld Gases) MAP. One each of a humerus, scapula, and thoracic vertebra, and 2 rib bone pieces were placed in each package. The PVC samples were packaged in 27.3 × 15.1 × 1.3 cm foam trays (No. 10S, Cryovac Sealed Air, Duncan, SC) with oxygen-permeable film (O<sub>2</sub> transmission = 23,250 mL/m<sup>2</sup>/24 h, 72 gauge; Resinite Packaging Films, Borden, Inc., North Andover, MA). High-oxygen and ultra-low-oxygen MAP packages were packaged (A10 Ross Jr.; Ross, Midland, VA) in rigid 27.3 × 17.3 × 7.6 cm plastic trays (CS11712, Cryovac Sealed Air) and covered with barrier lidding film (No. 1050, O<sub>2</sub> transmission = 20 mL/m<sup>2</sup>/24 h, 1.0 mil gauge; Cryovac Sealed Air). Each ultra-low-oxygen MAP had 1 acti-

vated oxygen scavenger (TSC0400; ActiveTech, Lake Forest, IL) added to the package. There were 2 replications of 18 packages of each system. Within each replication, 12 packages remained in the display case through d 4, whereas 6 packages were opened on d 2 of display (mid-display) for instrumental color readings.

### *Display*

Packages were displayed under continuous fluorescent lighting (1614 lux, 3,000K; Ultralume 30; Phillips, Bloomfield, NJ) for 5 d at 2°C. Packages were rotated twice daily to maintain a random display case (model DMF8; Tyler Refrigeration Corp., Niles, MI) placement.

### *Instrumental Color Measurements*

Instrumental Commission International d'Eclairage L\*, a\*, and b\* measurements were taken by using a 0.64-cm aperture (Illuminant A) on a Hunter Labscan 2 (Hunter Associates Laboratory, Inc., Reston, VA). Lightness (L\*) values are a measure of darkness to lightness (larger L\* values indicate a lighter color), a\* values measure the green to red spectrum (larger positive a\* values indicate a redder color), and b\* values measure the blue to yellow spectrum (larger positive b\* values indicate a more yellow color).

Each humerus, scapula, and thoracic-vertebra section was scanned twice, whereas 2 rib sections were each scanned once, and the values were averaged for statistical purposes. Within 1 min of opening each package, bones were scanned on d 0, 2, and 4 of display. Bones were scanned before packaging on d 0 and after visual color scores were taken on d 2 and 4. Six of each packaging type from both replications were opened on d 2 and were used for measuring mid-display instrumental color. Chroma was calculated as  $\sqrt{a^{*2} + b^{*2}}$ , and the resulting value was used to estimate the amount of graying/discoloration on each bone surface.

### *Visual Color Measurements*

Ten trained visual panelists (Hunt et al., 1991) scored bone-marrow color once each day for 5 days, beginning on d 0 (approximately 1 h after packaging). Mancini et al. (2004) developed the color scale used to score bones in high-oxygen MAP and PVC (1 = bright reddish-pink to red; 2 = dull pinkish-red; 3 = slightly grayish-pink/grayish-red; 4 = grayish-pink/grayish-red; 5 = moderately gray; 6 = all gray/grayish-black; and 7 = black discoloration). The 7-point scale used for ultra-low-oxygen MAP was developed during preliminary research (1 = bright purplish-red/purplish-pink; 2 = dull purplish-pink/purplish-red; 3 = slightly grayish purple/pink; 4 = grayish-purple/grayish-red; 5 = moderately gray; 6 = all gray/grayish-black; and 7 = black discoloration). Both color scales were used in half-point incre-

ments. Panelists were instructed to score the porous portion of the bone marrow.

### Sample Storage

Upon completion of display, bones were cleaned of all meat and connective tissue and were vacuum packaged (model A 300/16; Multivac, Kansas City, MO) in 3-mil nylon/polyethylene bags (75-gauge nylon/2 mil polyethylene; Koch Supplies, Inc., Kansas City, MO). Bones were then stored up to 1 mo at  $-80^{\circ}\text{C}$  until further analysis.

### Bone Marrow Collection

As determined by previous research in our laboratory, scapulas did not have enough bone marrow to extract for chemical analysis. Humeri, ribs, and thoracic-vertebrae marrow samples were pooled within bone and packaging type to obtain enough bone marrow to conduct the laboratory analyses. Marrow samples from humeri ( $n = 3$ ), ribs ( $n = 6$ ), and thoracic vertebrae ( $n = 6$ ) were pooled to provide adequate sample size.

### Bone Marrow Extraction

Bone marrow was extracted by using a modified procedure of Calhoun et al. (1998). Ribs and vertebrae were placed on top of a screen (type 304 stainless steel wire screen: wire diameter 0.119 cm, width opening 0.134 cm, open area 28.1%) fitted on 5-cm-long PVC adapter pipe (6.5- and 5.7-cm top and bottom diameters, respectively). The PVC adapter pipe, screen, and bone pieces were placed in a 500-mL plastic centrifuge bottle cut to 11.7 cm in height. Samples were centrifuged at  $8,075 \times g$  for 25 min (model J2-J21, Beckman Coulter, Fullerton, CA) at  $20^{\circ}\text{C}$ . The resulting bone marrow was placed in a 10.5-cm-long stainless steel cylinder (6.35 and 5.5 cm outer and inner diameters, respectively) fitted with a perforated (1-mm-diameter holes) screw-cap, which supported a 100- $\mu\text{m}$  nylon-mesh filter with 47% open area, 78- $\mu\text{m}$  thickness, and 55-mm diameter (Spectra/Mesh Nylon Filters, Spectrum Laboratories, Inc., Rancho Dominguez, CA). The marrow was forced through the nylon-mesh filter by a stainless-steel piston screwed down with a torque wrench (Craftsman 3/8-in Drive Microtork, Sears, Roebuck, and Co., Hoffman Estates, IL). The marrow was pressed once through the steel piston at 27.3 J of torque. The humeri marrow was scooped out from the bone and then forced through the same nylon-mesh filter by using the piston.

### 2-Thiobarbituric Acid Reactive Substances

Humeri (10 g), ribs (1 g), and vertebrae (1 g) marrow samples were used to determine 2-thiobarbituric acid reactive substances (TBARS) content on d 0 and 4 of display by using the modified method of Witte et al. (1970). Marrow samples were pulverized using a Waring blender (Dynamics Corps. of America, New Hart-

ford, CT) and combined with 7.2% perchloric acid and distilled water. Samples were blended and filtered through Whatman #2 filter paper (Whatman International Ltd., Maidstone, England), and TBARS reagent was added to the samples. Samples were stored at  $27^{\circ}\text{C}$  for 24 h, and the absorbance was read at 529.5 nm. Absorbancies were then compared with a standard curve to determine milligrams of malonaldehyde per kilogram of sample.

### Heme Pigment Analysis

Bone marrow samples for ribs (2.5 g), thoracic vertebrae (2.5 g), and humeri (15 g) were placed in a stomacher bag with 10, 10, or 25 mL, respectively, of 0.1 M sodium phosphate buffer (pH 7.0), and were stomached for 1 min. Contents were then poured into a centrifuge tube and centrifuged at  $21,500 \times g$  for 30 min at  $2^{\circ}\text{C}$ . The supernatant was then filtered through Whatman #1 filter paper, and a 2-mL aliquot was transferred to an amber vial containing 0.1 g of sodium hydrosulfite. This reaction was allowed to occur for a minimum of 10 minutes at room temperature ( $21^{\circ}\text{C}$ ), and then the sample was syringe-filtered through a 0.45- $\mu\text{m}$  filter into another amber vial.

Hydrophobic interaction HPLC (Hewlett Packard Series II, 1090 HPLC, Agilent Technologies, Palo Alto, CA) equipped with a 7.5-cm  $\times$  7.5-mm (10- $\mu\text{m}$  particle size) phenyl-5-TSK-gel PW hydrophobic interaction column (Supelco Inc., Bellefonte, PA) was used to determine myoglobin (Mb) and hemoglobin (Hb) pigment concentrations. A linear gradient from 100% A (1.7 M ammonium sulfate and 0.1 M sodium phosphate; pH 7.0) to 100% B (0.1 M sodium phosphate; pH 7.0) was used over a run time of 17 min (Oellingrath et al., 1990). Hemoglobin and Mb were detected at 420 nm by UV spectroscopy. A 50- $\mu\text{L}$  aliquot was injected into the column in a 20- $\mu\text{L}$  loop.

### Total Iron and Phosphorus

Ribs and vertebrae marrow samples were wet-ashed by using 1-g samples digested with 15 mL of nitric acid for 1.5 h on a hotplate (setting 5, Corning PC-100, Corning, Corning, NY). Humeri samples (2.5 g) were dry-ashed in a muffle furnace (model 85A, Neytech, Bloomfield, CT) at  $600^{\circ}\text{C}$  for 4 h and were digested by using 25 mL of 2.5 N hydrochloric acid for 50 min. Rib and vertebrae marrow samples were more liquid-like in composition, whereas humeri marrow samples resembled adipose tissue; therefore, the samples were ashed wet and dry, respectively. Digested samples were diluted to 50 mL with distilled-deionized water.

Total iron (method 968.08, AOAC, 2002) was measured with an atomic absorption spectrophotometer (AAAnalyst 100; Perkin Elmer, Norwalk, CT) at 248.3 nm. A 0.2-mL sample was mixed with 1 mL of 1% 4-methylaminophenol sulfate and 4.4 mL of molybdenum blue solution (0.02 M sodium molybdate and 36 N sulfu-

**Table 1.** Least squares means for visual color scores of bone marrow from bones in different packaging types during retail display (SE = 0.14)<sup>1</sup>

Bone	Package <sup>2</sup>	Day				
		0	1	2	3	4
Humeri	PVC <sup>3</sup>	1.5 <sup>v</sup>	2.0 <sup>w</sup>	2.2 <sup>x</sup>	2.3 <sup>y</sup>	2.6 <sup>z</sup>
Humeri	High <sup>3</sup>	1.4 <sup>w</sup>	1.8 <sup>x</sup>	2.1 <sup>y</sup>	2.2 <sup>y</sup>	2.5 <sup>z</sup>
Humeri	Ultra-low <sup>4</sup>	1.8 <sup>w</sup>	2.5 <sup>x</sup>	2.9 <sup>y</sup>	2.8 <sup>y</sup>	3.0 <sup>z</sup>
Ribs	PVC <sup>3</sup>	1.7 <sup>b, v</sup>	4.6 <sup>a, w</sup>	5.0 <sup>a, x</sup>	5.2 <sup>a, y</sup>	5.3 <sup>a, z</sup>
Ribs	High <sup>3</sup>	1.4 <sup>a, w</sup>	4.6 <sup>a, x</sup>	5.1 <sup>a, y</sup>	5.2 <sup>a, y</sup>	5.3 <sup>a, z</sup>
Ribs	Ultra-low <sup>4</sup>	2.1 <sup>w</sup>	2.5 <sup>x</sup>	2.8 <sup>y</sup>	3.0 <sup>z</sup>	3.1 <sup>z</sup>
Scapulas	PVC <sup>3</sup>	2.1 <sup>b, v</sup>	4.2 <sup>a, w</sup>	4.9 <sup>a, x</sup>	5.1 <sup>a, y</sup>	5.5 <sup>a, z</sup>
Scapulas	High <sup>3</sup>	1.8 <sup>a, v</sup>	4.6 <sup>b, w</sup>	5.2 <sup>b, x</sup>	5.4 <sup>b, y</sup>	5.6 <sup>a, z</sup>
Scapulas	Ultra-low <sup>4</sup>	2.3 <sup>v</sup>	3.1 <sup>w</sup>	3.4 <sup>x</sup>	3.6 <sup>y</sup>	3.7 <sup>z</sup>
Thoracic vertebrae	PVC <sup>3</sup>	2.2 <sup>b, w</sup>	5.3 <sup>a, x</sup>	5.8 <sup>a, y</sup>	5.8 <sup>a, y</sup>	6.1 <sup>a, z</sup>
Thoracic vertebrae	High <sup>3</sup>	1.6 <sup>a, w</sup>	5.2 <sup>a, x</sup>	5.6 <sup>a, y</sup>	5.8 <sup>a, yz</sup>	5.9 <sup>a, z</sup>
Thoracic vertebrae	Ultra-low <sup>4</sup>	2.7 <sup>w</sup>	3.1 <sup>x</sup>	3.3 <sup>y</sup>	3.4 <sup>yz</sup>	3.5 <sup>z</sup>

<sup>a,b</sup>Within bone type and column, least squares means lacking a common superscript letter differ,  $P < 0.05$ .

<sup>v-z</sup>Within bone type and row, least squares means lacking a common superscript letter differ,  $P < 0.05$ .

<sup>1</sup>No comparisons were made between bones packaged in PVC or high-oxygen and visual color of bones packaged in ultra-low oxygen because different color scales were used for the packaging methods.

<sup>2</sup>PVC = polyvinyl chloride overwrap film; high = high-oxygen modified atmosphere packaging; ultra-low = ultra-low-oxygen modified atmosphere packaging.

<sup>3</sup>1 = bright reddish-pink to red; 2 = dull pinkish-red; 3 = slightly grayish-pink/grayish-red; 4 = grayish-pink/grayish-red; 5 = moderately gray; 6 = all gray/grayish-black; 7 = black discoloration (Mancini et al., 2004).

<sup>4</sup>1 = bright purplish-red/purplish-pink; 2 = dull purplish-pink/purplish-red; 3 = slightly grayish-purple/pink; 4 = grayish-purple/grayish-red; 5 = moderately gray; 6 = all gray/grayish-black; 7 = black discoloration.

ric acid) and was allowed to stand for 45 min at room temperature to develop color. Phosphorus content was measured at 700 nm (Spectronic 21; Bausch & Lomb, Wilmington, MA). Fe and P (Assurance Spex CertiPrep, Metuchen, NJ) values of the samples were compared with a standard curve at 0, 2, 5, and 10 ppm, and 0, 20, 40, 60, 100, 150, and 200 ppm, respectively.

### Statistical Analysis

Statistical analysis was completed by using the PROC MIXED procedure of SAS (SAS Institute, Inc., Cary, NC). The experimental unit was animal. For visual and instrumental color, the fixed effects were package, bone, package  $\times$  bone, day, package  $\times$  day, bone  $\times$  day, and package  $\times$  bone  $\times$  day. For visual color, the random effects were panelist, replication, animal (replication), animal  $\times$  package (replication), animal  $\times$  day (replication), animal  $\times$  package  $\times$  day (replication), and animal  $\times$  bone (replication  $\times$  package). For instrumental color, the random effects were replication, animal (replication), animal  $\times$  package (replication), animal  $\times$  day (replication), animal  $\times$  package  $\times$  day (replication), and animal  $\times$  bone (replication  $\times$  package). For the TBARS analysis, the fixed effects were package, bone, package  $\times$  bone, day, package  $\times$  day, bone  $\times$  day, and package  $\times$  bone  $\times$  day, whereas the random effects included animal, animal  $\times$  package, animal  $\times$  bone (package). For the bone-analysis data, fixed effects were package, bone, and package  $\times$  bone, and animal and bone (pooled) were the random effects in the model. Least squares means were separated by using Fisher's Protected LSD, with Prasad-Rao-Jeske-Kackar-Harville SE and the

Kenward-Roger d.f. (SAS Institute, 2004) when  $F < 0.05$ . Highest-order interactions were reported; however, main effects were reported when there were no ( $P > 0.05$ ) interactions. Significance was determined to be present at probability values of  $P < 0.05$ .

## RESULTS AND DISCUSSION

### Visual Color Display

There was a bone  $\times$  package  $\times$  day interaction ( $P < 0.05$ ) for visual color scores (Table 1). Humeri bone marrow became darker ( $P < 0.05$ ) with each day of display, but, by d 4 of display, the visual score was still only dull pinkish-red for humeri packaged in PVC and high-oxygen MAP and slightly grayish purple/pink for those packaged in ultra-low-oxygen MAP (Table 1). This indicates that, visually, humeri did not turn gray or black during display. There were no differences ( $P > 0.05$ ) between PVC and high-oxygen MAP for humeri visual color scores (Table 1). No comparisons were made between visual color of bones packaged in PVC or high-oxygen MAP and ultra-low-oxygen MAP because different color scales were used for the packaging methods.

Visual scores for rib bone marrow increased ( $P < 0.05$ ) with increased display time in all packaging types (Table 1). In samples packaged in PVC and high-oxygen MAP, the ribs were grayish-pink/pinkish red by d 1 of display, and were moderately gray from d 2 to 4. Ribs packaged in ultra-low-oxygen MAP were only slightly grayish-purple/pink by the end of display, and PVC-packaged ribs received higher ( $P < 0.05$ ) color scores than high-oxygen MAP ribs on d 0 only.

**Table 2.** Least squares means for  $a^*$  values of bone marrow from bones in different packaging types during retail display

Bone	Package <sup>1</sup>	Day		
		0	2	4
Humeri	PVC	14.00 <sup>z</sup>	15.23 <sup>b, z</sup>	15.18 <sup>b, z</sup>
Humeri	High	13.65 <sup>y</sup>	15.01 <sup>b, yz</sup>	15.14 <sup>b, z</sup>
Humeri	Ultra-low	13.58 <sup>z</sup>	11.34 <sup>a, y</sup>	10.20 <sup>a, y</sup>
Ribs	PVC	26.60 <sup>z</sup>	18.09 <sup>a, y</sup>	16.70 <sup>a, y</sup>
Ribs	High	25.74 <sup>z</sup>	19.39 <sup>a, y</sup>	17.31 <sup>a, x</sup>
Ribs	Ultra-low	25.67 <sup>z</sup>	22.83 <sup>b, y</sup>	21.19 <sup>b, y</sup>
Scapulas	PVC	25.04 <sup>z</sup>	18.60 <sup>a, y</sup>	16.33 <sup>a, x</sup>
Scapulas	High	24.03 <sup>z</sup>	20.20 <sup>a, y</sup>	15.56 <sup>a, x</sup>
Scapulas	Ultra-low	24.56 <sup>z</sup>	23.86 <sup>b, z</sup>	24.01 <sup>b, z</sup>
Thoracic vertebrae	PVC	26.55 <sup>z</sup>	16.47 <sup>a, y</sup>	14.41 <sup>a, x</sup>
Thoracic vertebrae	High	25.72 <sup>z</sup>	18.69 <sup>a, y</sup>	14.25 <sup>a, x</sup>
Thoracic vertebrae	Ultra-low	26.24 <sup>z</sup>	22.60 <sup>b, y</sup>	22.70 <sup>b, y</sup>
SE		0.52	0.85	0.62

<sup>a,b</sup>Within bone type and column, means with different superscript letters differ ( $P < 0.05$ ).

<sup>x,y,z</sup>Within bone type and row, means with different superscript letters differ ( $P < 0.05$ ).

<sup>1</sup>PVC = polyvinyl chloride overwrap film; high = high-oxygen modified atmosphere packaging; ultra-low = ultra-low-oxygen modified atmosphere packaging.

Scapula bone marrow became darker ( $P < 0.05$ ) with increased display time in all 3 packaging types (Table 1). Visual color scores for bones packaged in PVC and high-oxygen MAP were grayish-pink/grayish-red beginning on d 1 of display, whereas those in ultra-low-oxygen MAP were only slightly grayish-pink/grayish-red. Furthermore, color scores were lower for bones packaged in PVC than for those packaged in high-oxygen MAP on d 1, 2, and 3 of retail display.

Visual color scores indicated that thoracic vertebrae bone marrow turned dark ( $P < 0.05$ ) by d 1 of display and became darker throughout the 4 days of display (Table 1). In vertebrae packaged in PVC and high-oxygen MAP, visual scores were already moderately gray by d 1, whereas vertebrae packaged in ultra-low-oxygen MAP were only slightly grayish-pink/grayish-red. On d 0 only, package differences ( $P < 0.05$ ) were found only between PVC and high-oxygen MAP.

All 4 bone types became darker to varying degrees during the display period in all 3 packaging types. Much of the darkening occurred within the first day of display; however, darkening was much more extensive in ribs, scapulas, and thoracic vertebrae than in humeri (an approximate 3 color score advantage), with a significant proportion of them described as black bone. Furthermore, the darkening was much more extensive for bones packaged in PVC and high-oxygen MAP than for those in ultra-low-oxygen MAP during retail display. In fact, mean visual color scores for bones packaged in the ultra-low-oxygen MAP were acceptable throughout display, with means ranging from 1.8 (dull purplish-pink/purplish-red) to 3.7 (grayish-purple/grayish-red). Noted differences in visual scores of bones packaged in PVC and high-oxygen MAP on d 0 of display may have been caused by the greater saturation of oxygen in the high-oxygen MAP resulting in a faster or more intense bloom compared with that of the PVC packages.

### Instrumental Color

In agreement with visual color scores, humeri had dramatically higher ( $P < 0.05$ )  $L^*$  values than did ribs, scapulas, and thoracic vertebrae (results not shown). The  $L^*$  values were similar ( $P < 0.05$ ) for all 3 packaging systems and did not show differences found with visual color scores.

### $a^*$ Values

There was a bone  $\times$  package  $\times$  day interaction ( $P < 0.05$ ) for  $a^*$  values. In general,  $a^*$  values decreased more for marrow from ribs, scapulas, and thoracic vertebrae in PVC and high-oxygen MAP than in ultra-low-oxygen MAP (Table 2). There were no differences ( $P > 0.05$ ) in  $a^*$  values during display for humeri marrow packaged in PVC (Table 2), but values for PVC and high-oxygen MAP were higher ( $P < 0.05$ ) for humeri and lower ( $P < 0.05$ ) for ribs, scapulas, and vertebrae than those of ultra-low-oxygen MAP at d 2 and 4.

Rib marrow was less red (lower  $a^*$  values;  $P < 0.05$ ) at d 2 and 4 than on d 0, as measured by  $a^*$ , but there was no change between d 2 and 4 for ultra-low-oxygen MAP and PVC (Table 2). Bone marrow from scapulas and thoracic vertebrae was less red ( $P < 0.05$ ) at d 2 and 4 than at d 0 when packaged in PVC and high-oxygen MAP, but  $a^*$  values did not change ( $P > 0.05$ ) when bones were packaged in ultra-low-oxygen MAP (Table 2). Humeri had lower  $a^*$  values (less red) than the other bones. The  $a^*$  values for ribs, scapulas, and thoracic vertebrae decreased over time, which corresponds to increased visual color scores. In addition,  $a^*$  value changes from bones packaged in ultra-low-oxygen MAP were smaller, matching much smaller visual color score changes.

Differences in  $a^*$  between bones packaged in either PVC or high-oxygen MAP and those in ultra-low-oxygen

**Table 3.** Least squares means for chroma values of bone marrow from bones in different packaging types during retail display

Bone	Package <sup>1</sup>	Day		
		0	2	4
Humeri	PVC	22.80 <sup>a, z</sup>	23.98 <sup>a, z</sup>	23.77 <sup>a, z</sup>
Humeri	High	22.84 <sup>a, z</sup>	23.73 <sup>a, z</sup>	23.67 <sup>a, z</sup>
Humeri	Ultra-low	22.73 <sup>a, z</sup>	19.38 <sup>b, y</sup>	19.45 <sup>b, y</sup>
Ribs	PVC	33.00 <sup>a, z</sup>	23.92 <sup>b, y</sup>	22.43 <sup>a, y</sup>
Ribs	High	31.91 <sup>a, z</sup>	25.88 <sup>ab, y</sup>	23.64 <sup>a, y</sup>
Ribs	Ultra-low	31.75 <sup>a, z</sup>	27.51 <sup>a, y</sup>	26.12 <sup>b, y</sup>
Scapulas	PVC	32.44 <sup>b, z</sup>	25.55 <sup>a, y</sup>	23.71 <sup>a, y</sup>
Scapulas	High	30.90 <sup>a, z</sup>	27.30 <sup>a, y</sup>	22.48 <sup>a, x</sup>
Scapulas	Ultra-low	31.60 <sup>ab, z</sup>	30.23 <sup>b, z</sup>	30.56 <sup>b, z</sup>
Thoracic vertebrae	PVC	33.61 <sup>a, z</sup>	23.03 <sup>a, y</sup>	21.84 <sup>a, y</sup>
Thoracic vertebrae	High	32.50 <sup>a, z</sup>	26.41 <sup>b, y</sup>	21.79 <sup>a, x</sup>
Thoracic vertebrae	Ultra-low	33.23 <sup>a, z</sup>	28.24 <sup>b, y</sup>	28.79 <sup>b, y</sup>
SE		0.58	0.96	0.69

<sup>a, b</sup>Within bone type and column, means with different superscript letters differ ( $P < 0.05$ ).

<sup>x, y, z</sup>Within bone type and row, means with different superscript letters differ ( $P < 0.05$ ).

<sup>1</sup>PVC = polyvinyl chloride overwrap film; high = high-oxygen modified atmosphere packaging; ultra-low = ultra-low-oxygen modified atmosphere packaging.

MAP at d 2 and 4 in all bone types would be expected because of the differences in oxygen composition in the packages. Initial instrumental color was measured before packaging; therefore, we would expect values at d 0 to be similar but values at d 2 and 4 to be different among the different packages.

Mancini et al. (2004) found similar visual color scores in untreated beef lumbar vertebrae packaged in high-oxygen MAP to those of our thoracic vertebrae packaged in PVC and high-oxygen MAP. In untreated beef lumbar vertebrae packaged in high-oxygen MAP,  $a^*$  values decreased from the initial value of 25.7 to 18.6 within 12 h (Mancini et al., 2004). These authors also found that 60 to 80% of the visual bone surface discoloration occurred during the first 24 h of display, supported by initial chroma values of beef lumbar vertebrae packaged in high-oxygen MAP being greater at 12 than 24 h after packaging (31.9 vs. 25.1).

### Chroma Values

There was a bone  $\times$  package  $\times$  day interaction ( $P < 0.05$ ) for chroma values. In general, chroma values decreased more for ribs, scapulas, and thoracic vertebrae in PVC and high-oxygen MAP than in ultra-low-oxygen MAP. Chroma has been used to measure surface discoloration (Hunter et al., 1991), in particular graying (Hunter, 1973). Chroma was similar ( $P > 0.05$ ) between humeri bone marrow packaged in PVC and high-oxygen MAP and did not change during display (Table 3). Ultra-low-oxygen MAP resulted in graying at d 2 and 4 of display (Table 3). Rib bone marrow had more graying ( $P < 0.05$ ) on d 2 and 4 than on d 0 in all 3 packaging types, but there was less graying in ribs packaged in ultra-low-oxygen MAP than PVC or high-oxygen MAP. Scapula bone marrow graying was detected in PVC and high-oxygen MAP ( $P < 0.05$ ), as indicated by a decrease

in chroma (Table 3); however, chroma values did not ( $P > 0.05$ ) change in scapulas packaged in ultra-low-oxygen MAP (Table 3). There was a decrease ( $P < 0.05$ ) in chroma on d 2 and 4 in thoracic vertebrae bone marrow with all 3 packaging types, but surface graying was most severe in vertebrae packaged in PVC and high-oxygen MAP.

Chroma showed that bone marrow discolored, or increased in graying, during display, but discoloration was noticeably less for all bones packaged in ultra-low-oxygen MAP and for humeri packaged in PVC and high-oxygen MAP. Minimal surface graying of humeri may have been caused by the varying fatty acid composition among bones (Mello et al., 1976; Miller et al., 1982) and/or the absence of oxygen in the ultra-low-oxygen MAP environment.

### Bone Marrow Analyses

There was a bone  $\times$  package  $\times$  day interaction ( $P < 0.05$ ) among means for TBARS values. Humeri TBARS values were quite low and were not different ( $P > 0.05$ ) initially or at the end of display, and there were no ( $P > 0.05$ ) differences in values among packaging methods (Table 4). Oxidation was considerably less for humeri marrow than for marrow from ribs and vertebrae. Marrow from ribs packaged in high-oxygen MAP had greater ( $P < 0.05$ ) TBARS values at d 4 of display than on d 0 (Table 4), but ribs packaged in ultra-low-oxygen MAP had lower ( $P < 0.05$ ) TBARS values at d 4 of display than at d 0, and the value was different ( $P < 0.05$ ) than those of ribs packaged in PVC and high-oxygen MAP (Table 4). Thoracic vertebrae marrow had greater ( $P < 0.05$ ) TBARS values than ribs packaged in PVC and high-oxygen MAP after 4 d of retail display. Additionally, vertebrae packaged in ultra-low-oxygen MAP had lower ( $P < 0.05$ ) TBARS values than those

**Table 4.** Least squares means for 2-thiobarbituric acid reactive substances (mg/kg of fresh weight) of bone marrow from bones in different packaging types during retail display

Bone	Package <sup>1</sup>	Day	
		0	4
Humeri	PVC	0.03 <sup>z</sup>	0.06 <sup>z</sup>
Humeri	High	0.03 <sup>z</sup>	0.06 <sup>z</sup>
Humeri	Ultra-low	0.03 <sup>z</sup>	0.04 <sup>z</sup>
SE		0.02	0.02
Ribs	PVC	0.74 <sup>z</sup>	0.77 <sup>b, z</sup>
Ribs	High	0.74 <sup>y</sup>	0.84 <sup>b, z</sup>
Ribs	Ultra-low	0.74 <sup>y</sup>	0.65 <sup>a, z</sup>
SE		0.03	0.03
Thoracic vertebrae	PVC	0.67 <sup>y</sup>	1.04 <sup>b, z</sup>
Thoracic vertebrae	High	0.67 <sup>y</sup>	1.01 <sup>b, z</sup>
Thoracic vertebrae	Ultra-low	0.67 <sup>y</sup>	0.75 <sup>a, z</sup>
SE		0.03	0.03

<sup>a,b</sup>Within bone type and column, means with different superscript letters differ ( $P < 0.05$ ).

<sup>y,z</sup>Within bone type and row, means with different superscript letters differ ( $P < 0.05$ ).

<sup>1</sup>PVC = polyvinyl chloride overwrap film; high = high-oxygen modified atmosphere packaging; ultra-low = ultra-low-oxygen modified atmosphere packaging.

packaged in PVC or high-oxygen MAP at the end of display.

#### Total Fe, P, Hb, and Mb

Rib and thoracic vertebrae marrow had dramatically more ( $P < 0.05$ ) total Fe and Hb than humeri marrow (Table 5). Marrow from humeri and ribs had more ( $P < 0.05$ ) P than thoracic vertebrae. Rib marrow had more ( $P < 0.05$ ) Mb than vertebrae marrow, but Mb was undetectable in humeri marrow. Dry ashing results in higher Fe levels than wet ashing (Windham and Field, 2000). In our study, ribs and thoracic vertebrae had much greater total Fe levels than humeri, even though humeri were dry ashed.

Field et al. (2002) found bone marrow from bovine cervical vertebrae had 17.4 mg/100 g of Fe. Lumbar vertebrae marrow from steers contained 30.12 mg of total pigment/g, of which 100% was Hb (Field et al., 1980). Muscle from the lumbar vertebrae region had 3.06 mg of total pigment/g, of which 12.85% was Hb and 87.15% was myoglobin (Field et al., 1980). Calhoun et al. (1998) reported that porcine femurs, ribs, and

vertebrae marrow had 2.67, 14.47, and 10.05 mg/100 g of total Fe, respectively, and 5.04, 17.7, and 13.67 mg of total pigment/g, respectively. The values reported by Calhoun et al. (1998) for ribs and vertebrae are much smaller than in our study. Concentrations of heme pigments in our study may be different because of differences in bone marrow recovery methods and pigment-extraction procedures.

The composition of marrow from round bones is about 90% fat in the shaft, and marrow lipids contain only minimal amounts of minerals (Gebault et al., 1998). This may largely explain the lower Fe and pigment concentrations in marrow from humeri than from ribs and vertebrae in our study. Approximately 4.5% of P content is accounted for as phospholipids, and phospholipids are most abundant in humeri, which do not have a bone-darkening problem, leading us to hypothesize that phospholipid content does not influence bone marrow discoloration. Thus, total Fe and Hb content in ribs and thoracic vertebrae marrow may be the cause of bone marrow discoloration.

Overall, the ribs, scapulas, and thoracic vertebrae turned dark (grayish-black) in PVC and high-oxygen MAP during 5 d of display. These bones turned dark in color within a 24-h period. Preliminary research from our laboratory showed that this happens approximately 5 to 24 h after packaging (results not shown). In contrast, the humeri remained acceptable in color throughout display. One possible explanation for humeri maintaining an acceptable color, in contrast to ribs, scapulas, and thoracic vertebrae, is the difference in bone marrow composition. Trubowitz and Davis (1982) classified 2 types of marrow: red marrow is the hemopoietically active marrow that is present in vertebrae and ribs, and yellow marrow is adipose tissue in bone marrow found in the distal portion of long bones. Thus, humeri bone marrow contains much more yellow marrow and lacks the abundance of red marrow and hemoglobin found in ribs and vertebrae that show more extreme discoloration. Humeri marrow should be comparable in composition to marrow from other round bones such as the femur. In beef animals more than 50 mo of age, femur marrow consisted of 9.2% water, 88.2% lipid, 2.6% residue, and 0.23% nitrogen (Dietz, 1949). If the major component of humeri marrow is lipid, then the extremely low TBARS values of humeri bone marrow indicated that lipid oxidation was not a primary cause of the bone marrow discoloration.

**Table 5.** Least squares means ( $\pm$ SE) for total Fe, P, hemoglobin (Hb), and myoglobin (Mb) of bone marrow from humeri, ribs, and thoracic vertebrae

Bone	Total Fe, ppm	P, ppm	Hb, mg/g	Mb, mg/g
Humeri	8.12 <sup>x</sup> $\pm$ 0.54	867.92 <sup>z</sup> $\pm$ 92.6	4.5 <sup>y</sup> $\pm$ 0.45	— <sup>1</sup>
Ribs	237.35 <sup>z</sup> $\pm$ 10.8	847.41 <sup>z</sup> $\pm$ 43.7	159.5 <sup>z</sup> $\pm$ 13.2	0.530 <sup>z</sup> $\pm$ 0.05
Thoracic vertebrae	219.08 <sup>y</sup> $\pm$ 8.93	574.32 <sup>y</sup> $\pm$ 42.1	153.0 <sup>z</sup> $\pm$ 6.36	0.313 <sup>y</sup> $\pm$ 0.02

<sup>x-z</sup>Within columns, means with different superscript letters differ ( $P < 0.05$ ).

<sup>1</sup>Not detectable in humeri bone marrow.

Some change in color occurred in bones packaged in ultra-low-oxygen MAP; however, marrow from these bones remains acceptable in color and will bloom to a reddish color when exposed to oxygen again. It is possible that the lack of oxygen in the ultra-low-oxygen MAP inhibits or greatly curtails oxidation of Hb and/or Mb in ribs, scapulas, and vertebrae, compared with being packaged in PVC or high-oxygen MAP.

### IMPLICATIONS

Bone marrow discoloration would be expected to occur in ribs, scapulas, and thoracic vertebrae packaged in polyvinyl chloride or high-oxygen modified atmosphere packaging, whereas bones packaged in ultra-low-oxygen modified atmosphere packaging should have minimal discoloration. Bone marrow discoloration is not an issue in humeri. It seems likely that bone marrow discoloration is caused primarily by oxidation of hemoglobin but may also be a result of heme-catalyzed lipid oxidation or by a combination of the 2.

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