Introduction

Failure to have a characteristic bright-red color at the cut surface of the ribeye muscle during grading results in discounted carcass value. The 2011 National Beef Quality Audit reported that 3% of carcasses sampled were graded as dark-cutters (Moore et al., 2012). Dark-cutting beef is an effect of a metabolic condition(s) due to chronic stress resulting in limited lactic acid formation in post-rigor muscles (Hendrick et al., 1959). A greater pH promotes muscle fibers to hold more water; therefore, less oxygen is diffused deep into the tissue (Lawrie, 1958; Offer and Trinick, 1983). Similarly, elevated muscle pH can enhance mitochondrial activity and deoxymyoglobin formation (Ashmore et al., 1971; Ashmore et al., 1972; Egbert and Cornforth, 1986; Tang et al., 2005; Mancini and Ramanathan, 2014; English et al., 2016a). Therefore, developing strategies to improve redness is essential to maximize the value of beef.

Various organic acids have been used to lower muscle pH and improve surface color of dark-cutting beef (Apple et al., 2011; Sawyer et al., 2009). Introduction of case-ready meat has allowed beef purveyors to modify the gas compositions within packages to improve shelf-life. Use of high-oxygen or carbon monoxide in modi-
The objective of the current study was to determine the effects of high-oxygen and carbon monoxide modified atmosphere packaging (HiOx-MAP and CO-MAP) on the surface color of dark-cutting beef muscle that had been aged for 21 d.

Materials and Methods

Raw materials processing, packaging, and retail display

Ten USDA Choice (normal-pH) and 10 no-roll dark cutting beef carcasses were selected 72 h after slaughter from a commercial packing plant, individually identified and tagged prior to carcasses fabrication. Both normal-pH (IMPS #180; North American Meat Processors Association, 2002) and dark-cutting strip loins (longissimus lumborum) were vacuum packaged and transported on ice to the Robert Kerr Food and Agricultural Products Center at Oklahoma State University. Two 2.5-cm thick steaks were cut from the anterior end of each normal-pH and dark-cutting loin to characterize pH, surface color, and lipid oxidation before aging (no packaging, display, or aging). The remaining loins were vacuum packaged (Prime Source vacuum pouches, 12 × 18 cm, 3 mil high barrier; oxygen partial pressure within package = 0.76%) and aged for 21 d in the dark at 2°C. After 21 d aging, four 2.5-cm thick steaks per loin were cut from the anterior end using a meat slicer (Bizerba USA Inc., Piscataway, NJ), and randomly assigned to 1 of 3 packaging conditions: PVC (oxygen-permeable polyvinyl chloride fresh meat film), HiOx-MAP (80% oxygen and 20% carbon dioxide), and CO-MAP (0.4% carbon monoxide, 69.6% nitrogen, and 30% carbon dioxide). The fourth steak was used to measure pH and lipid oxidation on d 0 of retail display.

Both HiOx-MAP and CO-MAP were performed using a MAP system utilizing Rock-Tenn DuraFresh rigid trays sealed with clear, multi-layer barrier film (LID 1050 film, Cryovac Sealed Air, Duncan, SC) in a Mondini semi-automatic tray-sealing machine (Model CV/VG-5, G. Mondini S.P.A. Cologne, Italy) and certified gas blends (Stillwater Steel and Welding Supply, Stillwater, OK). For PVC packaging, steaks were placed onto foam trays with absorbent pads, and over-wrapped with a PVC film (15,500 to 16,275 cm² O₂/m²/24 h at 23°C, E-Z Wrap Crystal Clear Polyvinyl Chloride Wrapping Film, Koch Supplies, Kansas City, MO). A headspace analyzer (Bridge 900131 O₂/CO₂/CO, Illinois Instruments, Ingleside, IL), was used to determine the percentage oxygen, carbon dioxide, and carbon monoxide in HiOx- and CO-MAP. Gas compositions were determined after 24 h of packaging using extra steaks not used in the study by a needle pierced into package. The average gas compositions in HiOx-MAP were 78 to 80% oxygen and 18 to 20% carbon dioxide and 0.3 to 0.4% carbon monoxide in CO-MAP. After packaging, steaks were placed in a coffin-style open display case maintained at 2°C ± 1 under continuous lighting (1612 to 2152 lx, Philips Delux Warm White Fluorescent lamps; Andover, MA; color rendering index = 86; color temperature = 3000 K). All packages were rotated daily to minimize variances in light intensity or temperature caused by the location.

Characterizing muscle pH, instrumental color, and lipid oxidation prior to aging

Normal-pH and dark-cutting steaks were over-wrapped with PVC film and allowed to bloom at 4°C for 2 h. The pH, instrumental color, and lipid oxidation of bloomed steaks were determined to characterize the muscle quality prior to aging.

The pH values of normal- and dark-cutting steaks were obtained by using an Accumet combination glass electrode connected to an Accumet 50 pH meter (Fisher Scientific, Fairlawn, NJ). Samples were blended in a Sorvall Omni tabletop mixer (Newton, CT). The pH was determined by combining 10-g sample with 100 mL of deionized water and homogenized for 30 s. Prior to pH measurements, the pH electrode was standardized with pH 4 and 7 buffers.

Surface color prior to aging was measured after 2 h of bloom at 4°C using a HunterLab MiniScan XE Plus spectrophotometer (Model 45/0 LAV, 2.5-cm diameter aperture, illuminant A, 10° observer; HunterLab, Reston, VA). The color measurements of the steaks
Muscle pH, color, and lipid oxidation of aged and modified atmosphere packaged steaks

After 21 d aging, each loin section was cut into 4 steaks. The first steak was used to determine pH and lipid oxidation prior to display (no packaging effect), and remaining 3 steaks were randomly packaged in PVC, HiOx-MAP, and CO-MAP. The steaks were displayed for 6 d and the surface color was evaluated using a HunterLab MiniScan XE Plus spectrophotometer every 24 h of display. For the steaks packaged in MAP, precautions were taken as recommended in the AMSA guidelines (Section VIII, American Meat Science Association, 2012). Following surface color measurement on d 6, the steaks were used to determine pH and lipid oxidation as described in the previous section.

Visual panel

A trained panel (n = 6) conducted daily visual color evaluations. All panelists passed the Farnsworth Munsell 100-hue test. Panelists were selected and trained according to the American Meat Science Association (1991) guidelines. Panelists scored each steak every 24 h of display to assess muscle color using a 7-point scale (1- no darkening, 2- little darkening, 3- slight darkening, 4- modest darkening, 5- moderate darkening, 6- extensive darkening, and 7- extreme darkening) and discoloration using a 7-point scale [1- no discoloration (0% metmyoglobin), 2- minimal discoloration (1 to 20%), 3- slight discoloration (11 to 20%), 4- small discoloration (21 to 40%), 5- modest discoloration (41 to 60%), 6- moderate discoloration (61 to 80%), and 7- extensive discoloration (81 to 100%)].

Statistical analysis

A split-plot design was used to evaluate the effects of packaging on dark-cutting beef color. Within the whole plot, 10 normal-pH *longissimus lumborum* and 10 dark-cutting *longissimus lumborum* muscles were considered experimental units (N = 10 for each muscle and N = 20 total muscles). Within the subplot, each longissimus muscle was divided into steaks and packaged in PVC, HiOx-MAP, or CO-MAP (sub-plot experimental unit). The fixed effects included muscle type, packaging, and their interactions. For the split-plot, random effects included loin, loin × whole plot treatments (Error A), and residual error (Error B). The most appropriate structure was determined using Akaike’s information criterion and Sawa’s Bayesian information criterion output. The repeated option in the Mixed Procedure of SAS (SAS Inst. Inc., Cary, NC) was used to assess covariance–variance structure among the repeated measures for instrumental and visual color data. Least squares means were separated using a pairwise t test (PDIFF option). Day 0 surface color and pH (prior to aging) was utilized only to characterize normal-pH and dark-cutting beef. Therefore, aging time was not included in the model.

Results

pH and surface color prior to aging

There were significant differences in pH and color between muscle types before aging (Table 1). As expected, dark-cutting beef had greater (P < 0.0001) pH and lower L*, a*, and chroma values than normal-pH beef.

pH and L* values of steaks aged for 21 d

Following 21 d aging, dark-cutting steaks had greater pH than normal-pH steaks (the average pHs of normal and dark-cutting beef were 5.50 and 6.36, respectively; SE = 0.03). There was a packaging × muscle type interaction for L* values (Fig. 1). Packaging in HiOx-MAP and CO-MAP increased lightness of dark-cutting steaks compared with dark-cutting steaks in PVC packaging (HiOx-MAP = CO-MAP > PVC; P < 0.001).
a* values and chroma

A packaging × muscle type × display time interaction resulted for a* values (redness) and chroma (red intensity; Table 2). On d 0 of the display, dark-cutting steaks in HiOx-MAP had greater a* values than dark-cutting PVC and CO-MAP. However, normal-pH and dark-cutting steaks in HiOx-MAP had identical (P = 0.35) redness values on d 3. By d 6 of the display, dark-cutting steaks had greater (P < 0.0001) redness values in HiOx-MAP and CO-MAP than dark-cutting steaks in PVC packaging. Compared to normal-pH steaks in PVC and CO-MAP, normal-pH steaks in HiOx-MAP were less red (P < 0.05) by d 4 of display. Both dark-cutting and normal-pH steaks in CO-MAP had greater a* values by d 4 of display than other packaging types.

Dark-cutting steaks in HiOx- and CO-MAP increased chroma (red intensity) compared with dark-cutting steaks in PVC on d 0 of display. Dark-cutting steaks attained the highest numerical chroma values on d 1 and 6 for HiOx- and CO-MAP, respectively compared with PVC dark-cutting steaks. By the end of display, both CO-MAP and HiOx-MAP steaks had greater chroma values compared with dark-cutting steaks in PVC.

Visual color

There was a packaging × muscle type × display time interaction (P < 0.0001) for trained color panel scores on muscle darkening and surface discoloration (Table 3). On d 0, dark-cutting steaks in HiOx-MAP had lower (P < 0.0001) muscle darkening scores than dark-cutting steaks in PVC and CO-MAP. By the end of display, both dark-cutting steaks in CO-MAP and HiOx-MAP had less muscle darkening than PVC dark-cutting steaks. On d 2 of display, dark-cutting steaks in HiOx- and CO-MAP had less surface discoloration compared with dark-cutting steaks in PVC packaging. CO-MAP had the least (P

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Table 1. Least squares means for pH, color, and lipid oxidation between muscle types prior to aging

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal-pH</th>
<th>Dark-cutting</th>
<th>SE</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>5.56</td>
<td>6.40</td>
<td>0.03</td>
<td>0.0001</td>
</tr>
<tr>
<td>L* value</td>
<td>38.2</td>
<td>25.2</td>
<td>0.60</td>
<td>0.0001</td>
</tr>
<tr>
<td>a* value</td>
<td>28.4</td>
<td>18.4</td>
<td>0.80</td>
<td>0.0001</td>
</tr>
<tr>
<td>Chroma</td>
<td>34.5</td>
<td>25.4</td>
<td>1.00</td>
<td>0.0001</td>
</tr>
<tr>
<td>Lipid oxidation (mg MDA/kg meat)</td>
<td>0.18</td>
<td>0.17</td>
<td>0.06</td>
<td>0.0600</td>
</tr>
</tbody>
</table>

1SE = standard error.

Table 2. Least squares means for a* and chroma (packaging × muscle type × day interaction) of steaks displayed for 6 d

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Packaging</th>
<th>Muscle type</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>a* value</td>
<td>PVC</td>
<td>Normal-pH</td>
<td>29.7a,u</td>
<td>23.4b,w</td>
<td>24.7c,v</td>
<td>22.9b,w</td>
<td>23.7bc,w</td>
<td>21.09d,w</td>
<td>19.7e,w</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dark-cutting</td>
<td>20.6b,y</td>
<td>21.5c,x</td>
<td>20.5d,b,x</td>
<td>19.9bc,x</td>
<td>18.8c,z</td>
<td>17.64d,x</td>
<td>16.8e,x</td>
</tr>
<tr>
<td></td>
<td>HiOx-MAP</td>
<td>Normal-pH</td>
<td>30.4a,u</td>
<td>28.4b,u</td>
<td>26.4c,wu</td>
<td>23.5d,w</td>
<td>20.4c,y</td>
<td>18.4d,x</td>
<td>15.4e,y</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dark-cutting</td>
<td>25.4b,v</td>
<td>27.4a,v</td>
<td>25.4b,vw</td>
<td>23.5c,w</td>
<td>22.5d,cx</td>
<td>21.5de,w</td>
<td>20.5e,w</td>
</tr>
<tr>
<td></td>
<td>CO-MAP</td>
<td>Normal-pH</td>
<td>24.5b,d</td>
<td>25.6c,v</td>
<td>27.5c,a</td>
<td>28.4bc,a</td>
<td>28.6a,b,cu</td>
<td>29.4ab,u</td>
<td>29.7a,bu</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dark-cutting</td>
<td>22.4c,x</td>
<td>23.5e,w</td>
<td>24.5c,d,v</td>
<td>25.4bc,v</td>
<td>26.4a,b</td>
<td>26.4a,bv</td>
<td>26.8a,bv</td>
</tr>
<tr>
<td>Chroma</td>
<td>PVC</td>
<td>Normal-pH</td>
<td>36.7a,v</td>
<td>30.4b,w</td>
<td>30.2b,x</td>
<td>29.9w</td>
<td>30.7h</td>
<td>28.1c,w</td>
<td>26.7d,w</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dark-cutting</td>
<td>27.0a,y</td>
<td>28.5b,x</td>
<td>27.4c,y</td>
<td>26.5ac,x</td>
<td>26.0c,d</td>
<td>25.5d,cx</td>
<td>24.5d,x</td>
</tr>
<tr>
<td></td>
<td>HiOx-MAP</td>
<td>Normal-pH</td>
<td>38.4a,u</td>
<td>36.4b,u</td>
<td>34.4c,a</td>
<td>31.5d,v</td>
<td>28.4c,d</td>
<td>26.4f,c</td>
<td>23.4g,x</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dark-cutting</td>
<td>32.4b,w</td>
<td>34.9c,v</td>
<td>32.4b,vw</td>
<td>30.5c,w</td>
<td>29.4d,sv</td>
<td>28.5a,b,cw</td>
<td>27.5e,w</td>
</tr>
<tr>
<td></td>
<td>CO-MAP</td>
<td>Normal-pH</td>
<td>30.5d,x</td>
<td>31.6c,dv</td>
<td>33.5acuv</td>
<td>34.4bc,a</td>
<td>34.6abc,u</td>
<td>35.4abh</td>
<td>35.7a,b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dark-cutting</td>
<td>29.4c,x</td>
<td>30.5d,sv</td>
<td>31.5c,dw</td>
<td>32.4bc,v</td>
<td>33.4a,b</td>
<td>33.4a,bv</td>
<td>34.2b,v</td>
</tr>
</tbody>
</table>

a–fLeast square means with different letters within a row are different (P < 0.05).

u–zuLeast square means with different letters within a column are different (P < 0.05).
< 0.001) amount of surface discoloration by the end of display than PVC and HiOx-MAP for both muscle types.

**Lipid oxidation**

There was a packaging × muscle type interaction for lipid oxidation (Fig. 2). Dark-cutting muscles showed less ($P < 0.001$) lipid oxidation in PVC and HiOx-MAP packaging when compared with normal-pH steaks. HiOx-MAP packaging had greater lipid oxidation for both muscle types than PVC and CO-MAP. However, there were no differences ($P = 0.25$) in lipid oxidation between normal-pH and dark-cutting when packaged in CO-MAP.

**Discussion**

Previous research from our laboratory has indicated that blooming properties of dark-cutting steaks were improved with extended aging (English et al., 2016a). Greater myoglobin oxygenation, in part, can be due to decreased mitochondrial activity. Cornforth and Egbert (1985) validated the role of mitochondria in bloom by the addition of rotenone (complex I electron-transport chain inhibitor) to increase redness of pre-rigor meat. In the current study, we combined 2 different post-harvest processing techniques (aging and modified atmosphere packaging) to enhance the appearance of dark-cutting beef. Greater oxygen content in HiOx-MAP (80% oxygen) can partially satisfy mitochondrial oxygen demand, leading to oxygenation of deoxymyoglobin. In addition, aging time can increase muscle proteolysis (Koohmaraie, 1994) and lower water holding capacity. Hence, more water will be available on the surface of the meat to reflect light. A higher oxygen partial pressure within the package can saturate both myoglobin and water, further enhancing oxymyoglobin formation. Earlier studies also demonstrated a bright-red color when normal-pH steaks packaged in HiOx-MAP compared with PVC (Jayasingh et al., 2001; Jakobsen and Bertelsen, 2002; John et al., 2005). Dark-cutting steaks in HiOx-MAP had increased redness than normal-pH HiOx-MAP on d 6. Less surface discoloration in dark-cutting steaks

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**Table 3. Least squares means for muscle darkening**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Packaging</th>
<th>Muscle type</th>
<th>Display, d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle darkening</td>
<td>PVC</td>
<td>Normal-pH</td>
<td>0 1 2 3 4 5 6</td>
</tr>
<tr>
<td></td>
<td>Dark-cutting</td>
<td>Normal-pH</td>
<td>1.46,1.59,1.79,2.46,2.56,4.13</td>
</tr>
<tr>
<td></td>
<td>HiOx-MAP</td>
<td>Normal-pH</td>
<td>1.36,1.24,1.21,2.06,2.54,3.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$SE = 0.26$</td>
</tr>
<tr>
<td>Surface discoloration</td>
<td>PVC</td>
<td>Normal-pH</td>
<td>2.98,2.42,2.33,2.31,1.34,1.54</td>
</tr>
<tr>
<td></td>
<td>Dark-cutting</td>
<td>Normal-pH</td>
<td>5.05,5.02,4.90,4.86,3.51,3.45</td>
</tr>
<tr>
<td></td>
<td>HiOx-MAP</td>
<td>Normal-pH</td>
<td>1.00,1.29,2.30,2.42,3.28,4.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$SE = 0.19$</td>
</tr>
</tbody>
</table>

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1 Least square means with different letters within a row, within a parameter, are different ($P < 0.05$).

2 Least square means with different letters within a column, within a parameter, are different ($P < 0.05$).

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$1 = $ no darkening, $3 = $ slight darkening, $5 = $ moderately dark, and $7 = $ very dark.

$2 = $ no discoloration, $2 = $ slight discoloration, $3 = $ small discoloration, $4 = $ modest discoloration, $5 = $ moderate discoloration, and $6 = $ extensive discoloration.
can be attributed to the protective effect of pH on oxy-
myoglobin and lipid oxidation. In-vitro experiments
utilizing purified myoglobin also demonstrated greater
stability of oxymyoglobin at pH 7.4 than at pH 5.6
(Suman et al., 2006). Previous research also reported
lower TBARS values in dark-cutting beef than nor-
mal-pH steaks (Sawyer et al., 2009). Increased aging
time coupled with high oxygen levels in normal-pH
conditions can promote lipid oxidation and decrease
metmyoglobin reducing activity, leading to lower col-
or-stability in normal-pH steaks packaged in HiOx-
MAP (English et al., 2016b).

Improved color stability of normal-pH steaks
in CO-MAP had been reported by various research-
ers (Jayasingh et al., 2001; Krause et al., 2003; Hunt
et al., 2004; John et al., 2005; English et al., 2016b).
However, limited studies have determined carbon
monoxide’s effects in high-pH meat conditions. In the
current study, dark-cutting steaks achieved the greatest
bright-red color in CO-MAP by the end of the display.
Carbon monoxide has a higher affinity for myoglobin
than oxygen and can form a strong bond with heme.
Moreover, carbon monoxide is a known mitochon-
drial inhibitor (Lanier et al., 1978; Cooper and Brown,
2008). Further, anaerobic condition within CO-MAP
can limit lipid oxidation. Hence, we speculate that a
greater affinity of myoglobin for carbon monoxide,
less oxidative stress due to anaerobic condition, and
effects on mitochondrial oxygen consumption may
have improved the surface color of dark-cutting beef.
In support, Hamling and Calkins (2008) reported CO-
MAP improved appearance of ammonium hydroxide-
enhanced (high-pH) beef.

Dark-cutting beef has more myoglobin concen-
tration than normal-pH beef (McKeith et al., 2016;
English et al., 2016a). With a greater myoglobin concen-
tration, one can speculate a bright-red color for
dark-cutting steaks in CO-MAP than normal-pH steaks
in CO-MAP. On the contrary, on d 6 of display,
dark-cutting steaks in CO-MAP had lower a* and chroma
values and greater muscle darkening than normal-pH
steaks, which suggests the effect of carbon monoxide
on high-pH meat is pH-dependent. This can be, in part,
due to impaired diffusion of carbon monoxide deep
into meat due to muscle swelling. Further, research
utilizing electrochemical techniques noted that affini-
ity of oxygen to myoglobin was greater at pH 7.4 than 5.6
(Nerimetla et al., 2017). However, limited information
is currently available on the pH-dependent effects of
carbon monoxide binding to myoglobin.

**Conclusion**

Surface color of dark-cutting beef color that had been
aged for 21 d was improved with the use of HiOx- and
CO-MAP. HiOx-MAP dark-cutting steaks were similar
in color to normal-pH steaks on d 1 of display. However,
dark-cutting steaks in CO-MAP resulted in a more stable
red color than HiOx-MAP. Lipid oxidation was greater
in HiOx-MAP dark-cutting steaks than in CO-MAP. An
elevated muscle pH enhances bacterial growth and can
affect flavor profile. Hence, characterizing the effects of
modified atmosphere packaging on microbial growth
and taste attributes are critical to maximizing the benefits
of improved surface color in dark-cutting beef.

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chuck and loin muscles with ammonium hydroxide and salt.


