Published August 8, 2019

Meat and Muscle Biology™


# Effects of Aging, Modified Atmospheric Packaging, and Display Time on Metmyoglobin Reducing Activity and Oxygen Consumption of High-pH Beef

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**Abstract:** The objective of current research was to determine the effects of extended aging, modified atmospheric packaging (MAP), and display time on metmyoglobin reducing activity (MRA) and oxygen consumption (OC) of high-pH beef using pH sensitive methodology for MRA and OC. Ten normal-pH (mean pH = 5.6) and 10 high-pH loins (mean pH = 6.4) were vacuum packaged on d 3 postmortem and aged for 0, 21, 42, and 62 d at 2°C. Following aging, 2.0-cm-thick steaks were cut from each of the normal- and high-pH loin sections and packaged in either PVC film, high-oxygen (HiOx-MAP), or carbon monoxide modified atmospheric (CO-MAP) packaging. Surface color, OC, and MRA were measured on d 0 and 6 of the respective aging periods. Steaks in HiOx-MAP and CO-MAP had similar ($P > 0.05$) L* values, which were greater ($P < 0.05$) than high-pH steaks packaged in PVC film. On 21-d of aging, steaks with at both pHs in CO-MAP and HiOx-MAP had greater ($P < 0.05$) a* values than steaks packaged in PVC. As aging time increased, MRA decreased ($P < 0.05$) for steaks with normal- and high-pH when packaged in PVC and HiOx-MAP. Steaks with a high-pH in CO-MAP had greater ($P < 0.05$) MRA than steaks with a normal-pH in CO-MAP at all aging periods. Steaks with a high-pH had greater ($P < 0.05$) OC on d 0 and 6 than normal-pH steaks. Steaks with a normal-pH aged for 21 d and packaged in PVC and HiOx-MAP had greater ($P < 0.05$) lipid oxidation than high-pH steaks aged for 21 d and packaged in PVC and HiOx-MAP. After 62 d of aging and 6 d of display, the greatest color stability chemistry (based on MRA and OC for all package types) were: high-pH meat > normal-pH meat; thus the MRA and OC methodology was useful in relative comparison of packaged meat color stability differences due to pH.

**Keywords:** aging, beef color, dark-cutter, high-pH beef, metmyoglobin reducing activity, oxygen consumption
*Meat and Muscle Biology* 3(1):276–288 (2019) **doi:10.22175/mmb2019.05.0017**
Submitted 22 May 2019 Accepted 30 June 2019

## Introduction

High-pH beef or dark-cutting beef is one of the most prominent beef quality defects worldwide (Moore et al., 2012; Mahmood et al., 2017; Zhang et al., 2018). Consumers often associate bright-red color with beef freshness and wholesomeness. Higher than normal-pH conditions are an example of a color deviation in which beef failed to have a bright-red color, leading to discounted carcasses and economic losses to the meat industry (Boykin et al., 2017). Therefore, elucidating the fundamental basis of high-pH beef is critical to developing post-harvest strategies to improve color and value of high-pH beef.

Metmyoglobin reducing activity (MRA) and oxygen consumption (OC) are inherent biochemical properties that influence beef color (Madhavi and Carpenter, 1993; Seyfert et al., 2007; Mancini and Ramanathan, 2014). Both processes can affect the proportion of myoglobin forms on the surface of beef steaks. Oxygen consumption is the ability of muscle

This research was in part supported by the Oklahoma Center for the Advancement of Science and Technology, Oklahoma Applied Research Support and the Agriculture and Food Research Institute Grant from the USDA National Institute of Food and Agriculture program.

 

to consume oxygen, primarily by mitochondria or oxygen-consuming enzymes, to a low partial pressure of oxygen so that metmyoglobin (MetMb) forms naturally and to deoxymyoglobin (DeoxyMb) depending on the reducing activity. Metmyoglobin reducing activity represents the ability of the postmortem muscle to donate an electron to MetMb ($Fe^{3+}$) to form deoxymyoglobin (DeoxyMb; $Fe^{2+}$). Current knowledge is that NADH and succinate are the 2 reducing equivalents that influence MRA by enzymatic-, non-enzymatic-, or electron transport chain mediated pathways (Tang et al., 2005a; Kim et al., 2006; Elroy et al., 2015). In addition, other substrates such as malate, lactate, and pyruvate can also reduce MetMb in concert with NADH (Mohan et al., 2010; Ramanathan et al., 2011; Bjelanovic et al., 2016). Increased postmortem time can limit the ability of muscle to regenerate NADH and succinate. In postmortem muscle, there is competition for the available oxygen between mitochondria and myoglobin. If mitochondria are active, there will be limited oxygenation of myoglobin resulting in darker meat due to predominant DeoxyMb. Various studies have shown that aging time decreased MRA and OC in normal-pH beef (English et al., 2016a; Mitacek et al., 2019). A greater muscle-pH can decrease oxidative changes and can increase the activity of enzymes involved in MRA and OC (Tang et al., 2005b; English et al., 2016b). However, limited knowledge is currently available on the effects of extended aging on MRA and OC of high-pH beef.

Although the mechanistic basis of high-pH is not totally clear, current knowledge implicates that a defective glycolytic pathway and limited glycogen storage antemortem leads to greater muscle-pH (Mahmood et al., 2017). Hence, the biochemical properties of high-pH beef may be different from normal-pH beef. McKeith et al. (2016) reported that mitochondrial content is greater in high-pH beef than normal-pH beef. Various post-harvest strategies such as high-oxygen modified atmospheric packaging (HiOx-MAP), carbon monoxide modified atmospheric packaging (CO-MAP), nitrite-embedded film packaging, antioxidant muscle enhancement, and acidification have improved the appearance of high-pH beef (Sawyer et al., 2009; Stackhouse et al., 2016; Wills et al., 2017; Mitacek et al., 2018; Ramanathan et al., 2018). However, potential interactions between inherently different muscle-pHs and numerous postmortem processing protocols on the color and color stability of beef have not been reported previously. Therefore, the objective of this research was to determine the effects of extended aging, 3 types of packaging, and display time on MRA and OC of beef longissimus muscle differing in pH. Also unique is the methodology for MRA and OC that more accurately represents the effects due to normal- and high-pH.

## Materials and Methods

### *Experimental design and raw material processing*

A split-split plot design was utilized to determine the effects of muscle-pH, aging period, MAP, and display time on MRA and OC. In the whole plot, 10 USDA Choice beef strip loins (average pH = 5.6, typical longissimus color, less than 30 mo old, and a USDA Small to Modest amount of marbling, approx. 5 to 7.5% intramuscular fat; United States Standards for Grades of Carcass Beef 2017; USDA, 2017) and 10 high-pH loins (No-Roll carcass, quality grade not known, average pH = 6.4; standard deviation = 0.1) were selected at 24 h postmortem and tag-identified at a commercial packing company. After fabrication, the strip loins (IMPS #180 *M. longissimus lumborum;* NAMI, 2014) were vacuum packaged and transported on ice to the Food and Agricultural Products Center at Oklahoma State University in Stillwater.

### *Allocation of steaks for aged 0 d measurements*

A 5-cm thick section was removed from the anterior end of each loin by a perpendicular cut through the long axis of the longissimus muscle. Samples from these pieces were used to determine the d 0 (no aging; the actual d 0 postmortem age of these samples was 72 h post-harvest) data for color and other biochemical analyses of both pH treatments. At time of analysis, two 2.0-cm-thick steaks were cut from each loin using a meat slicer (Bizerba USA Inc., Piscataway, NJ). One of these steaks was cut in half at the medial-lateral line resulting in two, 2-cm thick pieces. One piece was butterflied and the 2 fresh-cut surfaces were measured for either MRA or OC. The second piece of the first steak was used for lipid oxidation and pH measurements. The second 2.0-cm-thick steak was wrapped using PVC film. The steaks assigned to PVC packaging were used for repeated color measurements and also for d 6 biochemical assays.

### Allocation of sections for aging

The remaining portions of the loins were cut into 3 equal-length sections and vacuum packaged (Prime source vacuum pouches, 12 × 18 cm, 3 mil high barrier), and randomly assigned to 21-, 42-, or 62-d aging periods of dark storage at 2°C. After each respective aging period, four 2.0-cm-thick steaks per aged section were cut from the anterior end using a meat slicer (Bizerba USA Inc.). One of these steaks was randomly assigned for determination of color and other chemical analyses and the other 3 steaks were randomly allocated to 1 of 3 packaging systems: PVC, HiOx-MAP (high oxygen modified atmospheric packaging; 80% oxygen and 20% carbon dioxide), and CO-MAP (carbon monoxide modified atmospheric packaging; 0.4% carbon monoxide, 69.6% nitrogen, and 30% carbon dioxide). These aging periods were the sub-plot experimental units.

### Packaging and simulated retail display

Within the sub-sub plot, steaks from each aging period served as the experimental unit assigned to a packaging × display time combination of 3 packaging treatments (PVC, HiOx-MAP, and CO-MAP) and repeated color readings on 0, 2, 4, and 6 d. For PVC packaging, steaks were placed onto foam trays with absorbent pads, over-wrapped with a PVC film (oxygen-permeable polyvinyl chloride fresh meat film; 15,500 to 16,275 $cm^3$ $O_2$ $m^{-2}$ 24 $h^{-1}$ at 23°C, E-Z Wrap Crystal Clear Polyvinyl Chloride Wrapping Film, Koch Supplies, Kansas City, MO). 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). 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 and/or temperature caused by specific case locations. A headspace analyzer (Bridge 900131 $O_2$/$CO_2$/CO, Illinois Instruments, Ingleside, IL), was used to determine the percentage $O_2$, CO, and $CO_2$ in HiOx- and CO-MAP. Extra steaks not used in the study were packaged, stored in a display case, and gas compositions were determined after 24 h of packaging.

### Proximate composition and pH

An AOAC-approved (Official Method 2007.04; Anderson, 2007) near-infrared spectrophotometer (FOSS Food Scan 78800; Dedicated Analytical Solutions, DK-3400 Hillerod, Denmark) was utilized to determine protein, moisture, and fat content on d 0 of the initial aging period. The compositional values were reported on a percent (%) basis.

Ten-gram samples from all aging × muscle-pH × packaging × display time combinations were blended with 100 mL of deionized water and homogenized for 30 s in a Sorvall Omni tabletop mixer (Newton, CT). The pH of the muscle homogenates was obtained using an Accumet combination glass electrode connected to an Accumet 50 pH meter (Fisher Scientific, Fairlawn, NJ). The electrode was standardized using pH 4 and 7 buffer before use.

### Surface color measurements

All instrumental color measurements were performed using a HunterLab MiniScan XE Plus spectrophotometer (Model 45/0 large area view, 2.5-cm diameter aperture, Illuminant A, 10° Observer; HunterLab, Reston, VA) on respective aging, muscle-pH, packaging, and display time. Both reflectance spectra from 400 to 700 nm (10 nm increments) and CIE L*, a*, and b* values were measured on each steak at 3 random locations and the subsamples were averaged for statistical analyses. K/S ratios at isobestic points were used to estimate oxymyoglobin (OxyMb), DeoxyMb, and MetMb. For example, reflectance values were converted to K/S ratios using the equation: $K/S = (1 - R)^2 \div 2R$, where R represents the % reflectance expressed as a decimal. The ratio of K/S 474 ÷ K/S 525 and K/S 572 ÷ K/S 525 was used to estimate DeoxyMb and MetMb, respectively (AMSA color guide; AMSA, 2012). 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. The Commission Internationale de l'Eclairage (CIE, 1976) a* and b* values were used to calculate chroma and hue angle (AMSA, 2012).

### Quantification of metmyoglobin reducing activity and oxygen consumption

Several researchers have utilized reflectance methodology to quantify MRA and OC of intact steaks (Sammel et al., 2002; Nair et al., 2018). Light reflectance properties are influenced by pH. More specifically, greater pH can increase cell swelling

and biochemical activities, both of which can affect light reflectance properties (Hunt and Hedrick, 1977a; Ramanathan et al., 2010; McKeith et al., 2016). The MRA and OC calculations utilize changes in MetMb and OxyMb content, respectively. However, a greater pH can decrease initial bloom and limit nitrite-induced MetMb formation in comparison to normal-pH steaks. Hence, MRA and OC calculation using changes in MetMb and OxyMb will not provide a realistic value. Thus, recently modified procedures (described below) were used for both OC and MRA.

### *Metmyoglobin reducing activity*

The methodology described by English et al. (2016b) and McKeith et al. (2016) was modified to determine the effects of muscle-pH (normal- and high-pH), aging period (0, 21, 42, and 62 d), packaging (HiOx- MAP, CO- MAP, and PVC), and display time (0 and 6 d) on MRA. Samples from the interior of steak halves (approx. 3 × 3 × 1.5 cm tissue with no visible fat or connective tissue) were submerged in a 0.3% w/v solution of sodium nitrite (Sigma Aldrich, St. Louis, MO) for 20 min at 30°C (Fisher Scientific, Model 630F, Waltham, MA) to facilitate MetMb formation (Sammel et al., 2002). The sections were then removed and blotted to remove visible nitrite solution. The level of MetMb content on the surface was determined by using a Hunter Lab Miniscan. Resistance to myoglobin oxidation was a better indicator of MetMb reducing property than post-reduction values (O'Keeffe and Hood, 1982; Mancini et al., 2008). The resistance to myoglobin oxidation was reported as K/S572 ÷ K/S525. A greater K/S572 ÷ K/S525 ratio indicates lower MetMb formation, hence a greater MRA. To visualize MRA easily, K/S572 ÷ K/S525 ratio was converted to a relative percentage. The highest numerical MRA ratio was considered as 100% and other aging, MAP, and display time was reported relative to the highest MRA ratio.

### *Oxygen consumption*

Previous studies determined OC as changes in OxyMb level after incubating a bloomed steak in a vacuum package for a fixed period of time. A greater decrease in OxyMb level indicates greater OC. However, this method has 2 limitations in high-pH and in extended aged steaks. 1) During vacuum package, conversion of OxyMb to DeoxyMb is not a single step process. OxyMb will be first converted to MetMb, then to DeoxyMb (AMSA, 2012). Extended aging time can limit MRA, hence OxyMb is converted to MetMb and depending on the reducing activity, MetMb content can increase with aging time. 2) High-pH meat can limit initial OxyMb formation due to greater mitochondrial and oxygen consuming enzyme activity and a tighter more closed tissue structure that reduces OxyMb formation and the actual OC. Hence in the current study, OC was determined as the DeoxyMb level in vacuum packaged meat.

Samples from the interior of steak halves (approx. 3 × 3 × 1.5 cm tissue with no visible fat or connective tissue) were wrapped with PVC film (15,500 to 16,275 $cm^3$ $O_2$ $m^{-2}$ 24 $h^{-1}$ at 23°C, E-Z Wrap Crystal Clear Polyvinyl Chloride Wrapping Film, Koch Supplies) and stored at 4°C for 30 min. Bloomed steaks were vacuum packaged and incubated at 25°C for 30 min.; then DeoxyMb was quantified using the ratio of K/S474 ÷ K/S 525 (AMSA, 2012). A lower K/S474 ÷ K/S 525 ratio represents greater DeoxyMb or OC. The ratio was transformed using the equation of [1.5 – (K/S474 ÷ K/S 525)], which resulted in a larger number representing greater OC (AMSA, 2012). The transformed values were later converted to a percentage for easier visualization. To convert K/S474 ÷ K/S525 ratio to a relative percentage, the highest numerical OC ratio was considered as 100% and other treatment combinations were reported relative to the highest OC ratio.

### *Thiobarbituric acid reactive substances values (lipid oxidation)*

Thiobarbituric acid reactive substances (TBARS) values were measured on normal- and high-pH aged steaks packaged in PVC, HiOx-, and CO-MAP and displayed for 0 and 6 d using the procedure of Witte et al. (1970). From each steak, 5 g of the sample that contained both interior and surface (roughly 2 × 2 × 2.54 cm thick) was blended with 25 mL of 20% trichloroacetic acid (TCA) and 20 mL distilled water. Samples were homogenized using a Sorvall Omni mixer (Newton, CT) for 1 min and filtered through a Whatman (#1) filter paper. One mL of filtrate was mixed with 1 mL thiobarbituric acid (TBA) solution (20 mM) and incubated in a boiling water bath for 10 min. After incubation, samples were cooled and absorbance at 532 nm was measured using a Shimadzu UV-2600 PC spectrophotometer. The blank consisted of 2 mL TCA/distilled water (1:1 v/v) and 2 mL TBA solution. Thiobarbituric acid reactive substance values were reported as absorbance at 532 nm.

**Table 1.** Proximate composition of normal- and high-pH beef and gas compositions within modified atmospheric packaging

| Parameter | Trait | Normal-pH | High-pH | Standard error |
|---|---|---|---|---|
| Proximate composition[1], % | Moisture | 66.5[a] | 71.4[b] | 0.68 |
| | Protein | 23.6[a] | 22.8[a] | 0.12 |
| | Fat | 9.6[a] | 7.4[b] | 0.08 |
| Gas composition[2], % | Packaging | $O_2$ | $CO_2$ | CO |
| | HiOx-MAP | 78.4 ± 1.2 | 18 to 20 ± 1.2 | 0 |
| | CO-MAP | 0 | 27.62 ± 1.4 | 0.4 |

[a,b]Least squares means within a row with a different superscript are different ($P < 0.05$).

[1]*P*-values for moisture = 0.001; protein = 0.21; fat = 0.01.

[2]A headspace analyzer (Bridge 900131 $O_2$/$CO_2$/CO, Illinois Instruments, Ingleside, IL), was used to determine the percentage $O_2$, CO, and $CO_2$ in HiOx- and CO-MAP. Extra steaks not used in the study were packaged, stored in display case, and gas compositions were determined after 24 h of packaging. HiOx-MAP, high-oxygen modified atmospheric packaging; CO-MAP, carbon monoxide modified atmospheric packaging. Abbreviations: $O_2$, oxygen; $CO_2$, carbon dioxide; CO, carbon monoxide.

### *Statistical analysis*

Data were analyzed using the Mixed Procedure of SAS (SAS version 9.4, SAS Inst. Inc., Cary, NC; $n = 10$ replications for both normal- and high-pH). For display color, MRA, OC, and lipid oxidation, the fixed effects in the model included muscle-pH, aging period, packaging, display time, and their interactions. For the split-plot, random effects included loin, loin × whole plot treatments (Error A), and residual error (Error B). The repeated option in Proc Mixed was used to assess covariance–variance structure among the repeated measures for display data. The most appropriate structure was determined using Akaike's Information Criterion output. Least square means for protected F-tests ($P < 0.05$) were separated using the pdiff option and were considered significant at $P < 0.05$.

**Table 2.** Effects of muscle-pH, aging period, and display time on steak pH[1,2]

| | | Aging period, d | | | |
|---|---|---|---|---|---|
| Display time | Muscle-pH | 0 | 21 | 42 | 62 |
| 0 | Normal-pH | 5.54[a,x] | 5.65[b,x] | 5.55[a,x] | 5.52[a,x] |
| | High-pH | 6.41[a,y] | 6.45[a,y] | 6.41[a,y] | 6.44[a,y] |
| 6 | Normal-pH | 5.52[a,b,x] | 5.63[c,x] | 5.57[b,x] | 5.48[a,x] |
| | High-pH | 6.44[a,y] | 6.55[b,z] | 6.60[b,z] | 6.71[c,z] |

[a-c]Least squares means within a row with a different superscript letter are different ($P < 0.05$).

[x-z]Least squares means within a column with a different superscript letter are different ($P < 0.05$).

[1]Standard error for muscle-pH × display time × aging period = 0.03.

[2]*P*-values for display time < 0.0001; muscle-pH < 0.0001; aging period = 0.42; muscle-pH × aging period × display time = 0.002.

**Table 3.** Effects of muscle-pH, packaging, and display time on steak pH[1,2]

| | Muscle-pH | |
|---|---|---|
| Packaging and display time | Normal-pH | High-pH |
| d 0 PVC | 5.57 [a,y] | 6.43 [b,x] |
| d 6 PVC | 5.56 [a,y] | 6.82 [b,z] |
| d 6 HiOx-MAP | 5.57 [a,y] | 6.56 [b,y] |
| d 6 CO-MAP | 5.52 [a,x] | 6.43 [b,x] |

[a,b]Least squares means within a row with a different superscript are different ($P < 0.05$).

[x-z]Least squares means within a column with a different superscript letter are different ($P < 0.05$).

[1]Standard error for muscle-pH × display time × packaging = 0.02.

[2]*P*-values for display time < 0.0001; muscle-pH < 0.0001; packaging < 0.0001; muscle-pH × aging period × display time = 0.0001.

## Results

### *Proximate composition and pH*

High-pH beef had greater ($P < 0.05$) moisture content than normal-pH beef, while there were no differences ($P > 0.05$) between protein and fat content (Table 1). The modified atmospheric packaging data (Table 1) confirmed the gaseous environments for the HiOx- and CO-MAPs.

At all aging periods and display times, high-pH steaks had a greater ($P < 0.05$; Table 2) pH than normal-pH steaks. High-pH steaks aged for 62 d and displayed 6 d had greater ($P < 0.05$) pH than high-pH steaks displayed for 6 d and aged 0 d. Normal-pH CO-MAP steaks on d 6 had lower ($P < 0.05$) pH than normal-pH PVC and HiOx-MAP (Table 3). However, there were no differences between normal-pH PVC steaks and normal-pH HiOx-MAP steaks on d 6. Conversely, high-pH PVC had a greater pH followed by HiOx-MAP and CO-MAP on d 6 (PVC > HiOx-MAP > CO-MAP; $P < 0.05$).

### *Surface color*

**L* values.** A muscle-pH × packaging × aging period interaction resulted for L* values (lightness; Table 4). Initially (0 d aged), high-pH steaks had lower ($P < 0.05$) L* values than normal-pH. Packaging high-pH steaks in HiOx-MAP and CO-MAP improved ($P < 0.05$) lightness compared with high-pH PVC steaks (high-pH all aging periods; HiOx-MAP = CO-MAP

**Table 4.** Effects of muscle-pH, packaging, and aging period on L* values (lightness)[1,2,3]

| | | Aging period (d) | | | |
|---|---|---|---|---|---|
| Muscle-pH | Packaging | 0 | 21 | 42 | 62 |
| Normal-pH | PVC | 45.6 $^{a,w}$ | 41.7 $^{b,x}$ | 40.2 $^{c,x}$ | 40.6 $^{c,x}$ |
| | HiOx-MAP | | 42.9 $^{a,w}$ | 42.9 $^{a,w}$ | 42.8 $^{a,w}$ |
| | CO-MAP | | 42.9 $^{a,w}$ | 42.3 $^{a,w}$ | 41.5 $^{b,x}$ |
| High-pH | PVC | 35.4 $^{a,x}$ | 28.6 $^{c,z}$ | 32.1 $^{b,z}$ | 31.5 $^{b,z}$ |
| | HiOx-MAP | | 31.6 $^{c,y}$ | 35.6 $^{a,y}$ | 33.4 $^{b,y}$ |
| | CO-MAP | | 30.6 $^{b,y}$ | 34.5 $^{a,y}$ | 33.7 $^{a,y}$ |

$^{a-c}$Least squares means within a row with a different superscript letter are different ($P < 0.05$).

$^{w-z}$Least squares means within a column with a different superscript letter are different ($P < 0.05$).

[1]Standard error for muscle-pH × display time × aging period = 0.5.

[2]A lower number indicates darker color and a greater number represents lighter color.

[3]*P*-values for aging period < 0.0001; muscle-pH < 0.0001; packaging < 0.0001; muscle-pH × aging period × display time < 0.0001.

**Table 5.** Effects of muscle-pH, packaging, aging period, and display on a* values (redness)[1]

| Muscle-pH × packaging × aging period[2] | | | | | |
|---|---|---|---|---|---|
| | | Aging period, d | | | |
| Muscle-pH | Packaging | 0 | 21 | 42 | 62 |
| Normal-pH | PVC | 29.4 $^{a,x}$ | 24.1 $^{b,xy}$ | 20.3 $^{c,y}$ | 17.4 $^{d,y}$ |
| | HiOx-MAP | | 23.4 $^{a,y}$ | 20.8 $^{b,y}$ | 17.3 $^{c,y}$ |
| | CO-MAP | | 25.9 $^{a,w}$ | 25.8 $^{a,w}$ | 24.7 $^{a,w}$ |
| High-pH | PVC | 23.5 $^{a,y}$ | 18.4 $^{b,z}$ | 17.3 $^{b,z}$ | 16.8 $^{c,yz}$ |
| | HiOx-MAP | | 25.0 $^{a,wx}$ | 22.7 $^{b,x}$ | 15.7 $^{c,z}$ |
| | CO-MAP | | 24.5 $^{a,x}$ | 21.0 $^{b,y}$ | 20.3 $^{b,x}$ |
| Muscle-pH × display time × aging period[3] | | | | | |
| | | Display time, d | | | |
| Aging period, d | Muscle-pH | 0 | 2 | 4 | 6 |
| 0 | Normal-pH | 29.5 $^{a,v}$ | 28.4 $^{a,u}$ | 27.4 $^{b,u}$ | 23.8 $^{c,u}$ |
| | High-pH | 24.5 $^{a,y}$ | 23.2 $^{a,b,w}$ | 22.1 $^{b,w}$ | 18.4 $^{c,w}$ |
| 21 | Normal-pH | 29.7 $^{a,v}$ | 24.8 $^{b,v}$ | 23.7 $^{b,v}$ | 19.7 $^{c,v}$ |
| | High-pH | 27.7 $^{a,w}$ | 23.1 $^{b,wx}$ | 21.3 $^{c,w}$ | 18.3 $^{d,w}$ |
| 42 | Normal-pH | 28.3 $^{a,w}$ | 23.1 $^{b,wx}$ | 19.9 $^{c,x}$ | 18.1 $^{d,w}$ |
| | High-pH | 23.5 $^{a,yz}$ | 22.1 $^{b,x}$ | 18.9 $^{c,x}$ | 16.8 $^{d,x}$ |
| 62 | Normal-pH | 26.4 $^{a,x}$ | 20.6 $^{b,y}$ | 16.4 $^{c,y}$ | 15.7 $^{c,y}$ |
| | High-pH | 22.5 $^{a,z}$ | 18.8 $^{b,z}$ | 15.2 $^{c,z}$ | 13.9 $^{d,z}$ |

$^{a-d}$Least squares means within a row with a different superscript letter are different ($P < 0.05$).

$^{u-z}$Least squares means within a column with a different superscript letter are different ($P < 0.05$).

[1]*P*-values for aging period < 0.0001; muscle-pH < 0.0001; packaging < 0.0001; display time < 0.0001; muscle-pH × aging period × packaging < 0.0001; muscle-pH × aging period × display < 0.0001.

[2]Standard error for muscle-pH × packaging × aging period = 0.6.

[3]Standard error for muscle-pH × display time × aging period = 0.5.

> PVC; $P < 0.05$). However, 62 d aged normal-pH HiOx-MAP steaks were lighter in color than 62 d aged normal-pH CO-MAP steaks. High-pH steaks packaged in all 3 types of packaging were lighter on d 62 compared with aged 21 d. Nevertheless, aged 0 d steaks were lighter in color compared with aging × packaging combinations for normal- and high-pH steaks.

**a* values.** A muscle-pH × packaging × aging period interaction resulted for a* values (redness; Table 5). Steaks that were normal in pH were more red ($P < 0.05$) at display d 0 than were steaks with a high-pH (a* values 29.4 vs. 23.5). Furthermore, steaks with a normal-pH were redder at 21, 42, and 62 d of aging (Table 5) than similar high-pH steaks. Aged steaks with a high-pH and packaged in either HiOx-MAP or CO-MAP had improved ($P < 0.05$) redness scores compared with high-pH steaks in PVC. At all aging periods, normal-pH steaks in CO-MAP had greater redness than CO-MAP high-pH steaks. Packaging steaks with a normal-pH in CO-MAP stabilized the red color during the aging periods (Table 5); however, steaks with a high-pH and aged 21 d in CO-MAP had greater redness than high-pH steaks in CO-MAP for 42 and 62 d.

Values for a* (Table 5) were greater for steaks with a normal-pH at all aging times than high-pH steaks. In addition, all a* values declined as time in display increased ($P < 0.05$) and as the aging period increased ($P < 0.05$). On 21 d aging, packaging normal- and high-pH steaks in CO-MAP and HiOx-MAP improved redness ($P < 0.05$) compared with PVC packaging. Packaging normal-pH steaks in CO-MAP resulted in stable red color during aging periods; however, 21 d aged high-pH steaks in CO-MAP had greater redness than high-pH steaks in CO-MAP on 42 and 62 d.

## *Chroma and hue angle*

Chroma followed a similar pattern as a* values. Unaged steaks with a normal-pH were more intense in color than high-pH steaks (33.8 vs. 25.8, Table 6). In addition, aged steaks with a normal-pH in PVC were more intense in color than aged steaks with similar and high-pH at 21, 42, and 62 d. HiOx-MAP packaging of steaks had a detrimental effect on color intensity regardless of steak pH. Interestingly, there were no differences in chroma values between aging periods of 21, 42, and 62 d, when steaks with a normal-pH were packaged in CO-MAP. Over all aging periods, CO-MAP in normal-pH had greater redness than CO-MAP in high-pH steaks. The interaction of muscle-pH × display time × aging period (Table 6) clearly indicated

**Table 6.** Effects of muscle-pH, packaging, aging period, and display on chroma (red intensity)[1,2]

| Muscle-pH × packaging × aging period[3] | | | | | |
|---|---|---|---|---|---|
| | | Aging period, d | | | |
| Muscle-pH | Packaging | 0 | 21 | 42 | 62 |
| Normal-pH | PVC | 33.8 $^{a,u}$ | 31.5 $^{b,u}$ | 26.5 $^{c,v}$ | 23.5 $^{d,v}$ |
| | HiOx-MAP | | 29.2 $^{a,v}$ | 26.7 $^{b,v}$ | 23.1 $^{c,v,w}$ |
| | CO-MAP | | 29.8 $^{a,v}$ | 29.5 $^{a,u}$ | 28.3 $^{a,u}$ |
| High-pH | PVC | 25.8 $^{a,v}$ | 22.9 $^{b,x}$ | 20.9 $^{c,x}$ | 20.2 $^{c,x}$ |
| | HiOx-MAP | | 29.7 $^{a,v}$ | 26.5 $^{b,v}$ | 18.4 $^{c,y}$ |
| | CO-MAP | | 27.6 $^{a,w}$ | 23.3 $^{b,w}$ | 22.4 $^{b,w}$ |
| Muscle-pH × display time × aging period | | | | | |
| | | Display time, d | | | |
| Aging period, d | Muscle-pH | 0 | 2 | 4 | 6 |
| 0 | Normal-pH | 39.4 $^{a,u}$ | 33.4 $^{b,u}$ | 32.2 $^{c,u}$ | 28.4 $^{d,u}$ |
| | High-pH | 36.4 $^{a,v}$ | 29.4 $^{b,v}$ | 27.4 $^{c,w}$ | 24.5 $^{d,v}$ |
| 21 | Normal-pH | 36.5 $^{a,v}$ | 29.9 $^{b,v}$ | 29.5 $^{b,v}$ | 24.8 $^{c,v}$ |
| | High-pH | 34.0 $^{a,w}$ | 26.9 $^{b,x}$ | 24.9 $^{c,x}$ | 21.1 $^{d,x}$ |
| 42 | Normal-pH | 34.1 $^{a,w}$ | 28.2 $^{b,w}$ | 25.0 $^{c,x}$ | 23.0 $^{d,w}$ |
| | High-pH | 27.6 $^{a,y}$ | 25.5 $^{b,y}$ | 21.5 $^{c,y}$ | 19.6 $^{d,y}$ |
| 62 | Normal-pH | 31.9 $^{a,x}$ | 25.4 $^{b,y}$ | 21.4 $^{c,y}$ | 21.2 $^{c,x}$ |
| | High-pH | 26.2 $^{a,y}$ | 21.6 $^{b,z}$ | 17.4 $^{c,z}$ | 16.3 $^{d,z}$ |

$^{a-c}$Least squares means within a row with a different superscript are different ($P < 0.05$).

$^{u-z}$Least squares means within a column with a different superscript letter are different ($P < 0.05$).

[1]*P*-values for aging period < 0.0001; muscle-pH < 0.0001; packaging = 0.01; display time < 0.0001; muscle-pH × aging period × packaging < 0.0001; muscle-pH × aging period × display < 0.0001.

[2]Chroma was calculated as $\sqrt{(a^2+b^2)}$. Excel option was utilized to calculate chroma using the function = (SQRT((a*a)+(b*b))), where a and b represents *a** and b* values, respectively.

[3]Standard error for muscle-pH × packaging × aging period = 0.5; Standard error for muscle-pH × display time × aging period = 0.4.

**Table 7.** Effects of muscle-pH, packaging, aging period, and display on hue angle[1,2]

| Muscle-pH × packaging × aging period[3] | | | | | |
|---|---|---|---|---|---|
| | | Aging period, d | | | |
| Muscle-pH | Packaging | 0 | 21 | 42 | 62 |
| Normal-pH | PVC | 37.5 $^{a,u}$ | 40.4 $^{b,u}$ | 40.5 $^{b,u}$ | 42.7 $^{c,v}$ |
| | HiOx-MAP | | 38.1 $^{a,v}$ | 40.2 $^{b,u}$ | 44.3 $^{c,u}$ |
| | CO-MAP | | 29.2 $^{a,y}$ | 28.8 $^{a,x}$ | 29.1 $^{a,y}$ |
| High-pH | PVC | 34.2 $^{a,v}$ | 36.3 $^{b,w}$ | 34.2 $^{c,v}$ | 34.3 $^{c,w}$ |
| | HiOx-MAP | | 31.8 $^{a,x}$ | 30.9 $^{a,w}$ | 31.5 $^{a,x}$ |
| | CO-MAP | | 27.1 $^{a,z}$ | 25.3 $^{b,y}$ | 24.6 $^{b,z}$ |
| Muscle-pH × display time × aging period | | | | | |
| | | Display time, d | | | |
| Aging period, d | Muscle-pH | 0 | 2 | 4 | 6 |
| 0 | Normal-pH | 54.4 $^{a,u}$ | 51.2 $^{b,u}$ | 48.1 $^{c,u}$ | 48.2 $^{c,u}$ |
| | High-pH | 41.6 $^{a,v}$ | 42.1 $^{a,v}$ | 38.1 $^{c,w}$ | 39.5 $^{b,w}$ |
| 21 | Normal-pH | 34.4 $^{a,w}$ | 33.7 $^{a,x}$ | 36.7 $^{b,x}$ | 38.9 $^{c,w}$ |
| | High-pH | 34.3 $^{a,w}$ | 30.1 $^{b,y}$ | 31.2 $^{c,y}$ | 31.3 $^{c,x}$ |
| 42 | Normal-pH | 33.1 $^{a,x}$ | 35.0 $^{b,w}$ | 38.2 $^{c,w}$ | 40.0 $^{d,w}$ |
| | High-pH | 30.7 $^{b,y}$ | 29.3 $^{ab,y}$ | 28.6 $^{a,z}$ | 31.9 $^{c,x}$ |
| 62 | Normal-pH | 33.4 $^{a,x}$ | 36.1 $^{b,w}$ | 41.5 $^{c,v}$ | 43.8 $^{d,v}$ |
| | High-pH | 30.1 $^{a,b,y}$ | 29.1 $^{a,y}$ | 29.4 $^{a,z}$ | 32.1 $^{b,x}$ |

$^{a-c}$Least squares means within a row with a different superscript are different ($P < 0.05$).

$^{u-z}$Least squares means within a column with a different superscript letter are different ($P < 0.05$).

[1]*P*-values for aging period = 0.04; muscle-pH < 0.0001; packaging = 0.01; display time < 0.0001; muscle-pH × aging period × packaging < 0.0001; muscle-pH × aging period × display < 0.0001.

[2]Standard error for muscle-pH × packaging × aging period = 0.5; Standard error for muscle-pH × display time × aging period = 0.6

[3]Hue value was calculated as [arctangent (b*/a*)]. Excel function = ([ATAN(b/a)]/3.14)*180 was used to calculate hue angle, where a and b represents a* and b* values, respectively. Larger values indicate less red, more MetMb.

that, regardless of the packaging system, steaks with a normal-pH had greater chroma values than steaks with a high-pH, and the color intensity declined ($P < 0.05$) as both display and aging time increased.

Hue angle demonstrated that decreased redness in high-pH steaks (Table 7) was not due to discoloration or MetMb formation, which is often associated with increases in hue angles. Before aging, high-pH steaks had a smaller hue angle (37.5 vs.34.2) than normal-pH steaks. Irrespective of the packaging and display time, high-pH steaks had lower hue angle (indicating less discoloration) than normal-pH steaks (Table 7).

## *Metmyoglobin reducing activity*

Two significant interactions occurred for MRA: muscle-pH × packaging × aging period and muscle-pH × display time × aging period (Table 8). On d 0 of aging, steaks with a higher pH had greater ($P <$ 0.05) MRA than d 0 aged steaks with a normal-pH (% MRA = 100 vs. 86.4%, Table 8). As aging time increased, MRA decreased ($P < 0.05$) for steaks packaged in both PVC and HiOx-MAP regardless of the pH. Steaks with a high-pH in CO-MAP had greater ($P < 0.05$) MRA than steaks with a normal-pH at all aging periods. Both normal- and high-pH steaks in HiOx-MAP had the lowest ($P < 0.05$) MRA than other packaging formats at all aging periods.

Data in Table 8 clearly show that meat with a higher pH has a greater % MRA than meat at a normal-pH. Furthermore, there was a small decline of MRA during time in display at both normal and elevated pH treatments. For example, for unaged steaks (d 0 of display at d 0 of aging) the % MRA declined from 85.4 to 82.5% at normal-pH and from 100 to 97.1% for high pH steaks. In addition, the % MRA declined for both normal- and high-pH groups during postmortem aging of 0 to 62 d. On d 6 of display (Table 8), unaged steaks

**Table 8.** Effects of muscle-pH, packaging, and aging period on metmyoglobin reducing activity[1,2]

| Muscle-pH × packaging × aging period[3] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | MRA reported as K/S ratio | | | | Relative MRA (%) | | | |
| | | Aging period, d | | | | Aging period, d | | | |
| Muscle-pH | Packaging | 0 | 21 | 42 | 62 | 0 | 21 | 42 | 62 |
| Normal-pH | PVC | 0.89 $^{c,w}$ | 0.85 $^{b,x}$ | 0.82 $^{b,y}$ | 0.78 $^{a,y}$ | 86.4 $^{c,w}$ | 82.5 $^{b,x}$ | 79.6 $^{b,y}$ | 75.7 $^{a,y}$ |
| | HiOx-MAP | | 0.77 $^{c,y}$ | 0.70 $^{b,z}$ | 0.66 $^{a,z}$ | | 74.8 $^{c,y}$ | 68.0 $^{b,z}$ | 64.1 $^{a,z}$ |
| | CO-MAP | | 0.88 $^{a,x}$ | 0.85 $^{a,x}$ | 0.84 $^{a,x}$ | | 85.4 $^{a,x}$ | 82.5 $^{a,x}$ | 81.6 $^{a,x}$ |
| High-pH | PVC | 1.03 $^{c,v}$ | 0.95 $^{b,w}$ | 0.92 $^{ab,w}$ | 0.90 $^{a,w}$ | 100.0 $^{c,v}$ | 92.2 $^{b,w}$ | 89.3 $^{a,b,w}$ | 87.4 $^{a,w}$ |
| | HiOx-MAP | | 0.92 $^{c,w}$ | 0.85 $^{b,xy}$ | 0.79 $^{a,y}$ | | 89.3 $^{c,w}$ | 82.5 $^{b,xy}$ | 76.7 $^{a,y}$ |
| | CO-MAP | | 1.00 $^{a,v}$ | 0.99 $^{a,v}$ | 0.98 $^{a,v}$ | | 97.1 $^{a,v}$ | 96.1 $^{a,v}$ | 95.1 $^{a,v}$ |
| Muscle-pH × display time × aging period[4] | | | | | | Aging period, d | | | |
| Display time, d | Muscle-pH | 0 | 21 | 42 | 62 | 0 | 21 | 42 | 62 |
| 0 | Normal-pH | 0.88 $^{a,x}$ | 0.83 $^{b,x}$ | 0.79 $^{c,x}$ | 0.76 $^{d,x}$ | 85.4 $^{a,x}$ | 80.6 $^{b,x}$ | 76.7 $^{c,x}$ | 73.8 $^{d,x}$ |
| | High-pH | 1.03 $^{a,v}$ | 0.95 $^{b,v}$ | 0.92 $^{c,v}$ | 0.86 $^{d,v}$ | 100.0 $^{a,v}$ | 92.2 $^{b,v}$ | 89.3 $^{c,v}$ | 83.5 $^{d,v}$ |
| 6 | Normal-pH | 0.85 $^{a,y}$ | 0.78 $^{b,y}$ | 0.70 $^{c,y}$ | 0.72 $^{c,y}$ | 82.5 $^{a,y}$ | 75.7 $^{b,y}$ | 68.0 $^{c,y}$ | 69.9 $^{c,y}$ |
| | High-pH | 1.00 $^{a,w}$ | 0.91 $^{b,w}$ | 0.88 $^{c,w}$ | 0.82 $^{d,w}$ | 97.1 $^{a,w}$ | 88.3 $^{b,w}$ | 85.4 $^{c,w}$ | 79.6 $^{d,w}$ |

$^{a-c}$Least squares means within a row with a different superscript letter are different ($P < 0.05$).

$^{v-z}$Least squares means within a column with a different superscript letter are different ($P < 0.05$).

[1]MRA was calculated as resistance to form metmyoglobin after immersing in nitrite solution. A greater K/S represents lower metmyoglobin formation, hence more MRA. For easy visualization, the greatest K/S i.e. greatest value for MRA was considered as 100% and other K/S values were converted based on the greatest K/S value.

[2]*P*-values for aging period = 0.01; muscle-pH < 0.0001; packaging = 0.01; display time < 0.0001; muscle-pH × aging period × packaging < 0.0001; muscle-pH × aging period × display < 0.0001.

[3]Standard error for muscle-pH × packaging × aging period = 0.02 (MRA based on K/S); 1.94 (Relative MRA).

[4]Standard error for muscle-pH × display time × aging period = 0.01 (MRA based on K/S); 0.97 (Relative MRA).

with a normal-pH had lower MRA than unaged steaks of normal pH on d 0 of display (Table 8). Similarly, on d 6 of display, steaks with a higher pH aged for d 0 had lower MRA than high-pH steaks aged for d 0 and d 0 of display. However, at all aging periods, high-pH steaks had greater ($P < 0.05$) MRA both on d 0 and d 6 than normal-pH steaks.

## *Oxygen consumption*

Two significant interactions occurred for OC: muscle-pH × display time × aging period (Table 9) and muscle-pH × packaging × display time (Table 9). Both aging and display times decreased OC of both normal- and high-pH steaks (Tables 9 and 10).

At all aging periods, high-pH steaks had greater ($P < 0.05$) OC on display d 0 and 6 than normal-pH steaks (Table 9). However, OC decreased for steaks

**Table 9.** Effects of muscle-pH, display, and aging period on oxygen consumption[1,2,3]

| Display | | OC measured as K/S474÷K/525 | | | | Transformed OC [1.5-(K/S474÷K/S525)] | | | | Relative OC, % | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| time, d | Muscle- pH | 0 | 21 | 42 | 62 | 0 | 21 | 42 | 62 | 0 | 21 | 42 | 62 |
| 0 | Normal-pH | $0.51^{d,y}$ | $0.61^{c,y}$ | $0.70^{b,y}$ | $0.73^{a,y}$ | $0.99^{d,y}$ | $0.89^{c,y}$ | $0.80^{b,y}$ | $0.77^{a,y}$ | $81.8^{d,y}$ | $73.6^{c,y}$ | $66.1^{b,y}$ | $63.6^{a,y}$ |
| | High-pH | $0.29^{c,w}$ | $0.39^{b,w}$ | $0.56^{a,w}$ | $0.57^{a,w}$ | $1.21^{c,w}$ | $1.11^{b,w}$ | $0.94^{a,w}$ | $0.93^{a,w}$ | $100.0^{c,w}$ | $91.7^{b,w}$ | $77.7^{a,w}$ | $76.9^{a,w}$ |
| 6 | Normal-pH | $0.60^{d,z}$ | $0.70^{c,z}$ | $0.81^{b,z}$ | $0.85^{a,z}$ | $0.90^{d,z}$ | $0.80^{c,z}$ | $0.69^{b,z}$ | $0.65^{a,z}$ | $74.4^{d,z}$ | $66.1^{c,z}$ | $57.0^{b,z}$ | $53.7^{a,z}$ |
| | High-pH | $0.44^{c,x}$ | $0.54^{b,x}$ | $0.63^{a,x}$ | $0.65^{a,x}$ | $1.06^{c,x}$ | $0.96^{b,x}$ | $0.87^{a,x}$ | $0.85^{a,x}$ | $87.6^{c,x}$ | $79.3^{b,x}$ | $71.9^{a,x}$ | $70.2^{a,x}$ |

$^{a-d}$Least squares means within a row with a different superscript letter are different ($P < 0.05$).

$^{w-z}$Least squares means within a column with a different superscript letter are different ($P < 0.05$).

[1]Standard error for muscle-pH × display time × aging period = 0.01 (OC based on K/S); 1.4 (Relative OC).

[2]OC values were determined on bloom steaks that had been vacuum packaged and incubated at 25°C for 30 min. OC is reported as DeoxyMb present on vacuum packaged steaks. A lower K/S ratio represent greater DeoxyMb and OC. The ratio was transformed by subtracting (1.5 – (K/S474 ÷ K/S 525)), as result a larger number represents greater OC. The transformed values were converted to percentage for easier visualization. To convert K/S474 ÷K/S525 ratio to a relative percentage, the highest OC ratio was considered as 100% and other aging period, muscle-pH, and display time was reported relative to highest OC ratio.

[3]*P*-values for aging period < 0.0001; muscle-pH < 0.0001; display time < 0.0001; muscle-pH × aging period × display = 0.0015.

**Table 10.** Effects of muscle-pH, packaging, and display time on oxygen consumption[1,2,3]

| | OC measured as K/S474 ÷ K/525 | | Transformed OC [1.5-(K/S474÷K/S525)] | | Relative OC, % | |
|---|---|---|---|---|---|---|
| Parameter | Normal-pH | High-pH | Normal-pH | High-pH | Normal-pH | High-pH |
| D0 PVC | 0.65 [a,w] | 0.47 [b,w] | 0.85 [a,w] | 1.03 [b,w] | 82.5 [a,w] | 100.0 [b,w] |
| D6 PVC | 0.78 [a,y] | 0.61 [b,x,y] | 0.72 [a,y] | 0.89 [b,x,y] | 69.9 [a,y] | 86.4 [b,x,y] |
| D6 HiOx-MAP | 0.90 [a,z] | 0.62 [b,y] | 0.60 [a,z] | 0.88 [b,y] | 58.3 [a,z] | 85.4 [b,y] |
| D6 CO-MAP | 0.68 [a,x] | 0.59 [b,x] | 0.82 [a,x] | 0.91 [b,x] | 79.6 [a,x] | 88.3 [b,x] |

[a,b]Least squares means within a row with a different superscript letter are different ($P < 0.05$).

[w-z]Least squares means within a column with a different superscript letter are different ($P < 0.05$).

[1]Standard error for muscle-pH × display time × packaging = 0.01(OC based on K/S); 1.4 (Relative OC).

[2]OC values were determined on bloom steaks that had been vacuum packaged and incubated at 25°C for 30 min. OC is reported as DeoxyMb present on vacuum packaged steaks. A lower K/S ratio represent greater DeoxyMb and OC. The ratio was transformed by subtracting [1.5 – (K/S474 ÷ K/S 525)], as result a larger number represents greater OC. The transformed values were converted to percentage for easier visualization. To convert K/S474 ÷K/S525 ratio to a relative percentage, the highest OC ratio was considered as 100% and other MAP, muscle-pH, and display time was reported relative to highest OC ratio.

[3]*P*-values for muscle-pH < 0.0001; packaging < 0.0001; display time < 0.0001; muscle-pH × packaging × display < 0.0001.

for both normal- and high-pH groups as aging time increased.

On display d 0, high-pH steaks had a greater OC than those with a normal-pH (100% v/s 82.5%, Table 10). Steaks with a normal-pH and packaged in HiOx-MAP had lower ($P < 0.05$) OC than normal-pH steaks in PVC and CO-MAP. High-pH steaks in all 3 packagings on d 0 of display had greater ($P < 0.05$) OC than normal-pH steaks. High-pH steaks in CO-MAP on d 6 of display had greater ($P < 0.05$) OC than HiOx-MAP high-pH steaks on same day.

## *Thiobarbituric acid reactive substances values (lipid oxidation)*

A muscle-pH × packaging × aging period and display time × muscle-pH × aging period interactions (Table 11) resulted for TBARS values. Steaks with a normal-pH aged for 21 d and packaged in PVC and HiOx-MAP had greater ($P < 0.05$) TBARS values than steaks with a high-pH aged for 21 d and packaged in PVC and HiOx-MAP. However, there were no differences ($P > 0.05$) when packaged in CO-MAP for both pH steak groups. The maximum TBARS value was 0.42, which indicates high oxygen conditions in

**Table 11.** Effects of muscle-pH, packaging, and aging period on TBARS values (lipid oxidation)[1,2]

| | | | Aging period, d | | | |
|---|---|---|---|---|---|---|
| Interaction | Muscle-pH | Packaging | 0 | 21 | 42 | 62 |
| Muscle-pH × packaging × aging[3] | Normal-pH | PVC | 0.08 [a,w] | 0.14 [b,y] | 0.16 [bc,y] | 0.18 [c,x] |
| | | HiOx-MAP | | 0.19 [a,z] | 0.30 [b,z] | 0.42 [c,z] |
| | | CO-MAP | | 0.10 [ab,x] | 0.12 [bc,wx] | 0.15 [c,wx] |
| | High-pH | PVC | 0.06 [a,w] | 0.12 [b,xy] | 0.13 [b,wxy] | 0.15 [b,wx] |
| | | HiOx-MAP | | 0.13 [a,xy] | 0.15 [a,xy] | 0.22 [b,y] |
| | | CO-MAP | | 0.10 [a,x] | 0.11 [a,w] | 0.13 [a,w] |
| Display time × muscle-pH × aging period[4] | Display time | Muscle-pH | 0 | 21 | 42 | 62 |
| | 0 | Normal-pH | 0.08 [a,w] | 0.11 [b,w] | 0.18 [c,y] | 0.24 [d,y] |
| | | High-pH | 0.07 [a,w] | 0.12 [b,w] | 0.10 [b,w] | 0.18 [c,w] |
| | 6 | Normal-pH | 0.12 [a,x] | 0.18 [b,x] | 0.29 [c,z] | 0.37 [d,z] |
| | | High-pH | 0.09 [a,w] | 0.12 [b,w] | 0.15 [c,x] | 0.21 [d,x] |

[a-d]Least squares means within a row with a different superscript are different ($P < 0.05$).

[w-z]Least squares means within a column with a different superscript letter are different ($P < 0.05$).

[1]TBARS values were reported as absorbance at 532 nm.

[2]Standard error for muscle-pH × aging × packaging = 0.02.

[3]Standard error for muscle-pH × display time × packaging = 0.01.

[4]*P*-values for aging period = 0.03; muscle-pH < 0.0001; packaging < 0.0001; display time < 0.0001; muscle-pH × aging period × packaging < 0.0001; muscle-pH × aging period × display = 0.0003.

combination with normal-pH was conducive for lipid oxidation compared with high-pH at same conditions.

## Discussion

Quality variations in meat, such as a higher pH than normal, are intimately and complexly related to the chemical and physical changes in muscle that occur ante- and postmortem. The etiology of rapid glycolytic rates early postmortem in carcass meat ranges from the pale, soft, and exudative condition to dark, firm, and dry or dark-cutting if the stressors are prolonged until near glycogen depletion. Most of these aberrant properties are related to 2 fundamental chemical events in meat, OC and MRA (Ramanathan et al., 2019). Only recently, were comparative methodologies developed (English et al., 2016b) to more accurately study MRA and OC in high-pH steaks under various processing and packaging conditions.

The current research focused on a systems approach involving 2 pH levels, 3 packaging formats, 4 vacuum storage periods, and simulated display of steaks for up to 6 d. As expected, there were interactions for nearly every trait measured. All the chemical and physical data for the 2 pH groups were verified for pH. In addition, the proximate analyses and modified atmosphere gas compositions were typical to expectations for the various treatments listed in the materials and methods.

Aging time in combination with display time decreased pH of normal-pH beef when aged for 21 and 42 d. Conversely, aging time and 6 d display increased muscle-pH of high-pH steaks except for 0 d aging. CO-MAP, both in normal- and the higher-pH groups, resulted in the lower muscle-pH after 6 d display. Anaerobic conditions can promote more glycolytic activity and lower oxidative changes can result in lower muscle-pH compared with the high-oxygen and aerobic (PVC) packaging.

Muscle ultra-structure and biochemical properties vary between muscle with a normal vs. high-pH (Hunt and Hedrick, 1977b; Swatland, 2008). Hence, lightness and redness as indicated by L*, a*, and chroma values were lower in high-pH vs. normal-pH beef. A greater pH allows meat to hold more water, resulting in cell swelling and lower light reflectance. Conversely, meat with a greater pH is a more conducive environment for oxygen-consuming enzymes, leading to greater DeoxyMb (Ashmore et al., 1971; Tang et al., 2005a; English et al., 2016b). Hence, both conditions lead to a darker beef color. Aging time increased $L^*$ values of high-pH beef. Longer aging time can increase proteolysis (Huff-Lonergan and Lonergan, 2005); thus the meat holds less water, leads to greater reflectance. Previous research noted that an extended aging period increased reflectance properties of high-pH beef by 3% compared with d 0 aging (English et al., 2016b). However, this change was minimal compared to redness and lightness of normal-pH beef. Thus, in the current research utilized 2 MAP formats known to improve redness and lightness. Both HiOx-MAP and CO-MAP improved lightness of steaks with a high-pH than normal-pH beef compared to PVC packaging. Greater oxygen content can saturate more myoglobin and also increase oxygen penetration. The myoglobin form present can influence lightness. For example, predominant DeoxyMb will have lower $L^*$ values than COMb and OxyMb (Ramanathan et al., 2010). Carboxymyoglobin has similar spectral characteristics to OxyMb myoglobin (Suman et al., 2006), hence high-pH steaks in CO-MAP had a lighter color than did steaks in PVC packaging.

Previous research reported that aging more than 14 d can be detrimental to color stability (King et al., 2012; Mancini and Ramanathan, 2014; Kim et al., 2017). During 21 d aging, steaks with a normal-pH packaged in CO-MAP had greater redness than HiOx-MAP and PVC. Various studies have shown that COMb is more color stable that OxyMb (John et al., 2005; Liu et al., 2014). Hence, steaks with a normal-pH in CO-MAP had greater a* values and chroma during 42- and 62-d aging periods. However, there was a significant negative effect of aging time on the redness of HiOx-MAP steaks. Previous research also noted that increased aging time and display time decreased color stability of steaks in HiOx-MAP more than PVC packaging (English et al., 2016a).

Interestingly, steaks with a higher-pH in CO-MAP had lower a* values than steaks in HiOx-MAP when aged for 42 d. We speculate that 0.4% CO did not provide enough molecules of CO to saturate the available myoglobin, whereas there were a surplus of oxygen in the HiOx-MAP packages resulting in a deeper surface penetration by OxyMb. However, with aging extended to 62 d, steaks in HiOx-MAP had deteriorated in color in a more oxidative environment compared with steaks in CO-MAP. Overall, in the current research, changes during display (d 0 to d 6) were lower for steaks with a high-pH than normal-pH steaks as the anaerobic CO-MAP system had greater color stability. Hue angle values supported that a greater pH can limit myoglobin oxidation than normal-pH. Previous studies also reported a greater pH can stabilize myoglobin and other oxidative changes (Mancini et al., 2011;

Nerimetla et al., 2017). Hence, lower chroma and a* values in high-pH steaks can be a function of pH effects on reflectance and increased OC.

Extended aging time in conjunction with display effects decreased OC and MRA of steaks with normal- and a higher-pH. Various parameters such as decreased NADH, mitochondrial damage, and lower antioxidant capacity can be attributed to lower color stability of aged steaks (Mitacek et al., 2019). Both OC and MRA are interrelated processes. More specifically, mitochondria are key organelle involved in both MRA and OC. Mitochondria and oxygen-consuming enzymes can utilize oxygen in meat. A lower oxygen partial pressure is required for MRA. Further mitochondria can contribute to MRA. Hence, any process that affects mitochondrial function can impact MRA and OC. Previous research (Tang et al., 2005a; Mancini and Ramanathan, 2014) noted that aging time could increase mitochondrial damage in normal-pH meat. A greater pH can limit oxidative changes and mitochondrial damage (Ramanathan and Mancini, 2018). Hence, increased OC and MRA in high-pH steaks can be attributed, in part, to less extensive oxidative damages to mitochondria and enzymes involved in both processes in higher pH meat.

Atmospheric conditions within a package can influence MRA and OC. Steaks in HiOx-MAP steaks had lower MRA and OC than PVC, possibly due to the negative effects of oxygen concentration. Carbon monoxide-MAP creates anaerobic condition during storage and may represent the best packaging environment for color and oxidative stability, especially if the pH is greater than normal. Both MRA and OC depends on various factors such as enzyme activity, availability of reducing equivalents such as NADH or succinate (Lanier et al., 1978; Liu et al., 2014). Greater oxidative changes can limit the activity of enzymes involved in MRA and OC, and the ability to regenerate reducing equivalents. More specifically, previous in-vitro research has noted that lipid oxidation products such as aldehydes and peroxides can increase myoglobin oxidation, make myoglobin a poor substrate for enzymatic-MRA, and decrease activity of lactic dehydrogenase (Lynch and Faustman, 2000; Ramanathan et al., 2014; Elroy et al., 2015). Furthermore, incubation of 4-hydroxy-2-nonenal (a secondary lipid oxidation product) with bovine mitochondria decreased mitochondrial function and mitochondria-mediated MRA (Ramanathan et al., 2012). These effects were clearly demonstrated in CO-MAP compared with oxygen containing packages. Further, a greater pH in muscle can limit oxidative changes. Hence, enzymes involved in MRA and OC can retain more activity than normal-pH steaks. Therefore, OC and MRA were greater in higher-pH steaks, which may benefit from vacuum and other anaerobic systems.

### *Conclusion*

Extended aging decreased color stability of normal- and high-pH steaks packaged in PVC and HiOx-MAP. Greater oxidative conditions in the HiOx-MAP, in combination with longer aging time, decreased the redness of both normal and high-pH steaks compared with PVC. Utilization of CO-MAP can limit discoloration with extended aging for both in normal- and high-pH beef, providing there is strict cold-chain management. Use of appropriate myoglobin quantification methods are critical in determining biochemical properties of high-pH beef. Understanding the OC and MRA changes associated with aging can help beef processors to select packaging systems that will limit losses due to oxidative discoloration.

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