

Novel nitrite-embedded packaging improves surface redness of dark-cutting longissimus steaks

Ranjith Ramanathan,¹ Rachel M. Mitacek, Sabra D. Billups, Ravi Jadeja, Morgan M. Pfeiffer, Gretchen G. Mafi, and Deborah L. VanOverbeke

Department of Animal Science, Oklahoma State University, Stillwater, OK 74078

ABSTRACT: The objective of this research was to determine the effects of nitrite-embedded/FreshCase packaging on lean color of dark-cutting beef. Eight dark-cutting (pH > 6.0) and eight USDA Low Choice (normal-pH; mean pH = 5.6) beef strip loins (*longissimus lumborum*) were selected 3 day after harvest. Each dark-cutting loin was sliced into five 2.5-cm thick steaks and randomly assigned to 1) dark-cutting steak packaged in polyvinyl chloride film (PVC) overwrap, 2) dark-cutting steak packaged in nitrite-embedded film, 3) dark-cutting steaks dipped in 0.2% rosemary solution and packaged in nitrite-embedded film, and 4) dark-cutting steak dipped in deionized water and packaged in nitrite-embedded film. The fifth dark-cutting steak was used to determine pH and proximate composition. Normal-pH choice loins were used as a control and each loin was randomly assigned to either PVC overwrap for retail display or to determine pH and proximate composition. Packages were placed in coffin-style retail display cases under continuous fluorescent lighting for 3 days. A HunterLab MiniScan XE Plus spectrophotometer was utilized to characterize steak color every 24 h. There was a significant treatment × storage time interaction ($P < 0.05$) for

a^* values and nitric oxide myoglobin formation. On days 1, 2, and 3 of the display, nitrite-embedded treatment improved ($P < 0.05$) redness compared to other dark-cutting steaks in PVC. A 45% increase in redness ($P < 0.05$) was observed for nitrite-embedded rosemary treatment over dark-cutting steak in PVC on day 3 of display. Nitric oxide myoglobin formation on day 0 was less for all dark-cutting steaks in nitrite-embedded packaging. Metmyoglobin content was greater ($P < 0.05$) on day 0 for dark-cutting steaks packaged in nitrite-embedded treatments than dark-cutting steaks in PVC. However, metmyoglobin level in dark-cutting steaks packaged in nitrite-embedded treatments decreased ($P < 0.05$) on day 1 compared with day 0. Dark-cutting steaks packaged in PVC had greater ($P < 0.05$) L^* values on day 0 than other dark-cutting steaks in nitrite-embedded packaging. Conversely, on days 1, 2, and 3, there were no differences ($P > 0.05$) in L^* values between dark-cutting treatments. Dark-cutting steaks in nitrite-embedded packaging had lower total plate count ($P < 0.05$) than dark-cutting steak packaged in PVC. The current research indicated that nitrite-embedded packaging has the potential to improve surface color of dark-cutting beef.

Key words: beef color, dark-cutting beef, FreshCase, myoglobin, nitrite packaging, pH

© The Author(s) 2018. Published by Oxford University Press on behalf of the American Society of Animal Science.

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/4.0/>), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact journals.permissions@oup.com

Transl. Anim. Sci. 2018.2:135–143
doi: 10.1093/tas/txy006

¹Corresponding author: ranjith.ramanathan@okstate.edu

Received 24 January, 2018.

Accepted 21 February, 2018.

INTRODUCTION

Maximizing the value of fresh beef is important to recover lost revenue due to quality

defects. Dark-cutting beef is an example of a color deviation in which beef fails to have a characteristic bright-red color. Although this condition has worldwide occurrence (Boykin et al., 2017; Mahmood et al., 2017; Zhang et al., 2018); the mechanism is not clear. Various studies have concluded that depletion of glycogen prior to slaughter due to chronic stress (Hendrick et al., 1959), less efficient mitochondria (McKeith et al., 2016), and compromised glycolytic enzyme activity (Mahmood et al., 2018) can be attributed to limited decline of post-rigor muscle pH. A greater muscle pH can enhance mitochondrial oxygen consumption and increase muscle swelling (Ashmore et al., 1972; Hunt and Hedrick, 1977); both processes can decrease bloom.

Post-harvest techniques utilizing enhancement and modified atmospheric packaging have been used to improve the appearance of dark-cutting beef (Wills et al., 2017). Lactic acid-enhancement promotes localized muscle discoloration (Apple et al., 2011), while modified atmospheric packaging with high-oxygen or carbon monoxide can increase lipid oxidation and consumer concerns at the retail level, respectively (Cornforth and Hunt, 2008; English et al., 2016a). Nitrite-embedded/FreshCase packaging films offer an alternative strategy to improve surface color under anaerobic conditions. More specifically, nitric oxide formed from nitrite can bind with deoxymyoglobin to form bright-red nitric oxide myoglobin (Fox and Ackerman, 1968). This technique has been used to improve redness of aged beef *longissimus lumborum*, *psoas major*, and *semitendinosus* muscles (Claus and Du, 2013) and bison steaks (Roberts et al., 2017). A greater pH promotes mitochondrial oxygen consumption, hence dark-cutting beef will have more deoxymyoglobin on the surface than normal-pH beef (English et al., 2016b). Therefore, nitric oxide can bind with deoxymyoglobin and has the potential to improve redness of dark-cutting beef. The overall goal of the current study was to determine the effects of nitrite-embedded packaging on surface color of dark-cutting beef.

MATERIALS AND METHODS

Raw Materials and Processing

Eight dark-cutting beef carcasses (pH > 6.0) and eight USDA Low Choice (normal-pH; mean pH = 5.6) beef strip loins (*longissimus lumborum*; IMPS #180) were selected (visually displayed Small degree of marbling), individually identified, and marked prior to fabrication from the Tyson Fresh Beef Plant at Amarillo, TX, 3 d after harvest.

All carcasses displayed A skeletal maturity, and the normal-pH carcasses displayed A lean maturity. Carcasses were fabricated, strip loins were collected, vacuum packaged, and transported on ice to remain chilled to the Robert M. Kerr Food & Agricultural Products Center at the Oklahoma State University campus in Stillwater. Both dark-cutting and normal-pH were loins cut in half, packaged in 11 × 22 cm, 3-mil high barrier Cryovac vacuum bags utilizing a Multivac C500 vacuum packager and stored at 2°C in the dark until use.

Each dark-cutting loin was sliced into five 2.5-cm thick steaks from the anterior end using a meat slicer (Bizerba USA Inc., Piscataway, NJ) and randomly assigned to four treatments: 1) dark-cutting steak packaged in polyvinyl chloride film (PVC) overwrap, 2) dark-cutting steak packaged in nitrite-embedded film, 3) dark-cutting steaks dipped in rosemary solution and packaged in nitrite-embedded film, and 4) dark-cutting steak dipped in deionized water and packaged in nitrite-embedded film. The fifth dark-cutting steak was used to determine pH and proximate composition. Loins graded USDA Choice was utilized to characterize the color of normal-pH steak. Each normal-pH Choice loin was cut into two steaks from the anterior end and randomly assigned to either package in PVC overwrap for retail display or to determine pH and proximate composition.

pH and Proximate Composition Analysis

Normal-pH and dark-cutting steak pH was measured on day 0 of display by inserting a pH probe at four different locations within a section using a Mettler Toledo SevenGo pH meter (Mettler Toledo, Columbus, OH). Following pH measurement, steaks were ground and 200 gram samples from normal-pH steaks and dark-cutting beef were utilized to measure moisture, protein, and fat using an Association of Official Analytical Chemist approved FOSS Food Scan 78800 near-infrared spectrophotometer (Dedicated Analytical Solutions, DK-3400 Hillerod, Denmark). The proximate composition was recorded on a percentage basis.

Rosemary Dip Treatment, Packaging, and Simulated Retail Display

Previous research from our laboratory has indicated that a combination of rosemary enhancement and modified atmospheric packaging

improved surface redness of dark-cutting beef by 44.5% (Wills et al., 2017). Improved redness in rosemary enhancement was due to increased reflectance by water, modified gas composition within package, and antioxidant effect. Hence, a rosemary surface dip treatment was also included in this study. The methodology described by Mitsumoto et al. (1991) was utilized for rosemary dip treatment. Briefly, 2.5-cm thick longissimus steak was dipped in 0.2% rosemary solution for 20 s. The 0.2% rosemary-enhancement solution consisted of rosemary oleoresin (Herbalox oleoresin rosemary, Kalsec) and deionized water stored at 2°C. Rosemary oleoresin was mixed in deionized water using a hand-held mixer for 2 min. A deionized water control treatment (without rosemary) also was included. Following dipping, steaks were kept on an inclined rack for 2 min to drain excess rosemary. Following equilibration, steaks assigned to nitrite-embedded film (FreshCase; Curlon Grade A5106 Protective Packaging Film; approximately 115 mg/m² nitrite, 6 × 12 pouches; 7 mil thickness; <0.15 oxygen transmission rate cc/100 in²/24 Hrs @ 73°F, 0% RH, 1 atm; <0.5 water vapor transmission rate g/100 in²/24 Hrs @ 100°F, 90% RH, 1 atm; Bemis Innovation Center in Neenah, WI) were packaged using a Multivac C500 vacuum packager. Steaks assigned to PVC were placed onto foam trays with absorbent pads (Sealed Air—tray number 3; 22.2 cm × 17.1 cm × 3.2 cm; Elmwood Park, NJ) and overwrapped with PVC (15,500–16,275 cm³ O₂/m²/24 h at 23°C, E-Z Wrap Crystal Clear Polyvinyl Chloride Wrapping Film, Koch Supplies, Kansas City, MO) using a Winholt film wrap machine (Winholt WHSS-1, 115V; Woodbury, NY).

The amount of rosemary or distilled water uptake in each steak was measured individually by weighing prior to dipping and after draining for 2 min. Intake of rosemary or water was negligible (less than 0.001%). Dipping application resulted in a surface coating of either rosemary solution or distilled water. Packages were placed in coffin-style retail display cases under continuous fluorescent lighting (Philips Fluorescent lamps; 12 Watts, 48 inches; Philips, China; color temperature = 3,500°K) and maintained at 2 ± 1°C for 3 days. The light intensity within the display case ranged from 1,000 to 1,150 lx (Extech Instruments Corporation, Waltham, MA). The packages were rotated daily to minimize the variation due to a location within the display case.

Instrumental Color

A HunterLab MiniScan XE Plus spectrophotometer (2.5-cm aperture, illuminant A, and 10° standard observer angle; HunterLab Associates, Reston, VA) was used to measure surface color at two locations. Instrumental color readings were taken on days 0, 1, 2, and 3 of retail display. The objective measure of CIE L^* , a^* , and b^* values and spectral readings from 400 to 700 nm were utilized to characterize the surface color. L^* represents lightness on a scale of 0 to 100 and a^* indicates redness. The CIE a^* and b^* values were also used to calculate chroma [$\sqrt{(a^{*2} + b^{*2})}$] (AMSA, 2012), which represents strength and weakness of chromatic color (red intensity).

The ratio of reflectance values at 650 nm and 570 nm were calculated as an indicator for nitric oxide myoglobin formation (AMSA, 2012). A greater number indicates more nitric oxide myoglobin formation. In addition, absorbance spectra from 400 to 700 nm were also used to characterize nitric oxide- and metmyoglobin formation. Absorbance was calculated using reflectance values from 400 to 700 nm according to Faustman and Phillips (2001): $A = (2 - \log R)$, where A represents absorbance and R represents percent reflectance. The ratio of K/S 572 ÷ K/S 525 was used to estimate metmyoglobin (AMSA, 2012). Reflectance values were converted to K/S ratios using the following equation: $K/S = (1 - R)^2 \div 2R$, where R represents the % reflectance expressed as a decimal. K/S ratios were used to make the data more linear and to account for absorptive (absorbance coefficient, K) and scattering (scattering coefficient, S) properties. A lower ratio represents greater metmyoglobin formation.

Microbiology

Total plate count was determined on dark-cutting steak in PVC and nitrite-embedded treatment on day 3 of display. A sterile 5 × 5 cm² grid was utilized to swab the surface of each steak with a 3M Swab-Sampler with 10 mL buffered peptone water broth (3M Maplewood, MN). Swab containers were vortexed for 30 s utilizing a Fisher Scientific Vortex-Genie 2 (12–812; Hampton, NH). One mL of the swabbed sample was serially diluted in 9 mL of 0.1% sterile peptone water and one mL of each dilution was aseptically plated on 3M Petrifilm rapid aerobic count plates (Hampton, NH). Plates were incubated in a VWR Forced Air General

Incubator (5.4 ft³; VWR, Radnor, PA) at 37°C for 48 h. Following incubation, plates were counted on an Interscience Scan 100 pressure sensitive pad (Interscience, Woburn, MA) to determine total plate count per cm².

Statistical Analysis

The experimental design was a randomized complete block with repeated measure. Loins served as a block ($n = 8$) and steaks within each loin received one of four treatments (dark-cutting steak in PVC, dark-cutting steak in nitrite-embedded film, dark-cutting steak dipped in rosemary solution and packaged in nitrite-embedded film, dark-cutting steak dipped in water and packaged in nitrite-embedded film). Time of color measurement (0, 1, 2, and 3 d) was a repeated measurement. Fixed effects for total plate count had one-way treatment structure and instrumental color had a two-way treatment structure of packaging, display time, and their interactions. For the instrumental color, the fixed effects included packaging, display time, and their interactions; however, packaging was the fixed effect for total plate count. For both instrumental color and total plate count, the random term included loin (block) and unspecified residual error. For the instrumental color data, the repeated option in PROC MIXED was used to assess covariance-variance structure among the repeated measures. The most appropriate structure was determined using the Akaike's information criterion output. Type-3 tests of fixed effects for packaging, display time, and their interactions were performed using the Mixed Procedure of SAS (SAS 9.3). Least squares mean for the highest order interactions determined to be significant will be presented. Least squares means were separated using the PDIFF option and were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

pH and Proximate Composition

Dark-cutting steaks had greater ($P < 0.05$) pH and moisture content than normal-pH beef (Table 1). However, there were no differences ($P > 0.05$) in protein and fat content between dark-cutting and normal-pH steaks. Preharvest stress can decrease glycogen content in muscles, hence limited lactic acid is formed postmortem. Previous studies have also reported greater pH in dark-cutting beef

Table 1. pH and proximate composition (%) of normal-pH and dark-cutting steaks

Trait	Normal-pH	Dark-cutting beef	SE
pH	5.6 ^a	6.4 ^b	0.03
Moisture	67.5 ^a	71.4 ^b	0.61
Protein	22.4 ^a	21.5 ^a	0.20
Fat	7.77 ^a	7.25 ^a	0.50

Least square means within a row with different letters (a and b) differ ($P < 0.05$).

(Sawyer et al., 2009; Mitacek et al., 2018). A greater pH can increase cell swelling or fiber width (Barbut et al., 2005; Hughes et al., 2017), which can decrease light reflectance and oxygen diffusion into the meat. Nitrite-embedded/FreshCase technology uses a vacuum or low oxygen packaging to improve the appearance of fresh beef (Siegel, 2011). Although this packaging technique has been used to improve the appearance of low-color stable muscles such as *psoas major* or aged beef (Claus and Du, 2013), no research has determined its application in dark-cutting beef.

Surface Redness

There was a significant treatment \times storage time interaction for a^* values, chroma, ratio of R650 \div R570 nm, and metmyoglobin content (Figures 1–3, and Table 2). Dark-cutting treatments had lower redness ($P < 0.05$; a^* values) than normal-pH steaks on day 0 of display. Within the dark-cutting treatments, on day 0 of display, nitrite-embedded packaging treatments had lower redness ($P < 0.05$)

Table 2. Effects of nitrite-embedded packaging* and retail display on metmyoglobin formation[†]

Treatments	Days of retail display			
	0	1	2	3
Normal-pH PVC	1.391 ^{a,w}	1.325 ^{b,wxy}	1.230 ^{c,y}	1.134 ^{d,y}
Dark-cutter PVC	1.251 ^{a,x}	1.209 ^{ab,y}	1.192 ^{bc,y}	1.152 ^{c,y}
Dark-cutter nitrite	0.967 ^{c,z}	1.334 ^{b,wx}	1.357 ^{ab,x}	1.393 ^{ax}
Dark-cutter nitrite + rosemary	1.019 ^{c,y}	1.368 ^{b,w}	1.423 ^{a,w}	1.440 ^{a,w}
Dark-cutter nitrite water control	1.037 ^{c,y}	1.309 ^{b,xy}	1.415 ^{a,w}	1.437 ^{a,wx}

Least square means within a row with different letters (a–d) differ ($P < 0.05$). Least square means within a column with different letters (w–z) differ ($P < 0.05$). SE = 0.025.

*Treatments included normal-pH steak packaged in PVC, dark-cutting steak packaged in PVC, dark-cutting packaged in nitrite-embedded, dark-cutting steak dipped in 0.2% rosemary solution and packaged in nitrite-embedded film, and dark-cutting steak dipped in distilled water and packaged in nitrite-embedded film.

[†]Metmyoglobin formation was calculated as K/S572 \div K/S525 nm. A lower number indicates greater metmyoglobin formation.

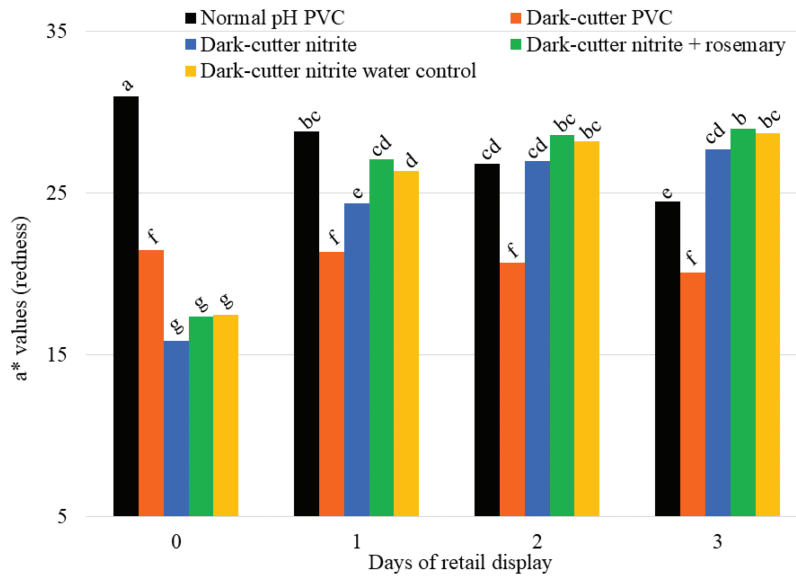


Figure 1. Effects of nitrite-embedded packaging¹ and retail display on a^* values. Least square means with different letters (a–g) differ ($P < 0.05$). SE for treatment \times days of retail display interaction = 0.98. ¹Treatments included normal-pH steak packaged in PVC, dark-cutting steak packaged in PVC, dark-cutting packaged in nitrite-embedded, dark-cutting steak dipped in 0.2% rosemary solution and packaged in nitrite-embedded film, and dark-cutting steak dipped in distilled water and packaged in nitrite-embedded film.

than dark-cutting steaks in PVC. Nitrite is a potent oxidizing agent, hence myoglobin can be oxidized to form nitric oxide metmyoglobin. The absorbance spectra (peak at 630 nm; Figure 4) and a lower ratio of $K/S\ 572 \div K/S\ 525$ (Table 2) indicated greater metmyoglobin on day 0 in nitrite-embedded packaging compared with dark-cutting beef in PVC. Research using normal-pH ground beef and the nitrite-embedded film also reported that the formation of red color is not immediate and it took 5 d to have redder color (Yang et al., 2016). Meat has an inherent reducing capacity to reduce

metmyoglobin to deoxymyoglobin, and a greater pH can accelerate this conversion (Zhu and Brewer, 1998; Djimsa et al., 2017). Dark-cutting beef has greater metmyoglobin reducing activity than normal-pH beef (English et al., 2016b; McKeith et al., 2016); hence, the formation of bright-red nitric oxide myoglobin was faster in the current study. From day 1 onward, nitrite-embedded treatments had greater a^* values and chroma than dark-cutting steaks in PVC (Figure 5). On day 3 of display, nitrite-embedded treatment with rosemary had greater numerical a^* values compared with

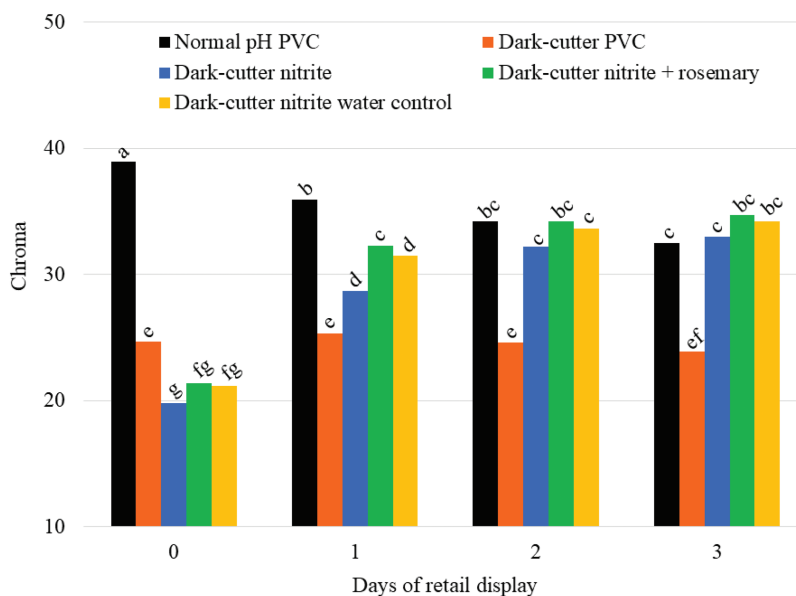


Figure 2. Effects of nitrite-embedded packaging¹ and retail display on chroma. Least square means with different letters (a–g) differ ($P < 0.05$). SE for treatment \times days of retail display interaction = 1.4. ¹Treatments included normal-pH steak packaged in PVC, dark-cutting steak packaged in PVC, dark-cutting packaged in nitrite-embedded, dark-cutting steak dipped in 0.2% rosemary solution and packaged in nitrite-embedded film, and dark-cutting steak dipped in distilled water and packaged in nitrite-embedded film.

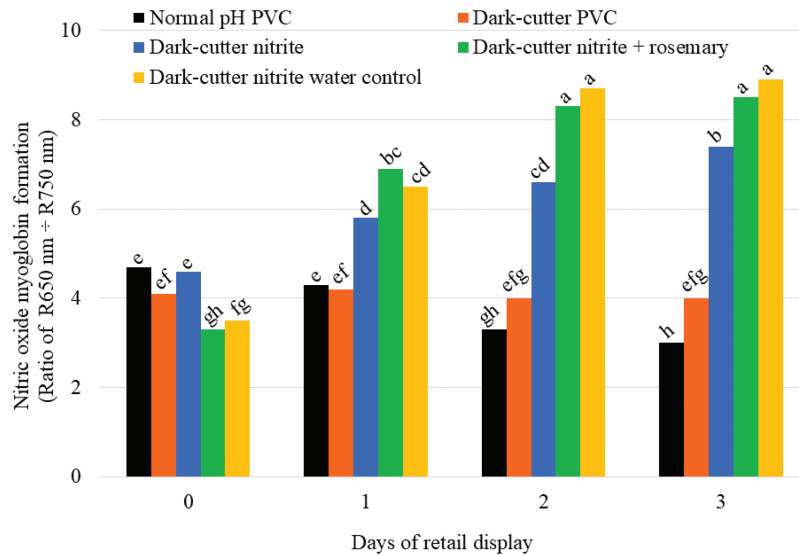


Figure 3. Effects of nitrite-embedded packaging¹ and retail display on nitric oxide myoglobin formation². Least square means with different letters (a–h) differ ($P < 0.05$). SE for treatment \times days of retail display interaction = 0.35. ¹Treatments included normal-pH steak packaged in PVC, dark-cutting steak packaged in nitrite-embedded, dark-cutting steak dipped in 0.2% rosemary solution and packaged in nitrite-embedded film, and dark-cutting steak dipped in distilled water and packaged in nitrite-embedded film. ²Nitric oxide formation was calculated as the ratio of R650 \div R750 nm. A greater number indicates more nitric oxide formation.

other dark-cutting treatments. The ratio of R650 \div R750 nm for nitrite-embedded treatments increased with storage time, indicating more nitric oxide myoglobin formation. In support, absorbance spectrum also indicated more nitric oxide myoglobin with storage time (Figure 6; increase in absorbance at 550 and 570 nm). On days 2 and 3, both rosemary and distilled water treatments had greater nitric oxide formation than control dark-cutting steak in nitrite-embedded packaging.

In the current study, both rosemary and distilled water treatments in nitrite-embedded film had greater redness than control nitrite-embedded film

treatment. Although the mechanism of improved color stability is not clear, we speculate that the antioxidant effect of rosemary may have limited nitric oxide myoglobin oxidation. More specifically, nitric oxide myoglobin is sensitive to light-induced photo-oxidation, hence the addition of rosemary may have increased redox stability. Previous research indicated that light-exposed steaks packaged in nitrite-embedded film had lower redness than dark-storage steaks packaged in nitrite-embedded film (Claus and Du, 2013). The antioxidant effects of rosemary in beef were noted by previous studies (Sánchez-Escalante et al., 2003; Wills et al.,

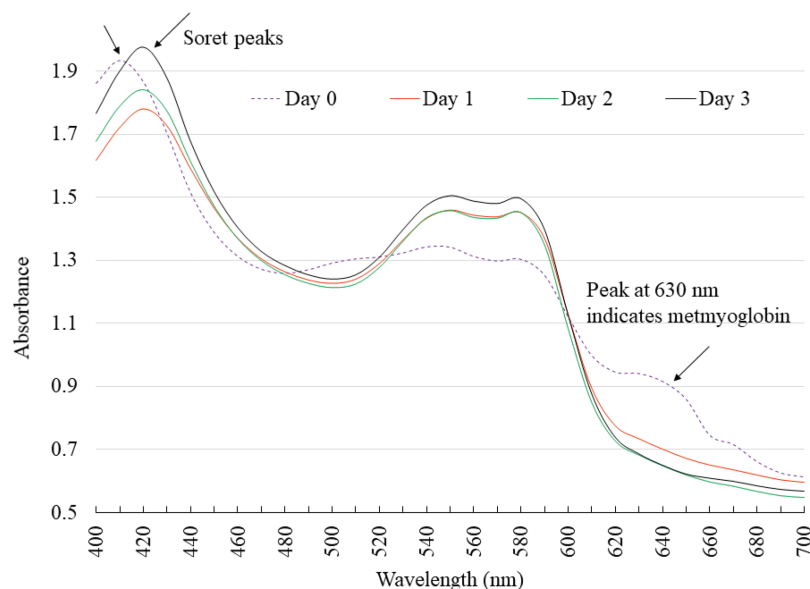
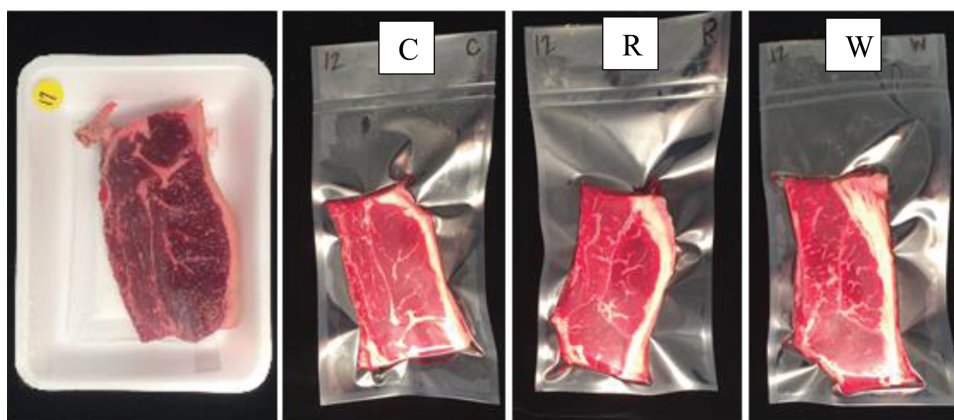


Figure 4. Changes in absorbance spectra of dark-cutting steaks packaged in nitrite-embedded packaging during 3-d retail display.



Dark-cutting
steak in PVC

Dark-cutting steaks in nitrite-embedded packaging

Figure 5. Pictorial representation of dark-cutting steaks packaged¹ in nitrite-embedded film on day 2 of retail display. ¹Treatments included dark-cutting steak packaged in PVC, dark-cutting packaged in nitrite-embedded (C), dark-cutting steak dipped in 0.2% rosemary solution and packaged in nitrite-embedded film (R), and dark-cutting steak dipped in distilled water and packaged in nitrite-embedded film (W).

2017). Further, dark-cutting beef has lower moisture content than normal-pH beef. Hence, water in rosemary and distilled water treatment may have increased diffusion of nitrite from the packaging material to meat surface.

Redness of normal-pH steaks decreased ($P < 0.05$) with storage time, while no changes in a^* values ($P > 0.05$) were observed for dark-cutting steak packaged in PVC during 3-day display. There were no differences ($P > 0.05$) in ratio of $R_{650} \div R_{570}$ nm for dark-cutting steaks packaged in PVC. However, ratio of $R_{650} \div R_{570}$ nm decreased for normal-pH steak packaged in PVC between days

1 and 2, which can be attributed to metmyoglobin formation.

Surface Lightness (L^* Values)

There was a significant treatment \times storage time interaction for L^* values ($P < 0.05$; Figure 6). Normal-pH steaks packaged in PVC were lighter ($P < 0.05$) in color than dark-cutting treatments. Many studies have shown that dark-cutting steaks have lower L^* values than normal-pH steaks (English et al., 2016b; Apple et al., 2011). On day 0 of display, dark-cutting steaks packaged in PVC

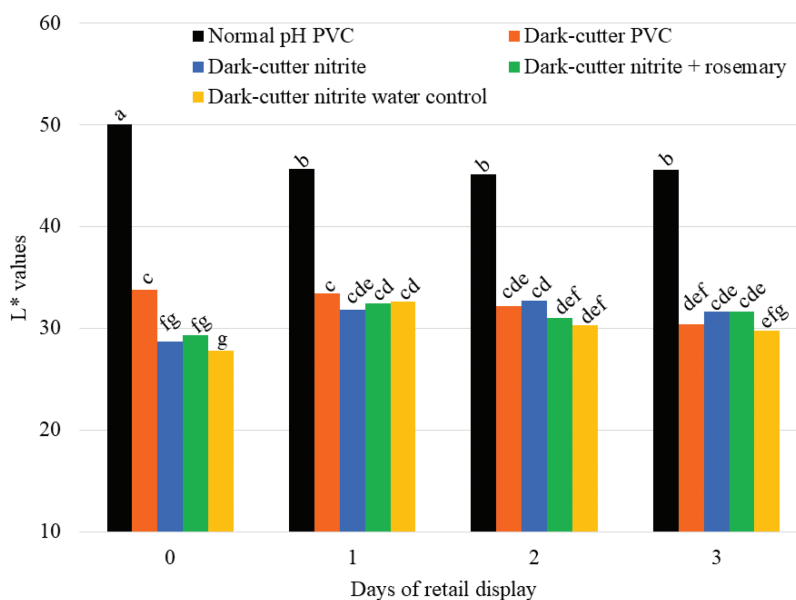


Figure 6. Effects of nitrite-embedded packaging¹ and retail display on L^* values. Least square means with different letters (a–g) differ ($P < 0.05$). SE for treatment \times days of retail display interaction = 1.2. ¹Treatments included normal-pH steak packaged in PVC, dark-cutting steak packaged in PVC, dark-cutting packaged in nitrite-embedded, dark-cutting steak dipped in 0.2% rosemary solution and packaged in nitrite-embedded film, and dark-cutting steak dipped in distilled water and packaged in nitrite-embedded film.

had greater L^* values than dark-cutting steaks in nitrite-embedded packaging. Although redness was improved on days 1, 2, and 3, there were no differences ($P > 0.05$) in L^* values between dark-cutting treatments observed. In the present study, water treatment was included to determine the effects of water on reflectance properties. Water-enhancement increased lightness or L^* values of ground beef (Seyfert et al., 2007), normal-pH longissimus steaks (Ramanathan et al., 2010), and dark-cutting steaks (Wills et al., 2017); conversely minimal effect on lightness (L^* values) was observed in the current study.

Total Plate Count

Dark-cutting steaks in nitrite-embedded packages had lower ($P < 0.05$) total plate counts than dark-cutting steaks packaged in PVC on day 3 of display (average total plate count per cm² for dark-cutting steaks in nitrite-embedded packaging = 5.61 and dark-cutting steaks in PVC = 6.72; SE = 0.24). A greater pH favors the growth of spoilage bacteria (Gill and Newton, 1979). One log decrease in total plate count can be attributed to lower oxygen content within the package. For example, nitrite-embedded packaging creates a low or anaerobic condition, while PVC is an aerobic packaging. In support, previous research also reported nitrite-embedded packaging had an approximate 1.5-log reduction in psychrophilic bacteria than PVC (Narváez-Bravo et al., 2017).

Although current research indicated that nitrite-embedded packaging increase surface redness, limited knowledge is available on sustained redness after exposure to oxygen. Cooked color can be another potential issue with high-pH and nitrite packaging. For example, a combination of greater pH and thermostable nitric oxide myoglobin can predispose to persistent pinking. Currently, research is on-going in our laboratory to determine the effects of re-packing on sustained redness and cooked color. Greater muscle pH and reducing activity make dark-cutting beef a good model to study factors affecting nitric oxide myoglobin formation in raw beef.

CONCLUSION

Surface redness and chroma were greater for steaks packaged in nitrite-embedded film than dark-cutting steaks in PVC. A greater muscle pH accelerated the formation of bright-red nitric oxide myoglobin. Improved redness in nitrite-embedded

treatment was not supported by an increase in L^* values. Rosemary-dipped steaks packaged in nitrite-embedded film were the most effective in improving surface of dark-cutting steaks. Therefore, understanding fundamental myoglobin chemistry has the potential to develop effective post-harvest strategies that can improve surface color and value of dark-cutting beef.

LITERATURE CITED

- American Meat Science Association. 2012. Meat color measurement guidelines. Chicago (IL): American Meat Science Association.
- Apple, J. K., J. T. Sawyer, J. F. Meullenet, J. W. Yancey, and M. D. Wharton. 2011. Lactic acid enhancement can improve the fresh and cooked color of dark-cutting beef. *J. Anim. Sci.* 89:4207–4220. doi:10.2527/jas.2011-4147
- Ashmore, C. R., W. Parker, and L. Doerr. 1972. Respiration of mitochondria isolated from dark-cutting beef: postmortem changes. *J. Anim. Sci.* 34:46–48.
- Barbut, S., L. Zhang, and M. Marcone. 2005. Effects of pale, normal, and dark chicken breast meat on microstructure, extractable proteins, and cooking of marinated fillets. *Poult. Sci.* 84:797–802.
- Boykin, C. A., L. C. Eastwood, M. K. Harris, D. S. Hale, C. R. Kerth, D. B. Griffin, A. N. Arnold, J. D. Hasty, K. E. Belk, D. R. Woerner, et al. 2017. National beef quality audit-2016: in-plant survey of carcass characteristics related to quality, quantity, and value of fed steers and heifers. *J. Anim. Sci.* 95:2993–3002. doi:10.2527/jas.2017.1543
- Claus, J. R., and C. Du. 2013. Nitrite-embedded packaging film effects on fresh and frozen beef color development and stability as influenced by meat age and muscle type. *Meat Sci.* 95:526–535. doi:10.1016/j.meatsci.2013.05.029
- Cornforth, D., and M. Hunt. 2008. Low-oxygen packaging of fresh meat with carbon monoxide. Meat quality, microbiology, and safety. AMSA White Paper Series, (number 2). Savoy (IL): American Meat Science Association; p. 1–10.
- Djimsa, B. A., A. Abraham, G. G. Mafi, D. L. VanOverbeke, and R. Ramanathan. 2017. Effects of metmyoglobin reducing activity and thermal stability of NADH-dependent reductase and lactate dehydrogenase on premature browning in ground beef. *J. Food Sci.* 82:304–313. doi:10.1111/1750-3841.13606
- English, A. R., G. G. Mafi, D. L. VanOverbeke, and R. Ramanathan. 2016a. Effects of extended aging and modified atmospheric packaging on beef top loin steak color. *J. Anim. Sci.* 94:1727–1737. doi:10.2527/jas.2015-0149
- English, A. R., K. M. Wills, B. N. Harsh, G. G. Mafi, D. L. VanOverbeke, and R. Ramanathan. 2016b. Effects of aging on the fundamental color chemistry of dark-cutting beef. *J. Anim. Sci.* 94:4040–4048. doi:10.2527/jas.2016-0561
- Faustman, C., and A. L. Phillips. 2001. Measurement of discoloration in fresh meat. In: Schwartz, S.J., editor. Current protocols in food analytical chemistry. New York (NY): Wiley (Ch. F3 Unit F3.3); p. 139–151.
- Fox, J. B., Jr., and S. A. Ackerman. 1968. Formation of nitric oxide myoglobin: mechanisms of the reaction with various reductants. *J. Food Sci.* 33:364–370.

- Gill, C. O., and K. G. Newton. 1979. Spoilage of vacuum-packaged dark, firm, dry meat at chill temperatures. *Appl. Environ. Microbiol.* 37:362–364.
- Hendrick, H. B., J. B. Boillot, D. E. Brady, and H. D. Naumann. 1959. Etiology of dark-cutting beef. *Res. Bull.* 717. Columbia: University MO Agric. Exp. Stn.
- Hughes, J., F. Clarke, P. Purslow, and R. Warner. 2017. High pH in beef longissimus thoracis reduces muscle fibre transverse shrinkage and light scattering which contributes to the dark colour. *Food Res. Int.* 101:228–238. doi:10.1016/j.foodres.2017.09.003
- Hunt, M. C., and H. B. Hedrick. 1977. Profile of fiber types and related properties of 5 bovine muscles. *J. Food Sci.* 42:513–517. doi:10.1111/j.1365-2621.1977.tb01535.x
- Mahmood, S., B. C. Roy, I. L. Larsen, J. L. Aalhus, W. T. Dixon, and H. L. Bruce. 2017. Understanding the quality of typical and atypical dark cutting beef from heifers and steers. *Meat Sci.* 133:75–85. doi:10.1016/j.meatsci.2017.06.010
- Mahmood, S., N. Turchinsky, F. Paradis, W. T. Dixon, and H. L. Bruce. 2018. Poteomics of dark cutting longissimus thoracis muscle from heifer and steer carcasses. *Meat Sci.* 137:47–57.
- McKeith, R. O., D. A. King, A. L. Grayson, S. D. Shackelford, K. B. Gehring, J. W. Savell, and T. L. Wheeler. 2016. Mitochondrial abundance and efficiency contribute to lean color of dark cutting beef. *Meat Sci.* 116:165–173. doi:10.1016/j.meatsci.2016.01.016
- Mitacek, R. M., A. R. English, G. G. Mafi, D. L. VanOverbeke, and R. Ramanathan. 2018. Modified atmospheric packaging improves the surface color of dark-cutting beef. *Meat and Muscle Bio.* Accepted, 2:57–63. doi:10.22175/mmb2017.04.0023.
- Mitsumoto, M., R. G. Cassens, D. M. Schaefer, R. N. Arnold, and K. K. Scheller. 1991. Improvement of color and lipid stability in beef longissimus with dietary vitamin E and vitamin C dip treatment. *J. Food Sci.* 56:1489–1492.
- Narváez-Bravo, C., A. Rodas-González, C. Ding, O. López-Campos, J. Galbraith, I. L. Larsen, J. Ye, D. Siegel, and J. L. Aalhus. 2017. Effects of novel nitrite packaging film on the bacterial growth of bison strip-loin steaks. *J. Food Proc. Preserv.* 41:e13311.
- Ramanathan, R., R. A. Mancini, B. M. Naveena, and M. K. Konda. 2010. Effects of lactate-enhancement on surface reflectance and absorbance properties of beef longissimus steaks. *Meat Sci.* 84:219–226. doi:10.1016/j.meatsci.2009.08.027
- Roberts, J. C., A. Rodas-González, J. Galbraith, M. E. R. Dugan, I. L. Larsen, J. L. Aalhus, and Ó. López-Campos. 2017. Nitrite embedded vacuum packaging improves retail color and oxidative stability of bison steaks and patties. *Meat Mus. Bio.* 1:169–180.
- Sánchez-Escalante, A., D. Dkenane, G. Torrescano, J. A. Beltrán, and P. Roncales. 2003. Antioxidant action of borage, rosemary, oregano, and ascorbic acid in beef patties packaged in modified atmosphere. *J. Food Sci.* 68:339–344.
- Sawyer, J. T., J. K. Apple, Z. B. Johnson, R. T. Baublits, and J. W. Yancey. 2009. Fresh and cooked color of dark-cutting beef can be altered by post-rigor enhancement with lactic acid. *Meat Sci.* 83:263–270. doi:10.1016/j.meatsci.2009.05.008
- Seyfert, M., M. C. Hunt, M. Lundesjö Ahnström, and D. E. Johnson. 2007. Efficacy of lactic acid salts and sodium acetate on ground beef colour stability and metmyoglobin-reducing activity. *Meat Sci.* 75:134–142. doi:10.1016/j.meatsci.2006.06.022
- Siegel, D. G. 2011. An update on packaging fresh meat with nitrite containing film. 64th Reciprocal Meat Conference. Kansas State University.
- Wills, K. M., R. M. Mitacek, G. G. Mafi, D. L. VanOverbeke, D. Jaroni, R. Jadeja, and R. Ramanathan. 2017. Improving the lean muscle color of dark-cutting beef by aging, antioxidant-enhancement, and modified atmospheric packaging. *J. Anim. Sci.* 95:5378–5387. doi:10.2527/jas2017.1967
- Yang, X., D. R. Woerner, J. D. Hasty, K. R. McCullough, I. Geornaras, J. N. Sofos, and K. E. Belk. 2016. An evaluation of the effectiveness of freshcase technology to extend the storage life of whole muscle beef and ground beef. *J. Anim. Sci.* 94:4911–4920. doi:10.2527/jas.2016-0508
- Zhu, L. G., and M. S. Brewer. 1998. Metmyoglobin reducing capacity of fresh normal, PSE and DFD pork during retail display. *J. Food Sci.* 63:390–393.
- Zhang, Y., L. Qin, Y. Mao, D. L. Hopkins, G. Han, L. Zhu, and X. Luo. 2018. Carbon monoxide packaging shows the same color improvement for dark cutting beef as high oxygen packaging. *Meat Sci.* 137:153–159. doi:10.1016/j.meatsci.2017.11.016