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Effects of postmortem storage time on color and mitochondria in beef

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ABSTRACT

To assess the effects of aging time (0, 15, 30, and 45 d) and temperature (0 or 5 °C) on beef mitochondria and steak color, vacuum packaged *longissimus* (n = 15) and cardiac muscles were assigned to 1 of 6 temperature × time combinations. As time increased, initial red color intensity increased whereas both mitochondrial oxygen consumption and color stability decreased. The decrease in mitochondrial oxygen consumption associated with longer aging times will increase initial color intensity. However, this improvement in color development will be negated by the decreased color stability that results from the effects of storage on mitochondria.

1. Introduction

In fresh meat, myoglobin can exist in any of 3 redox states: deoxymyoglobin, oxymyoglobin, and metmyoglobin. The color of deoxymyoglobin is purplish-red; whereas oxymyoglobin is responsible for the consumer-preferred bright cherry-red color of fresh meat. As a result, the formation of oxymyoglobin via oxygenation of deoxymyoglobin, determines initial red color intensity. Development of brown colored metmyoglobin on the surface of beef products results from myoglobin oxidation. Thus, metmyoglobin reducing activity, which regenerates ferrous myoglobin, is critical for meat color stability (Bekhit & Faustman, 2005; Faustman & Cassens, 1990; Ledward, 1985).

Meat color, in particular intensity and stability, is significantly influenced by mitochondrial activity via two mechanisms: oxygen consumption and metmyoglobin reducing activity. Oxygen consumption decreases initial red color development and intensity (decreases myoglobin oxygenation) when mitochondrial respiration outcompetes myoglobin for oxygen. This maintains myoglobin in a deoxy-state and results in dark colored muscle (Ledward, 1992). Furthermore, mitochondria have the ability to influence color stability via mitochondria-mediated metmyoglobin reducing activity (Ramanathan, Mancini, & Naveena, 2010). As a result, factors that influence mitochondrial activity can affect color. The practical implications of this are commonly associated with the role of postmortem aging in beef color during subsequent display.

Lindahl (2011) reported that initial color development of *longissimus* and *semimembranosus* steaks increased as time postmortem increased because of changes in oxygen consumption. Lee, Apple, Yancey, Sawyer, and Johnson (2008a) reported that aging time in vacuum influenced initial color development of *gluteus medius* steaks due to changes in oxygen-consuming enzyme activity. Young, Priolo, Simmons, and West (1999) reported that as storage time of beef strip loins increased, the time required for initial color development decreased due to changes in oxygen consumption. Lee, Apple, Yancey, Sawyer, and Johnson (2008b) concluded that further research assessing factors that influence initial color development of vacuum packaged beef is needed. Lagerstedt, Lundström, and Lindahl (2011) reported that the color stability of *longissimus* steaks aerobically packaged decreased as aging time in vacuum increased. As a result, although initial color development is greater when mitochondrial oxygen-consuming processes are disrupted, subsequent color is often negatively impacted (Young et al., 1999).

Tang et al. (2005) assessed mitochondrial morphology and function at 2 h, 6 h, and 60 d postmortem and reported that mitochondrial oxygen consumption decreased as time increased. To date, additional research has not assessed the effects of aging times less than 60 d and temperature on mitochondrial function. Therefore, the objectives of this research were to assess the effects of aging time (0, 15, 30, and 45 d) and temperature (0 or 5 °C) on beef mitochondrial oxygen consumption and metmyoglobin reduction, as well as steak color intensity and color stability. Two separate experiments, using skeletal and cardiac

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muscles, were conducted to better understand the role of mitochondria and myoglobin in meat color during extended aging.

2. Materials and methods

2.1. Experiment 1 (effects of aging on beef color intensity and stability)

2.1.1. Experimental design

A split plot design was used to evaluate the effects of aging temperature and time on color intensity, metmyoglobin reducing activity, and lipid oxidation. The whole plot consisted of a randomized complete block, with USDA Select *Longissimus lumborum* (NAMP 180; NAMP, 2002; n = 15) muscles serving as blocks. Two steaks were removed from each muscle and used to measure color intensity and stability prior to storage (0 d of storage, no aging). The remaining portion of each *longissimus* was divided into 6 roasts (5 cm thick) and vacuum packaged (4 mil, standard barrier nylon/polyethylene, 0.6 cm^3 O_2/645.16 cm^2/24 h at 0 °C; Bunzl Koch Supplies Inc., Kansas City, MO). Three of the 6 roasts within each loin were aged at either 0 °C or 5 °C. Of the 3 roasts within each aging temperature, 1 roast was stored in the dark in vacuum packaging for either 15, 30, or 45 d. As a result, a 2-way + 1 factorial treatment structure of storage temperature × time was used, resulting in a no storage/aging control and 6 treatment combinations (1 = 15 d at 0 °C; 2 = 15 d at 5 °C; 3 = 30 d at 0 °C; 4 = 30 d at 5°; 5 = 45 d at 0 °C; and 6 = 45 d at 5 °C).

Within the subplot, each 5 cm-roast was bisected after aging. Of the 2 steaks resulting from each storage time × temperature combination (in addition to the 2 steaks per loin removed prior to storage/aging), 1 steak was used for initial day 0 measurements (prior to display) and the second steak was used to measure color stability during display. Red color intensity, metmyoglobin reducing activity, and lipid oxidation was measured prior to display immediately after each aging period after samples were removed from vacuum. The second steak was over-wrapped in PVC aerobic packaging (over-wrapped with oxygen-permeable polyvinyl chloride fresh meat film; 15,500–16,275 cm^3 O_2/m^2/24 h at 23 °C, E-Z Wrap Crystal Clear Polyvinyl Chloride Wrapping Film, Koch Supplies, Kansas City, MO) and displayed for 7 days at 4 °C (fluorescent lighting at 3000 K and 1614 lumens). This steak was used to assess metmyoglobin reducing activity and lipid oxidation on day 7 of display. The surface color of this steak also was measured daily during display to determine color stability. As a result, a randomized complete block with repeated measures was used to assess aging effects on color stability.

2.1.2. Color measurements

Surface color was measured at three random locations on each steak using a HunterLab MiniScan XE Plus spectrophotometer (HunterLab Associates, Reston, VA, USA) with a 2.54-cm diameter aperture, Illuminant A, and 10° standard observer. Reflectance at isobestic wavelengths from 400 to 700 nm was used to quantify myoglobin redox forms on the surface of steaks. Myoglobin 100% reference standards were determined using extra steaks and substituted into the appropriate equation outlined in AMSA (2012). These values also were used to calculate metmyoglobin reduction (metmyoglobin reducing activity).

To assess initial color intensity, the fresh-cut surface of each steak was scanned immediately after aging when each roast was bisected to determine pre-oxygenation a* and oxymyoglobin values. Each steak was then oxygenated at 1 °C for 1 h. During this time, a* and oxymyoglobin were measured every 5 min. The increase in a* and oxymyoglobin that occurred during the 1-h oxygenation period was used to determine initial color intensity (myoglobin oxygenation). Surface color, including a* and metmyoglobin, was measured daily for 7 days after aerobic packaging. Color stability was characterized using a* and % metmyoglobin; measurements of redness and discoloration, respectively.

2.1.3. Metmyoglobin reducing activity

Metmyoglobin-reducing activity was determined after aging for 0, 15, 30, and 45 d, and on day 7 after packaging in PVC according to the procedures described by Sammel, Hunt, Kropf, Hachmeister, and Johnson (2002). Samples from the interior of steak halves were submerged for 20 min in a 0.3% solution of sodium nitrite (Sigma, St. Louis, MO) to facilitate metmyoglobin formation, and then removed, blotted dry, vacuum packaged, and scanned with a HunterLab MiniScan XE Plus Spectrophotometer to determine pre-incubation metmyoglobin values (AMSA, 2012). Each sample was incubated at 30 °C for 2 h to induce metmyoglobin reduction. Upon removal from the incubator, samples were rescanned to determine the percentage of remaining surface metmyoglobin. The following equation was used to calculate metmyoglobin reducing activity: [(% surface metmyoglobin pre-incubation − % surface metmyoglobin post-incubation) ÷ % surface metmyoglobin pre-incubation] × 100. Increased metmyoglobin reducing activity is associated with improved color stability.

2.1.4. Lipid oxidation

Thiobarbituric acid reactive substance values were measured according to the procedure of Witte, Krause, and Bailey (1970). From each steak, 5 g of sample was blended with 25 mL TCA solution (20%) and 20 mL distilled water. Samples were homogenized using a Waring table-top blender (Dynamics Corp. of America, New Hartford, CT, USA) for 1 min and filtered through Whatman (#1) filter paper. One milliliter of filtrate was mixed with 1 mL TBA solution (20 mM), incubated at 25 °C for 20 h, and absorbance at 532 nm was measured using a Shimadzu UV-2101 PC spectrophotometer (Shimadzu Inc., Columbia, MD, USA). Blank samples for the spectrophotometer consisted of 2 mL TCA/distilled water (1:1 v/v) and 2 mL TBA solution.

2.1.5. Statistical analysis

Data were analyzed using the Mixed Procedure of SAS (SAS, 2011). Fixed effects included aging temperature, aging time, post-aging/post-PVC packaging time, and their interactions. For the split plot, random effects included loin, loin × whole plot treatments (Error A), and residual error (Error B). For color stability data, the random term included loin and the repeated option was used to assess covariance–variance structure amongst the repeated measures. The most appropriate structure was determined using AIC and BIC output. Least square means for protected *F*-tests ($P < 0.05$) were separated using the diff option and were considered significant at $P < 0.05$.

2.2. Experiment 2 (effects of aging on mitochondria)

2.2.1. Experimental design

Myoglobin and mitochondria isolated from beef cardiac muscles (n = 15) aged at different temperatures and times were used to provide a better fundamental understanding of the mechanism by which aging influences color chemistry. More specifically, samples were used to assess the combined effects of aging time and temperature on mitochondria-mediated oxygen consumption and metmyoglobin reduction. In the current study, mitochondria were isolated from fresh bovine hearts because the mitochondrial content in cardiac muscle is greater than that in skeletal muscle; therefore, cardiac muscle has been used as a model for mitochondrial respiration (Tang et al., 2005). Fresh bovine cardiac muscles were procured on the day of slaughter and 150 g was removed for day 0 analyses (0 d of storage, no aging). The remaining portion of each sample was divided into six equal portions. Using a randomized complete block design where each cardiac muscle served as a block, 1 of the 6 portions within each block was vacuum packaged and stored at either 0 or 5 °C for 15, 30, and 45 d (6 time × temperature combinations outlined in experiment 1). After aging, mitochondria were isolated and used to assess the effects of aging temperature and time on oxygen consumption (determinant of initial color intensity) and metmyoglobin reduction (factor influencing color stability).