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CHARACTERIZATION, EFFECTS OF PACKAGING, AND ANTIOXIDANT PREVENTION OF BEEF BONE MARROW DISCOLORATION

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Introduction

Bone marrow discoloration to a gray-black color and its occurrence in modified-atmosphere packaged (MAP), bone-in, beef retail cuts has been observed by meat retailers. Consumers could perceive bone discoloration as unwholesome, and it might affect their acceptance 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).

Gill (1996) suggested that bone blackening occurs 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).

Mancini et al. (2004) found that treating beef lumbar vertebrae with 1.5 or 2.5% ascorbic acid was effective in minimizing marrow discoloration, with the 2.5% ascorbic acid treatment being the most effective through a 5-d display.

Objectives

The objectives of Experiment 1 were to determine the prevalence of marrow discoloration in beef humeri, ribs, thoracic vertebrae, and scapulas in different packaging systems and to determine factors that may cause marrow discoloration. Experiment 2 was designed to evaluate the effects of applying different antioxidant treatments in preventing beef lumbar vertebrae marrow discoloration from occurring in different packaging systems.

Methodology

Experiment 1: Thirty-six beef humeri, ribs, scapulas, and thoracic vertebrae from two 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. 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. Bones were packaged into one of three package types: 1) PVC overwrap; 2) high-oxygen (80% O_2, 20% CO_2) MAP; and 3) ultra-low-oxygen (70% N_2,

30% CO_2) MAP. One each of a humerus, scapula, and thoracic vertebra, and two rib bone sections were placed in each package.

Experiment 2: Seventy-two beef backbones containing lumbar vertebrae from USDA Select and Choice carcasses were obtained from a commercial abattoir and held at 2°C in a cardboard box for either 6 or 14 d postmortem. Vertebrae were then cut similar to Experiment 1. Before packaging, bone sections were treated with one of the following antioxidant treatments: control with no treatment application; 1.25% or 2.5% ascorbic acid; 0.1% or 0.2% rosemary; or a combination treatment of 0.15% Origanox™ and 0.3% ascorbic acid. Origanox™ is a natural antioxidant extracted from the edible-herb family *Labitae*. A 1-ml aliquot of the given antioxidant solution, dissolved in distilled-deionized water, was pipetted onto the marrow cut surface of individual bones.

Packages were displayed under continuous fluorescent lighting (Experiment 1: 1614 lux, 3000K; Experiment 2: 2153 lux, 3000K) for 5 d at 2˚C.

Ten trained visual panelists scored bone-marrow color once daily for 5 d, beginning on d 0 about 1 h after packaging for both experiments. Mancini et al. (2004) developed the 7-point color scale used to score bones in high-oxygen MAP and PVC. Bone sections in high-oxygen MAP and PVC packages were scored according to the seven-point scale: 1) bright reddish-pink to red, 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, and 7) black discoloration. We developed the 7-point scale for ultra-low-oxygen MAP bones: 1) bright purplish-red or purplish-pink, 2) dull purplish-pink or purplish-red, 3) slightly grayish purple or pink, 4) grayish-purple or grayish-red, 5) moderately gray, 6) all gray or grayish-black, and 7) black discoloration. Panelists were instructed to score the porous portion of the bone marrow in half-point increments.

Instrumental CIE L*a*b* measurements were taken by using a 0.64-cm aperture (Illuminant A) on a Hunter labscan 2 in both experiments. Instrumental color measurements were taken on d 0, 2, and 4 of display.

Upon completion of display, bones were cleaned of all meat and connective tissue, vacuum packaged, and stored at -80°C until further analysis. As determined by previous research in our laboratory, scapulas did not have enough bone marrow to extract for chemical analysis. In Experiment 1, humeri, ribs, and thoracic-vertebrae marrow samples were pooled (three humeri, six ribs, or six thoracic vertebrae marrow samples) within bone and packaging type to obtain enough marrow to conduct the laboratory analyses. Marrow was not pooled in Experiment 2.

Bone marrow was extracted by using a modified procedure of Calhoun et al. (1998). Humeri, ribs, and vertebrae marrow (10-g, 1-g, and 1-g samples, respectively) were used to determine TBARS content on d 0 and 4 of display by using a slightly modified method described by Witte et al. (1970). Hydrophobic interaction HPLC was used to determine myoglobin and hemoglobin (Hb) pigment concentrations. Total iron (AOAC method 968.08) was measured with an atomic absorption spectrophotometer at 248.3 nm. Phosphorus content was measured at 700 nm. In Experiment 2, marrow was extracted and TBARS were measured on d 0, 2, and 4 of display.

Statistical analysis was completed by using the PROC MIXED procedure of SAS®. Means were separated by using Fisher's Protected LSD with Prasad-Rao-Jeske-Kackar-Harville standard errors and the Kenward-Roger degrees of freedom. Highest-order

interactions were reported; main effects were reported when no interactions were significant. Significance was determined at probability values of $P < 0.05$.

Results & Discussion

Ribs, scapulas, and thoracic vertebrae marrow discolored in PVC and high-oxygen MAP packaging according to visual color scores (Table 1). Oxidation was considerably less for humeri marrow than for ribs and thoracic vertebrae marrow and did not change over display time (Table 2). Marrow from humeri had much less ($P < 0.05$) total iron and Hb than marrow from ribs and thoracic vertebrae. Myoglobin was greater ($P < 0.05$) in marrow from ribs than in thoracic vertebrae and was undetected in humeri (Table 3). Lumbar vertebrae treated with 0.1 and 0.2% rosemary, and control treatments discolored ($P < 0.05$) in PVC and high-oxygen MAP. The combination of 0.15% Origanox™ + 0.3% ascorbic acid maintained desirable color through d 2 of display in PVC and high-oxygen MAP. Ascorbic acid treatments of 1.25 and 2.5% were equally effective ($P > 0.05$) in preventing bone discoloration in lumbar vertebrae packaged in high-oxygen MAP (Tables 4, 5, and 6). In all three packaging systems, bones held 14 d postmortem discolored more and had larger TBARS values than did those held 6 d. Instrumental color results, especially a* and chroma, supported visual color scores for both experiments (data not shown). Overall, ascorbic acid treatments were most effective in minimizing TBARS changes throughout display (data not shown).

Conclusions

Distinct bone marrow discoloration occurred in ribs, scapulas, and thoracic vertebrae packaged in PVC or high-oxygen MAP but was minimal in ultra-low oxygen MAP. Discoloration was not an issue in humeri. Bone marrow discoloration is likely caused primarily by oxidation of Hb but also by heme-catalyzed lipid oxidation. Untreated lumbar vertebrae discolored to relatively dark gray when packaged in PVC and high-oxygen MAP, but not when packaged in ultra-low-oxygen MAP. Ascorbic acid treatments, particularly the 2.5% application, were very effective in preventing bone-marrow discoloration, and were superior to other treatments.

References

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Tables and Figures

Table 1. Means[a] of visual color scores[b] for bone marrow from different bones in different package types from d 0 to 4 of display

		Day				
Bone	**Package[c]**	**0**	**1**	**2**	**3**	**4**
Humeri	PVC	1.5^{v}	2.0^{w}	2.2^{x}	2.3^{y}	2.6^{z}
Humeri	High	1.4^{w}	1.8^{x}	2.1^{y}	2.2^{y}	2.5^{z}
Humeri	Ultra-low	1.8^{w}	2.5^{x}	2.9^{y}	2.8^{y}	3.0^{z}
Ribs	PVC	1.7^{ev}	4.6^{dw}	5.0^{dx}	5.2^{dy}	5.3^{dz}
Ribs	High	1.4^{dw}	4.6^{dx}	5.1^{dy}	5.2^{dy}	5.3^{dz}
Ribs	Ultra-low	2.1^{w}	2.5^{x}	2.8^{y}	3.0^{z}	3.1^{z}
Scapulas	PVC	2.1^{ev}	4.2^{dw}	4.9^{dx}	5.1^{dy}	5.5^{dz}
Scapulas	High	1.8^{dv}	4.6^{ew}	5.2^{ex}	5.4^{ey}	5.6^{dz}
Scapulas	Ultra-low	2.3^{v}	3.1^{w}	3.4^{x}	3.6^{y}	3.7^{z}
Thoracic vertebrae	PVC	2.2^{ew}	5.3^{dx}	5.8^{dy}	5.8^{dy}	6.1^{dz}
Thoracic vertebrae	High	1.6^{dw}	5.2^{dx}	5.6^{dy}	5.8^{dyz}	5.9^{dz}
Thoracic vertebrae	Ultra-low	2.7^{w}	3.1^{x}	3.3^{y}	3.4^{yz}	3.5^{z}

[a]Standard error for all means = 0.14

[b]High-oxygen and PVC color scale: 1=bright reddish-pink to red, 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, and 7=black discoloration; Ultra-low-oxygen color scale: 1=bright purplish-red or purplish-pink, 2=dull purplish-pink or purplish-red, 3=slightly grayish purple or pink, 4=grayish-purple or grayish-red, 5=moderately gray, 6=all gray or grayish-black, 7=black discoloration

[c]PVC = polyvinyl choloride overwrap film; High = high-oxygen modified atmosphere packaging; and Ultra-low = ultra-low oxygen modified atmosphere packaging

[d,e]Means with different superscript letters within bone type and within columns (PVC vs High) differ ($P < 0.05$)

[v,w,x,y,z]Means with different superscript letters across rows (days) differ ($P < 0.05$)

Table 2. Means[a] of 2-thiobarbituric reactive substances[b] for bone marrow from different bones in different package types from d 0 and 4 of display

		Day	
Bone	**Package[c]**	**0**	**4**
Humeri	PVC	0.03^{z}	0.06^{z}
Humeri	High	0.03^{z}	0.06^{z}
Humeri	Ultra-low	0.03^{z}	0.04^{z}
Ribs	PVC	0.74^{z}	0.77^{ez}
Ribs	High	0.74^{y}	0.84^{ez}
Ribs	Ultra-low	0.74^{y}	0.65^{dz}
Thoracic vertebrae	PVC	0.67^{y}	1.04^{ez}
Thoracic vertebrae	High	0.67^{y}	1.01^{ez}
Thoracic vertebrae	Ultra-low	0.67^{y}	0.75^{dz}

[a]Standard error for humeri on d 0 and d 4 = 0.02; for ribs on d 0 and d 4 = 0.03; and for thoracic vertebrae on d 0 and d 4 = 0.03

[b]mg malonaldehyde/ 1000 g sample

[c]PVC = polyvinyl choloride overwrap film; High = high-oxygen modified atmosphere packaging; and Ultra-low = ultra-low oxygen modified atmosphere packaging

[d,e]Means with different superscript letters within bone types and within columns differ ($P < 0.05$)

[y,z]Means with different superscript letters across rows (days) differ ($P < 0.05$)

Table 3. Main-effects means[a] of total iron, phosphorus (P), hemoglobin (Hb), and myoglobin (Mb) for humeri, ribs, and thoracic vertebrae bone marrow

Bone	**Total Iron ppm**	**P ppm**	**Hb mg/g**	**Mb mg/g**
Humeri	8.12^{x}	867.92^{z}	4.5^{y}	–[b]
Ribs	237.35^{z}	847.41^{z}	159.5^{z}	0.530^{z}
Thoracic Vertebrae	219.08^{y}	574.32^{y}	153.0^{z}	0.313^{y}

[a]Standard error for humeri total iron = 0.54, P = 92.59, and Hb = 0.45; for ribs total iron = 10.8, P = 43.72, Hb = 13.2, and Mb = 0.05; for thoracic vertebrae total iron = 8.93, P = 42.10, Hb = 6.36, and Mb = 0.02

[b]Not detected in humeri bone marrow

[x,y,z]Means with different superscript letters within columns differ ($P < 0.05$)

Table 4. Means[a] of visual-color scores[b] for different antioxidant treatments of lumbar vertebrae packaged at 6 or 14 d postmortem in polyvinyl chloride overwrap and displayed from d 0 to 4

Antioxidant treatment	6 d Postmortem				
	Display day				
	0	**1**	**2**	**3**	**4**
Control	2.3^{ew}	5.1^{ewx}	5.3^{fx}	5.7^{fy}	5.9^{ez}
1.25% Ascorbic acid	1.5^{cdv}	2.0^{cdw}	2.5^{dx}	3.7^{dy}	4.6^{dz}
2.5% Ascorbic acid	1.5^{cdw}	1.7^{cwx}	2.0^{cx}	2.7^{cy}	3.1^{cz}
0.1% Rosemary	2.0^{ew}	4.9^{ex}	5.2^{fy}	5.4^{fyz}	5.8^{ez}
0.2% Rosemary	1.9^{dex}	5.0^{ey}	5.1^{fy}	5.5^{fz}	5.8^{ez}
0.15% Origanox™ + 0.3% Ascorbic acid	1.3^{cv}	2.3^{dw}	3.8^{ex}	4.7^{ey}	5.1^{dz}
Antioxidant treatment	**14 d Postmortem**				
	Display day				
	0	**1**	**2**	**3**	**4**
Control	3.7^{ew}	5.1^{fx}	5.4^{gy}	5.7^{eyz}	5.9^{fz}
1.25% Ascorbic acid	1.7^{cdv}	2.2^{cw}	3.3^{dx}	4.2^{dy}	4.9^{dz}
2.5% Ascorbic acid	1.8^{cdw}	2.1^{dwx}	2.3^{cx}	3.1^{cy}	3.9^{cz}
0.1% Rosemary	3.2^{ew}	4.8^{efy}	5.3^{fgz}	5.4^{ez}	5.4^{efz}
0.2% Rosemary	3.0^{dew}	4.5^{ex}	4.9^{fy}	5.3^{ey}	5.7^{fz}
0.15% Origanox™ + 0.3% Ascorbic acid	1.4^{cv}	2.8^{dw}	4.0^{ex}	4.6^{dy}	5.1^{dez}

[a]Standard error for all means = 0.20

[b]See footnote [b] Table 1

[c,d,e,f,g]Means with different superscript letters within columns within postmortem age differ ($P < 0.05$)

[v,w,x,y,z]Means with different superscript letters across rows within postmortem age differ ($P < 0.05$)

Table 5. Means[a] of visual-color scores[b] for different antioxidant treatments of lumbar vertebrae packaged at 6 or 14 days postmortem in high-oxygen modified-atmosphere packaging and displayed from d 0 to 4

Antioxidant treatment	6 d Postmortem				
	Display day				
	0	1	2	3	4
Control	1.4^{cx}	5.1^{ey}	5.5^{fz}	5.6^{ez}	5.8^{ez}
1.25% Ascorbic acid	1.3^{cw}	1.5^{cwx}	1.8^{cxy}	1.8^{cyz}	2.2^{cz}
2.5% Ascorbic acid	1.4^{cx}	1.5^{cxy}	1.8^{cxyz}	1.8^{cyz}	2.0^{cz}
0.1% Rosemary	1.4^{cx}	4.7^{dey}	5.2^{efz}	5.2^{ez}	5.5^{ez}
0.2% Rosemary	1.4^{cw}	4.5^{dx}	5.0^{ey}	5.3^{eyz}	5.5^{ez}
0.15% Origanox™ + 0.3% Ascorbic acid	1.4^{cv}	1.9^{cw}	3.1^{dx}	3.6^{dy}	4.1^{dz}
Antioxidant treatment	**14 d Postmortem**				
	Display day				
	0	1	2	3	4
Control	2.4^{ew}	4.4^{dx}	5.1^{ey}	5.5^{ez}	5.8^{ez}
1.25% Ascorbic acid	1.9^{cdx}	1.9^{cx}	2.0^{cxy}	2.4^{cyz}	2.6^{cz}
2.5% Ascorbic acid	1.7^{cx}	1.8^{cxy}	2.0^{cxy}	2.1^{cyz}	2.4^{cz}
0.1% Rosemary	2.4^{dew}	4.6^{dx}	5.1^{ey}	5.4^{eyz}	5.6^{ez}
0.2% Rosemary	2.3^{dew}	4.8^{dx}	5.4^{ey}	5.6^{eyz}	5.8^{ez}
0.15% Origanox™ + 0.3% Ascorbic acid	1.8^{cw}	1.9^{cw}	2.6^{dx}	3.2^{dy}	3.7^{dz}

[a]Standard error for all means = 0.20

[b]See footnote [b] Table 1

[c,d,e,f]Means with different superscript letters within columns within postmortem age differ ($P < 0.05$)

[v,w,x,y,z]Means with different superscript letters across rows within postmortem age differ ($P < 0.05$)

Table 6. Means[a] of visual-color scores[b] for different antioxidant treatments of lumbar vertebrae packaged at 6 or 14 d postmortem in ultra-low oxygen modified-atmosphere packaging and displayed from d 0 to 4

Antioxidant treatment	6 d Postmortem				
	Display day				
	0	1	2	3	4
Control	2.2^{cw}	2.5^{cwx}	2.6^{cxy}	2.9^{cyz}	3.2^{cz}
1.25% Ascorbic acid	2.1^{cx}	2.4^{cxy}	2.5^{cyz}	2.7^{cyz}	2.9^{cz}
2.5% Ascorbic acid	2.1^{cx}	2.3^{cxy}	2.4^{cxy}	2.7^{cyz}	2.9^{cz}
0.1% Rosemary	2.1^{cx}	2.5^{cy}	2.6^{cyz}	2.7^{cyz}	2.9^{cz}
0.2% Rosemary	2.1^{cw}	2.4^{cwx}	2.5^{cxy}	2.8^{cyz}	3.0^{cz}
0.15% Origanox™ + 0.3% Ascorbic acid	2.0^{cw}	2.2^{cwx}	2.5^{cxy}	2.7^{cy}	3.0^{cz}
Antioxidant treatment	**14 d Postmortem**				
	Display day				
	0	1	2	3	4
Control	2.7^{dy}	3.7^{dz}	3.7^{ez}	3.6^{ez}	3.6^{dz}
1.25% Ascorbic acid	2.1^{cx}	2.3^{cxy}	2.5^{cy}	3.0^{cdz}	2.9^{cz}
2.5% Ascorbic acid	2.1^{cx}	2.4^{cxy}	2.5^{cy}	2.7^{cyz}	2.9^{cz}
0.1% Rosemary	2.6^{dy}	3.5^{dz}	3.5^{dez}	3.5^{ez}	3.4^{dz}
0.2% Rosemary	2.8^{dy}	3.5^{dz}	3.5^{dez}	3.7^{ez}	3.5^{dz}
0.15% Origanox™ + 0.3% Ascorbic acid	2.3^{cdx}	2.5^{cx}	3.1^{dy}	3.4^{dez}	3.5^{dz}

[a]Standard error for all means = 0.20

[b]See footnote [b] Table 1; standard error for all means = 0.20

[c,d,e]Means with different superscript letters within columns within postmortem age differ ($P < 0.05$)

[w,x,y,z]Means with different superscript letters across rows within postmortem age differ ($P < 0.05$)