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Comparison of ascorbic acid and sodium erythorbate: Effects on the 24 h display colour of beef lumbar vertebrae and *longissimus lumborum* packaged in high-oxygen modified atmospheres ☆

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Abstract

Sodium erythorbate and ascorbic acid were compared as a means to stabilize surface colour of bone-in beef steaks in high-oxygen modified atmosphere (80% oxygen and 20% carbon dioxide). Bone-in strip loins ($n = 8$) were fabricated into 1.9 -cm thick steaks, of which both the lumbar vertebrae and *longissimus lumborum* were topically treated with either ascorbic acid or sodium erythorbate (0, 0.05, 0.1, 0.5, 1.0, or 1.5%, wt/wt basis). Colour ($L^*a^*b^*$) was evaluated before treatment and 24 h after packaging (display at 1 °C). Sodium erythorbate was as effective as ascorbic acid for inhibiting vertebrae discolouration ($P > 0.05$). Either reducing agent at 0.5, 1.0, or 1.5% improved ($P < 0.05$) vertebrae redness (compared with 0%, 0.05% and 0.1%). No detrimental effects on muscle colour were observed. When selecting antioxidants intended for bone-in beef steaks displayed in high-oxygen packaging, sodium erythorbate may be a cost effective substitute for ascorbic acid.

Keywords: Bone marrow colour; High-oxygen modified atmosphere; Ascorbic acid; Sodium erythorbate; Lumbar vertebrae discolouration

1. Introduction

Discolouration of marrow on the surface of cut bones influences the shelf life of bone-in beef cuts (Grobbel, Dikeman, Smith, Kropf, & Milliken, 2006; Grobbel et al., 2006; Mancini, Hunt, Hachmeister, Kropf, & Johnson, 2004, 2005). Although industry has adopted case-ready, high-oxygen, modified-atmosphere packaging (MAP) to promote muscle-colour stability (Seyfert et al., 2004), it appears that increased storage time and high-oxygen MAP tend to accelerate bone discolouration (Grobbel, Dikeman, & Smith, 2006; Grobbel et al., 2006; Lanari et al., 1995; Mancini et al., 2004, 2005; Warren et al., 1992). As a result, the beef industry has expressed an interest in postmortem technologies aimed at improving the colour stability of marrow from cut bones. Ascorbic acid in amounts of 0.5% or greater applied directly to the marrow of exposed cut surfaces inhibits beef rib and vertebrae marrow discolouration (Grobbel et al., 2006; Mancini et al., 2004). Therefore, ascorbic acid may be adopted by industry as a means of improving marrow colour stability because, when used at the correct levels, ascorbic acid can also stabilize muscle colour (Harbers, Harrison, & Kropf, 1981; Lee, Hendricks, & Cornforth, 1999; Mitsumoto, Cassens, Schaefer, & Scheller, 1991; Shivas et al., 1984).

Previous research demonstrated that application of ascorbic acid to inhibit bone discolouration did not

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adversely affect lean tissue discolouration (Mancini et al., 2004). In addition to effectiveness, industry is also seeking methodologies that are cost efficient. Ascorbic acid is more expensive than its salt isomer, sodium erythorbate, whose effects on bone marrow colour have not yet been evaluated. In addition to a difference in cost, the two isomers differ in solution pH, with erythorbate being closer to normal meat pH.

Our objectives were to compare the utility of two reducing agents, ascorbic acid and sodium erythorbate, at five levels (0%, 0.05%, 0.1%, 0.5%, 1.0% or 1.5%) for improving the colour stability of beef lumbar vertebrae packaged in high-oxygen MAP at 24 h after packaging. The effects of the two reducing agents on *Longissimus lumborum* colour also were evaluated.

2. Materials and methods

2.1. Experimental design

The experimental design used to compare the effects of ascorbic acid and erythorbate on marrow and muscle colour was a randomized complete block with repeated measures. Bone-in strip loins ($n = 8$, 6-d postmortem; IMPS#175; NAMP, 1997; USDA, 1996) served as blocks, and 1.9 -cm thick bone-in steaks within each loin received one of eleven treatments. The treatments had a 2-way treatment structure of reducing agent (ascorbic acid and sodium erythorbate) and concentration (0.05–1.5%). In addition, a negative control (0% ascorbic acid / erythorbate) was tested. Preliminary data (not shown) from our lab suggested that a positive control using distilled water has no effect on marrow colour, resulting in similar discolouration when compared with untreated vertebrae (negative controls). Following treatment, steaks were individually packaged in 80% oxygen, and displayed for 24 h at 1 °C. This display time was chosen because previous research (Mancini et al., 2004) has suggested that untreated lumbar vertebrae will discolour significantly within 24 h after packaging in high-oxygen.

The instrumental colour of both the porous bone marrow of lumbar vertebrae and *longissimus lumborum* muscle was measured prior to treatment (initial colour; 0 h) and again at 24 h post packaging on each of the 11 steaks taken from the bone-in strip loins, resulting in 176 total observations (2 evaluation times × 8 loins × 11 steaks per loin). Thus, time of colour evaluation (0 or 24 h) during display (1 °C) in high-oxygen modified atmosphere packaging was a repeated measure (Milliken & Johnson, 1992). Only fresh-cut steak surfaces with at least 2.54-cm^2 of freshly exposed lumbar marrow were selected for treatment and colour analysis.

2.2. Experimental treatments

Each steak within a loin was assigned randomly to 1 of 11 treatments, which consisted of a negative control (0% antioxidant) and 0.05%, 0.1%, 0.5%, 1.0%, and 1.5% ascorbic acid or sodium erythorbate. Both ascorbic acid and sodium erythorbate treatments (Sigma, St. Louis, MO) were prepared on a wt/wt basis using distilled water. Previous work in our laboratory (Mancini et al., 2004) indicated that for short display periods, there was little advantage to using greater than 1.5% ascorbic acid; thus, this amount was selected as the maximum for this study.

The assigned treatment (5 mL) was pipetted over the entire fresh cut surface of each steak, including the bone surface. To insure even treatment distribution, ascorbic acid was spread over both the muscle and marrow surfaces using disposable, L-shaped sterile cell spreaders (Heathrow Scientific, Vernon Hills, IL). Due to the porous nature of the marrow, some ascorbic acid was absorbed by the marrow, while some acid remained on the surface.

2.3. Packaging

Steaks were packaged individually in pre-formed trays (polypropylene, 0.1 cc oxygen/tray/24 h at 22.7 °C/0% relative humidity, 2.0 g water vapour/64,516 cm^2/24 h at 37.8 °C/100% relative humidity; Sealed Air Corp., Duncan, SC) containing 80% oxygen and 20% carbon dioxide (Certified Standard; Airgas Specialty Gases, Los Angeles, CA) and sealed with MAP-Shield AF shrinkable barrier film (0.02 cc oxygen/645.16 cm^2/24 h at 10 °C and 80% relative humidity, 0.92 g water vapor/645.16 cm^2/24 h at 37.8 °C and 100% relative humidity; Honeywell, Morristown, NJ) using a Ross Inpack Jr. (Model S3180; Ross Industries, Inc., Midland, VA). Due to the headspace within the high-oxygen packages, bone did not come into contact with the packaging film.

2.4. pH and package gas composition

Treatment solution pH values were determined using an Accumet® combination electrode (Fischer Scientific, Fair Lawn, NJ) attached to an Accumet® 50 pH meter (Fischer Scientific, Fair Lawn, NJ). The surface pH of each steak (*Longissimus lumborum* muscle) was measured using a flat surface epoxy body combination pH electrode (Fisher Scientific, Fair Lawn, NJ) attached to an Accumet® 50 pH meter. Oxygen and carbon dioxide concentrations in MAP packages were determined immediately after packaging (extra packages) and 24 h after packaging (all displayed packages) using a MOCON PacCheck (Model 650 Dual Head Space Analyzer; MOCON, Minneapolis, MN).

2.5. Display conditions

Packages were displayed for 24 h at 1 °C in open-top display cases (Unit Model DMF8; Tyler Refrigeration Corp., Niles, MI) under continuous 1614 lux of fluorescent light (34 W, Ultralume 30, 3000 K; Phillips, Bloomfield, NJ). To simulate proper cold chain management, 1 °C was chosen for display. Case temperatures were monitored

at the package level using temperature loggers (RD-TEMP-XT; Omega Engineering, Inc., Stamford, CT).

2.6. Instrumental colour

Instrumental colour (CIE $L^*a^*b^*$, Illuminant A) of the *longissimus lumborum* muscle and lumbar vertebrae from each steak was evaluated at 0 and 24 h after packaging in high-oxygen. Initial colour (0 h) was evaluated 30 min after fabrication, prior to topical antioxidant treatment and packaging. Vertebrae colour was measured using a Hunter-Lab MiniScan™ XE Spectrophotometer (Model 45/0 SAV, 0.64 cm aperture, 10° standard observer; Hunter Associates Laboratory, Inc., Reston, VA). Muscle colour was measured using a HunterLab MiniScan™ XE Spectrophotometer (Model 45/0 LAV, Illuminant A, 3.18 cm aperture, 10° standard observer; Hunter Associates Laboratory, Inc., Reston, VA). Chroma $[(a^{*2} + b^{*2})^{1/2}]$ was used to determine the amount of surface graying on vertebrae sections. Graying was calculated according to the following: 24 h chroma value−initial chroma value. A negative number indicated an increase of surface graying and a positive number represents an increase in red colour intensity. From each steak, three subsamples per vertebrae and muscle were averaged for statistical analysis.

2.7. Statistical analysis

Muscle and bone data were analyzed separately, and thus *longissimus lumborum* and vertebrae sections within a loin were each considered experimental units. Using a 1-way means model with 11 treatments, type-3 tests of fixed effects for treatment, time, and treatment × time were evaluated using the MIXED procedure of SAS (SAS Institute, Inc., Cary, NC). *F*-test denominator degrees of freedom were estimated using the Satterthwaite adjustment. Loin was considered a random effect. The covariance–variance structure for time and treatments within a loin was modeled, and the most appropriate structure for the repeated measure was determined using Akaike's information criterion, Schwartz's Bayesian information criterion, and the null likelihood ratio test.

Least square means for protected *F*-tests ($P < 0.05$) were separated according to the following approach. Single degree of freedom orthogonal contrasts were first used to statistically verify that raw material initial colour prior to treatment application was similar. As expected, initial instrumental colour prior to treatment was similar ($P = 0.96$ for a^* and $P = 0.89$ for chroma) for marrow from all vertebrae. Because there was no treatment effect on colour prior to ascorbic or erythorbate addition, initial values were averaged to obtain the best estimate of true sample means. Average initial colour values from each treatment within each block were then compared with 24 h treatment effects using single degree of freedom orthogonal contrasts. Treatment and concentration effects at 24 h also were determined using contrasts.

3. Results and discussion

3.1. Treatment solution and meat pH

The pH values of sodium erythorbate solutions (0.05% = 4.59; 0.1% = 5.36; 0.5% = 5.60; 1.0% = 5.89; 1.5% = 6.17) were greater than those for ascorbic acid solutions (0.05% = 3.13; 0.1% = 2.99; 0.5% = 2.72; 1.0% = 2.66; 1.5% = 2.58). The pH of sodium erythorbate solutions increased as concentration increased, whereas ascorbic acid solutions became more acidic with increasing concentration. Treatment had little influence on muscle surface pH (data not shown), likely because of the meat's buffering ability. Package gas composition before display was 79.3% oxygen and 19.9% carbon dioxide. At the end of display (1 d at 1 °C), there was little to no change in gas composition.

3.2. Reducing agent effects on vertebrae colour

Vertebrae treated with ascorbic acid or sodium erythorbate were similar in redness ($P > 0.05$) after 24 h of display, regardless of concentration (Fig. 1). There was a significant concentration effect on 24 h redness, independent of antioxidant type; implying that ascorbic acid and sodium erythorbate performed similarly ($P > 0.05$).

3.3. Concentration effects on vertebrae colour

Compared with initial redness, untreated vertebrae discoloured significantly (lower a^* values) within the first

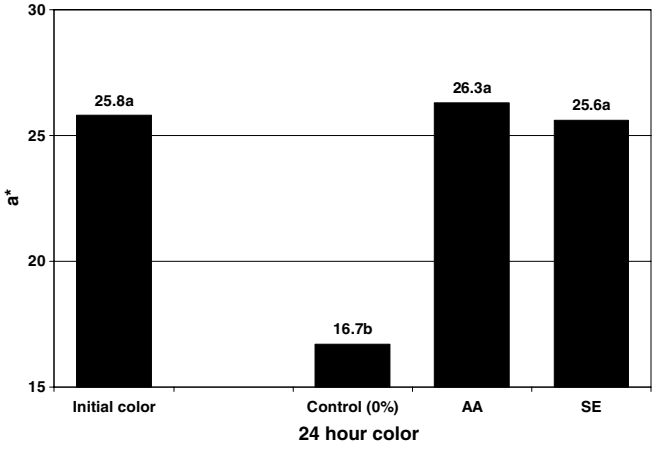

Fig. 1. Comparison of ascorbic acid (AA) and sodium erythorbate (SE) effects on the surface redness (a^*) of marrow from beef lumbar vertebrae packaged in high-oxygen (80% oxygen and 20% carbon dioxide) modified-atmosphere packaging and displayed for 1 d at 1 °C. Initial vertebrae surface colour was evaluated 30 min after fabrication, but prior to treatment and packaging. Treatment effects on a^* values were evaluated 24 h after treatment and packaging. Control refers to untreated samples (0% antioxidant). [ab]Least square means with a different letter differ ($P < 0.05$). Standard errors for comparisons of initial and 24 h colour LSMeans = 0.38. Standard errors for comparing 24 h colour LSMeans = 0.52.

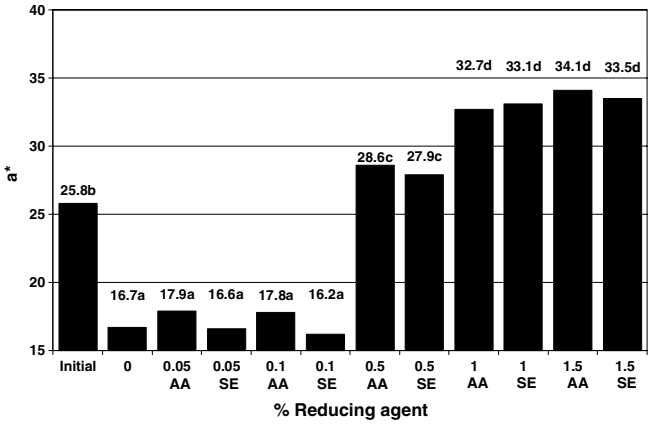

Fig. 2. Effects of ascorbic acid (AA) and sodium erythorbate (SE) concentration on the surface redness (a^*) of marrow from beef lumbar vertebrae packaged in high-oxygen (80% oxygen and 20% carbon dioxide) modified-atmosphere packaging and displayed for 1d at 1 °C. Initial vertebrae surface colour was evaluated 30 min after fabrication, but prior to treatment and packaging. All other data represent treatment effects on a^* values 24 h after packaging. Negative controls at 24 h of display are represented by 0%: other concentrations represent aqueous wt/wt amounts. [abcd]Least square means with a different letter differ ($P < 0.05$). The standard error for comparisons of initial and 24 h colour LSMeans = 0.59. The standard error for comparing 24 h color LSMeans = 0.77.

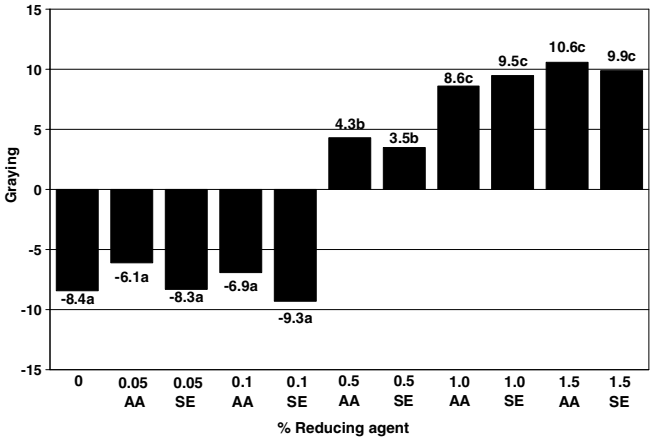

Fig. 3. Effects of ascorbic acid (AA) and sodium erythorbate (SE) concentration on the surface graying values of marrow from beef lumbar vertebrae packaged in high-oxygen (80% oxygen and 20% carbon dioxide) modified-atmosphere packaging and displayed for 1d at 1 °C. Negative controls at 24 h of display are represented by 0%: other concentrations represent aqueous wt/wt amounts. Graying = 24 h chroma value–initial chroma value. A negative value indicates an increase of surface graying and a positive value represents an increase in red colour intensity. Initial vertebrae surface colour was evaluated 30 min after fabrication, but prior to treatment and packaging. [abc]Least square means with a different letter differ ($P < 0.05$). The standard error for comparing 24 h colour = 1.72.

24 h of high-oxygen MAP display (Fig. 2). Conversely, vertebrae treated with either ascorbic acid or sodium erythorbate at 0.5, 1.0, or 1.5% were more red ($P < 0.05$) than both fresh-cut vertebrae prior to packaging and untreated controls after 24 h in high-oxygen MAP. Using only 0.05% or 0.1% ascorbic acid or sodium erythorbate did not prevent vertebrae discolouration. Previous research noted a similar increase in bone marrow colour stability when 0.5% ascorbic acid or greater was used (Grobbel et al., 2006; Mancini et al., 2004) and no effect when lesser amounts of ascorbic acid were applied (Mancini et al., 2004). However, the authors in the aforementioned studies did not evaluate sodium erythorbate.

Similar to control product, marrow from vertebrae treated with 0.05% or 0.1% antioxidant also had significant surface graying (decrease in chroma) after packaging in high-oxygen MAP ($P < 0.05$; Fig. 3). Conversely, vertebrae treated with 1.0% or 1.5% antioxidant demonstrated no surface graying (no chroma decrease) after packaging and became more "intense red" (increased in chroma) during display ($P < 0.05$ change in chroma after packaging). Treatments that showed no effect in preventing vertebrae discolouration (0%, 0.05% and 0.1% antioxidant) also resulted in the lowest b^* values (data not shown). Sodium erythorbate, the less expensive salt isomer of ascorbic acid, performed similarly to ascorbic acid with respect to colour, regardless of their distinct difference in pH.

Although marrow discolouration is often referred to as "bone darkening" or "bone blackening" by the meat industry, our data suggests that marrow lightness increased during display (initial L^* of 47.2 compared with an L^* of 50.7 on d 1; data not shown). It is likely that the changes in L^* were representative of "bone lightening" rather than "bone darkening" because vertebrae treated with 0, 0.05, and 0.1% antioxidant demonstrated a significant surface graying instead of a blackening. This graying paralleled a loss of redness intensity (numerical decrease in chroma; Aberle, Forrest, Gerrard, & Mills, 2001). Longer display may have resulted in further discolouration from gray to black. Although vertebrae treated with 0.5%, 1.0% and 1.5% antioxidant also lightened after packaging, there was no surface graying or discolouration evident in these samples.

3.4. Reducing agent effects on longissimus lumborum redness

Following 24 h in high oxygen MAP, *longissimus lumborum* treated with ascorbic acid or erythorbate tended to be less red ($P > 0.05$) than untreated steaks (24 h negative control $a^* = 30.5$; ascorbic acid $a^* = 29.7$; erythorbate $a^* = 29.6$; standard error = 0.48). Thus, the difference in redness between untreated control steaks and steaks treated with ascorbic acid or sodium erythorbate was not significant and treated steaks showed no signs of discolouration (metmyoglobin accumulation). No significant effect on *longissimus lumborum* redness when ascorbic acid was applied agrees with previous research (Mancini et al., 2004). This demonstrates that using sodium erythorbate to limit bone discolouration would not be expected to have a negative effect on lean tissue redness. Steaks from all concentrations of ascorbic acid or sodium erythorbate had higher ($P < 0.05$; standard error = 0.37) a^* values than freshly cut steaks prior to packaging (initial $a^* = 28.1$)

likely because the high-oxygen atmosphere (80% oxygen) promoted a deeper layer of oxymyoglobin compared to initial surface colour prior to packaging (Seyfert, Hunt, Mancini, Kropf, & Stroda, 2004; Taylor, 1990).

Ascorbic acid is both an antioxidant and a prooxidant; thus, the optimal level of use in meat products varies with several factors (Lee & Hendricks, 1997; Lee et al., 1999; Shivas et al., 1984). In our work, ascorbic acid and sodium erythorbate had relatively no affect on muscle redness, but significantly improved lumbar vertebrae 24 h colour stability when used at either 0.5%, 1.0%, or 1.5%. Other work has suggested that ascorbic acid levels between 1% and 10% may be effective for stabilizing muscle colour (Harbers et al., 1981; Mitsumoto, Cassens, Schaefer, & Scheller, 1991; Mitsumoto et al., 1991).

Although the mechanism responsible for marrow discolouration was not evaluated, it is likely that ascorbic acid and sodium erythorbate maximized colour stability on the surface of lumbar vertebrae by suppressing hemoglobin oxidation (Mancini et al., 2005). Although numerous modes of action are possible, reduction of oxidized heme pigments is most likely. Nevertheless, neutralization of radicals, oxygen scavenging, hydrogen donation, and metal chelation may have played a role in each reducing agent's effectiveness.

4. Conclusions

Treating bone-in beef steaks with ascorbic acid or sodium erythorbate at levels between 0.5 and 1.5% will inhibit vertebrae discolouration, while also having no oxidizing effects on *longissimus lumborum* colour early in display. Applying sodium erythorbate to the surface of cut lumbar vertebrae is as effective as treatment with ascorbic acid and may have a cost advantage. Because the *longissimus lumborum* portion of bone-in beef steaks is relatively colour stable, the colour life benefits associated with case-ready, high-oxygen MAP may be somewhat negated by bone discolouration if a reducing agent such as ascorbic acid or sodium erythorbate is not utilized.

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