EFFECTS OF ANTIOXIDANTS ON BONE MARROW DISCOLORATION IN BEEF LUMBAR VERTEBRAE IN DIFFERENT PACKAGING SYSTEMS

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Summary

To evaluate how antioxidants might prevent bone marrow discoloration, beef lumbar vertebrae held at 35.6°F for 6 or 14 days postmortem before packaging were cut into 1-inch-thick sections and packaged into 1) PVC overwrap; 2) high-oxygen (80% O_2, 20% CO_2) modified atmosphere packages (MAP); or 3) ultra-low-oxygen (70% N_2, 30% CO_2) MAP. Before packaging, bones were treated with: no treatment application (control); 1.25% or 2.5% ascorbic acid; 0.1% or 0.2% rosemary; or a combination treatment of 0.15% Origanox™ + 0.3% ascorbic acid. Packages were displayed under continuous fluorescent lighting for 4 days at 35.6°F. Untreated lumbar vertebrae and those treated with 0.1 or 0.2% rosemary discolored to gray or grayish-black, as measured by visual color scores and instrumental a* values, in PVC and high-oxygen MAP. The 1.25% ascorbic acid and 0.15% Origanox™ + 0.3% ascorbic acid were able to maintain desirable color scores through day 2 of display in PVC and high-oxygen MAP, but not after 4 days. The 2.5% ascorbic acid treatment was most effective in preventing discoloration and maintaining initial color in both PVC and high-oxygen MAP. In ultra-low-oxygen MAP, the 1.25% ascorbic acid treatment was as effective as the 2.5% ascorbic acid treatment in preventing bone marrow discoloration. In general, discoloration tended to be greater in bones held 14 days postmortem before packaging than in those held 6 days. Ascorbic acid treatments, particularly the 2.5% application, were effective in preventing bone marrow discoloration.

Introduction

Bone marrow discoloration, and its occurrence in modified atmosphere packaged (MAP) bone-in beef retail cuts, has been observed by industry personnel, especially meat retailers. Consumers may perceive bone discoloration as unwholesome, and it might affect their acceptance of a fresh meat product. Bone marrow discoloration has been reported in high-oxygen MAP beef and pork and also in cuts packaged in polyvinyl chloride film (PVC).

Some researchers have found that supplementing pigs with vitamin E (198 and 207 ppm) for 105 days increased a* (redness) values of lumbar vertebrae over non-supplemented pigs in a 5-day display. Other published literature found that treating beef lumbar vertebrae with 1.5 or 2.5% ascorbic acid was effective in minimizing lumbar vertebrae discoloration, with the 2.5% ascorbic acid treatment being the most effective through a 5-day display.

Ascorbic acid (vitamin C) is a generally recognized as safe (GRAS) substance and can be applied at no more than 500 ppm to delay discoloration. Several studies have indicated that ascorbic acid can extend beef color stability. Rosemary in powder, extract, or oleoresin form has also been shown to improve beef color stability and inhibit oxidation. The Food and Drug Administration has given rosemary GRAS status as well.

The objectives of this experiment were to evaluate the effects of applying antioxidant

treatments in preventing bone marrow discoloration from occurring in beef lumbar vertebrae.

Procedures

Seventy-two beef lumbar vertebrae from USDA Select and Choice carcasses obtained from a commercial abattoir were held at 35.6°F for either 6 or 14 days postmortem. Lumbar vertebrae were cut into 1-inch-thick sections and packaged into one of three packages: 1) polyvinyl chloride film (PVC) overwrap; 2) high-oxygen (80% O_2, 20% CO_2) modified atmosphere package (MAP); and 3) ultra-low-oxygen (70% N_2, 30% CO_2) MAP. 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 extract; or a combination treatment of 0.15% Origanox™ WS and 0.3% ascorbic acid. Origanox™, a natural antioxidant that is extracted from edible herbs, is easily dissolved in water. An aliquot of the given antioxidant solution was pipetted onto the marrow cut surface of individual bones. In each package, there was one vertebra section for each of the antioxidant treatments and a control. The PVC samples were packaged in foam trays overwrapped with oxygen-permeable film. Bones assigned to high-oxygen and ultra-low-oxygen MAP packages were packaged in rigid plastic trays and covered with barrier lidding film. Each ultra-low-oxygen MAP had one activated oxygen scavenger added to the package. Within each individual package, lumbar vertebrae sections were from the same animal.

Packages were displayed under continuous fluorescent lighting for 4 days at 35.6°F. Packages were rotated twice daily to maintain a random sample placement.

Instrumental CIE a* measurements were collected by using a Hunter labscan 2. CIE a* measures red (+) to green (-). Immediately after opening packages, bone sections were scanned. Instrumental color scores were taken on day 0, 2, and 4 of display.

Ten trained panelists scored the porous portion of bone marrow for visual color once each day for 5 days, beginning on day 0. High-oxygen MAP and PVC packages were scored according to a 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. Ultra-low-oxygen MAP bones were scored according to a different seven-point 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, and 7) black discoloration.

Lumbar vertebrae marrow was extracted and analyzed for 2-thiobarbituric acid reactive substances (TBARS), a measure of oxidation.

Results and Discussion

The effects of antioxidant treatments on lumbar vertebrae packaged in PVC are shown in Table 1. For lumbar vertebrae held for 6 days postmortem before being cut and displayed, the control and both rosemary treatments were distinctly gray by day 1 and stayed gray or grayish-black throughout display in PVC packages. Vertebrae treated with 1.25% ascorbic acid turned ‘slightly grayish-pink or -red’ on day 3 of display, and those treated with 2.5% ascorbic acid turned only ‘slightly grayish-pink or -red’ by the last day of display in PVC packages. Vertebrae treated with the combination of 0.15% Origanox™ + 0.3% ascorbic acid did not turn ‘grayish-pink or -red’ until day 2 of display in PVC packages, but were ‘moderately gray’ at the end of display. Visual color differences between lumbar vertebrae held 6 vs. 14 days and packaged in PVC suggest that bones held for

longer times before packaging for display tended to discolor a little faster.

Treatment comparisons within high-oxygen MAP are presented in Table 2. When vertebrae were held 6 days postmortem before cutting and packaging, the control and both rosemary treatments were 'grayish-pink or -red' or 'moderately gray' by day 1 and stayed 'moderately gray' or 'grayish-black' throughout display in high-oxygen MAP. Vertebrae remained 'reddish-pink' throughout the 4-day display when treated with 1.25 or 2.5% ascorbic acid. Vertebrae treated with the combination of 0.15% Origanox™ + 0.3% ascorbic acid did not turn 'grayish-pink or -red' until day 2 of display when packaged in high-oxygen MAP. Lumbar vertebrae packaged in high-oxygen MAP held 14 days postmortem showed similar results to those held 6 days postmortem.

Table 3 lists treatment comparisons for lumbar vertebrae held 6 or 14 days in ultra-low-oxygen MAP. Lumbar vertebrae held 6 days and packaged in ultra-low-oxygen MAP remained 'purplish-red or -pink' for all treatments except control, 0.2% rosemary, and the combination of 0.15% Origanox™ + 0.3% ascorbic acid on day 4, when the latter became 'slightly grayish-purple or pink.' Lumbar vertebrae held 14 days and packaged in ultra-low-oxygen MAP were either a 'dull purplish-pink or -red' or 'slightly grayish-purple or pink' throughout the 4-day display. Visual color differences between lumbar vertebrae held 6 and 14 days packaged in ultra-low-oxygen MAP generally indicated that bones held 14 days discolored more than bones held 6 days postmortem. Although antioxidant treatments are not needed as much in ultra-low-oxygen MAP, the 1.25% ascorbic acid treatment was as effective as the 2.5% ascorbic acid treatment.

Control lumbar vertebrae darkened dramatically in PVC and high-oxygen MAP; discoloration in ultra-low-oxygen MAP was much less extensive. In general, 0.1 and 0.2% rosemary treatments were not effective in preventing discoloration in PVC and high-oxygen MAP. The 2.5% ascorbic acid treatment was most effective in preventing discoloration and maintaining initial color in both PVC and high-oxygen MAP. The 1.25% ascorbic acid and the combination of 0.15% Origanox™ + 0.3% ascorbic acid were able to maintain desirable color scores through day 2 of display in PVC and high-oxygen MAP but not through day 4 of display.

Other research indicates that ascorbic acid does not have any effect on the color of the ribeye muscle. This is important because the positive effects of ascorbic acid application to bone to prevent discoloration should not cause negative effects on the muscle color.

Overall, mean a* values for lumbar vertebrae held 6 and 14 days and packaged in PVC, high-oxygen MAP, and ultra-low-oxygen MAP corresponded to visual color score trends (data not shown). Lumbar vertebra treated with ascorbic acid had higher or no change in a* values over display time.

Lumbar vertebrae held 6 days and packaged in PVC had smaller TBARS values for most treatments on day 2 and(or) day 4 than did those held 14 days (data not shown). The ascorbic acid treatments (1.25%, 2.5%, and combination) generally were effective in minimizing changes in TBARS during display. The rosemary treatments and control resulted in distinct increases in TBARS when vertebrae were held 14 days before display. For all treatments and days, including the initial samples on day 0, lumbar vertebrae held 6 days and packaged in high-oxygen MAP had smaller TBARS values than did those held 14 days (data not shown). In all three packaging systems, bones held 14 days postmortem had larger TBARS values than did those held 6 days. Overall, ascorbic acid treatments were most effective in minimizing TBARS changes throughout display.

Although lipid and pigment oxidation are closely related, it is not completely understood exactly how they relate to each other. It is suggested that lipid oxidation produces a radical that, in turn, acts directly to encourage pigment oxidation and/or causes the pigment-reducing systems to be indirectly damaged.

Bone darkening may be due to an oxidation reaction or a combination of oxidation reactions. When bones become discolored, this could be through oxidation of the hemoglobin and myoglobin present in bone marrow. Ascorbic acid can reduce methemoglobin in both aerobic and anaerobic solutions. Also, the heme proteins present could catalyze lipid oxidation reactions. Vertebrae bone marrow has a lot of iron and hemoglobin present and seems to discolor more quickly and severely than other bones. The presence of more polar lipid and cell membranes in such bone marrow may also provide a better environment for lipid oxidation to take place, especially with iron present. Previous research in our laboratory indicated that TBARS values in arm bones did not increase throughout display. Because arm bone marrow is mostly yellow (adipose) marrow, this suggests that lipid oxidation is not the primary form of oxidation taking place to cause bone marrow discoloration. One possibility is that bone marrow discoloration could be caused by lipid oxidation that is catalyzed by iron. As a consequence, bone marrow discoloration may be caused by a combination of oxidation reactions.

In summary, untreated lumbar vertebrae discolored when packaged in PVC and high-oxygen MAP. Rosemary treatments were not effective in preventing bone discoloration in lumbar vertebrae packaged in PVC and high-oxygen MAP. Ascorbic acid treatments, particularly the 2.5% application, were very effective in preventing bone marrow discoloration, and were superior to other treatments of our study.

Table 1. Visual Color Score[ab] for Different Antioxidant Treatments of Lumbar Vertebrae Packaged at 6 or 14 Days Postmortem in Polyvinyl Chloride Overwrap from Day 0 to 4 of Display at 35.6°F

		Display Day				
Antioxidant Treatment	Days Postmortem	0	1	2	3	4
Control	6	2.3^{ev}	5.1^{ewx}	5.3^{fx}	5.7^{fy}	5.9^{ez}
1.25% Ascorbic acid	6	1.5^{cdv}	2.0^{cdw}	2.5^{dx}	3.7^{dy}	4.6^{dz}
2.5% Ascorbic acid	6	1.5^{cdw}	1.7^{cwx}	2.0^{cx}	2.7^{cy}	3.1^{cz}
0.1% Rosemary	6	2.0^{ew}	4.9^{ex}	5.2^{fy}	5.4^{fyz}	5.8^{ez}
0.2% Rosemary	6	1.9^{dex}	5.0^{ey}	5.1^{fy}	5.5^{fz}	5.8^{ez}
0.15% Origanox™ + 0.3% Ascorbic acid	6	1.3^{cv}	2.3^{dw}	3.8^{ex}	4.7^{ey}	5.1^{dz}
Control	14	3.5^{ew}	5.1^{fx}	5.4^{gy}	5.7^{eyz}	5.9^{fz}
1.25% Ascorbic acid	14	1.7^{cdv}	2.2^{dw}	3.3^{dx}	4.2^{dy}	4.9^{dz}
2.5% Ascorbic acid	14	1.8^{cdw}	2.1^{cwx}	2.3^{cx}	3.1^{cy}	3.9^{cz}
0.1% Rosemary	14	3.2^{ew}	4.8^{efy}	5.3^{fgz}	5.4^{ez}	5.4^{efz}
0.2% Rosemary	14	3.0^{dew}	4.5^{ex}	4.9^{fy}	5.3^{ey}	5.7^{fz}
0.15% Origanox™ + 0.3% Ascorbic acid	14	1.4^{cv}	2.8^{dw}	4.0^{ex}	4.6^{dy}	5.1^{dez}

[a]Standard error for all means = 0.20.

[b]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.

[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 differ ($P<0.05$).

Table 2. Visual Color Scores[ab] for Different Antioxidant Treatments of Lumbar Vertebrae Packaged at 6 or 14 Days Postmortem in High-oxygen Modified Atmosphere Packaging from Day 0 to 4 of Display at 35.6°F

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

[a]Standard error for all means = 0.20.

[b]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.

[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 differ ($P<0.05$).

Table 3. Visual Color Score[ab] for Different Antioxidant Treatments of Lumbar Vertebrae Packaged at 6 or 14 Days Postmortem in Ultra-low-oxygen Modified Atmosphere Packaging from Day 0 to 4 of Display at 35.6°F

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

[a]Standard error for all means = 0.20.

[b]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,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 differ ($P<0.05$).