



## Change in Myoglobin Denaturation and Physiochemical Properties Among Three Degrees of Doneness and Three Beef Whole Muscles

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<sup>1</sup>Contribution no. 24-015-J of the Kansas Agricultural Experiment Station, Manhattan, KS 66506.

**Abstract:** While the factors impacting raw meat color have been extensively explored, the factors affecting cooked meat color have been largely ignored, leaving a gap in research. Establishing a baseline for the changes that accompany different degrees of doneness (DOD) could serve as the foundation for future studies to further understand what factors are impacting differences in cooked color of whole muscles. Beef strip loins ( $n = 12$ ) and top butts ( $n = 12$ ) were used to evaluate the *Longissimus dorsi* (LD), *Biceps femoris* (BF), and *Gluteus medius* (GM) between 3 DOD: medium rare (62.8°C), medium (71.1°C), and well-done (76.7°C). Immediately after cooking, steaks were sliced, evaluated for  $L^*$ ,  $a^*$ ,  $b^*$ , frozen in liquid nitrogen, and blended into a powder for lab assays including myoglobin denaturation, metmyoglobin-reducing activity (MRA), surface hydrophobicity, proximate analysis, and lipid oxidation. An additional steak was cooked for shear force and cook loss. As expected, the  $a^*$  values decreased ( $P < 0.05$ ) and myoglobin denaturation percentages increased ( $P < 0.05$ ) with increasing DOD, while muscle source did not impact ( $P > 0.05$ ) either. Surface hydrophobicity, which is a measure of exposed hydrophobic groups caused by denaturation, increased ( $P < 0.05$ ) with increasing DOD, while MRA decreased ( $P < 0.05$ ) with each DOD. However, the BF and GM muscles resulted in greater ( $P < 0.05$ ) MRA compared to the LD. There was an interaction ( $P < 0.05$ ) for lipid oxidation. As DOD increased past the raw state, differences in oxidation between muscles emerged. While the factors evaluated among the DOD were impacted, muscle type surprisingly affected most assays evaluated as well. This study can serve as a foundational paper to support future cooked color research to further understand what factors impact cooked color and cooked color stability of whole muscle cuts.

**Key words:** cooked color, myoglobin, color stability, beef

*Meat and Muscle Biology* 8(1): 16919, 1–12 (2024)

doi:10.22175/mmb.16919

Submitted 25 August 2023

Accepted 19 December 2023

## Introduction

Cooked meat color is the visual external and internal appearance of a steak, which drives the consumer's perception of quality and food safety (Cox et al., 1994; Prill et al., 2019). The mechanisms that cause the changes a steak undergoes from the raw state to the cooked state are vast. The color change associated with cooking is largely attributed to complex changes in myoglobin, even though other factors are involved (Mancini and Hunt, 2005; Suman and Joseph, 2013).

Myoglobin undergoes a series of intricate post translational changes through the aging and cooking processes altering the pigment (Hughes et al., 2020; Salim et al., 2020). While the mechanisms of color change within raw meat and ground beef have been intensely studied, the same depth has not been achieved for cooked color of whole muscles.

The heating application of the cooking process changes the overall appearance and eating experience of a steak, categorized as different degrees of doneness (DOD; Tornberg, 2005; Prill et al., 2019). While

heat is necessary for cooking, it is also a common method to denature proteins, changing their solubility and functionality (Tornberg, 2005). These changes can impact the water-holding capacity, oxidation, aggregation, tenderness, and the pigment form for optically active proteins such as myoglobin (Tornberg, 2005). While the physical changes that occur during cooking between different DOD have been investigated (Lorenzen et al., 1999; Lucherik et al., 2016; Drey et al., 2019), the mechanism of change for cooked color remains unexplored for whole muscles.

The factors impacting raw meat color and ground beef color have been researched extensively (Mancini and Hunt, 2005). A multitude of factors impact raw meat color, including muscle source, packaging, inclusion of antioxidants or antimicrobials, age of the animal, aging period of the meat, level of oxidation, and pre-harvest factors (Mancini and Hunt, 2005; Suman and Joseph, 2013). Due to food safety concerns of premature browning, ground beef cooked color has also been robustly evaluated. Hunt et al. (1999) determined the ratio of myoglobin forms at the time of cooking will dictate the final pigment of ground beef. Therefore, any factor impacting the raw color stability has the potential to impact cooked meat color such as muscle source, oxidation, and metmyoglobin-reducing activity (MRA) (Hunt et al., 1999; Suman and Joseph, 2013).

While evaluating factors impacting raw meat color has been a priority of research for decades, the factors affecting the cooked color of whole muscles have been largely ignored. Therefore, the objective of this study was to determine the changes in myoglobin denaturation and physiochemical properties of 3 different muscles cooked to a medium rare (MR), medium (MED), or a well-done (WD) DOD.

## Materials and Methods

### Product collection and fabrication

Beef strip loins ( $n = 12$ , IMPS # 180) and top butts ( $n = 12$ , IMPS # 184) grading USDA Select were collected from a Midwestern beef processor and transported to Kansas State University (KSU) by KSU personnel. The subprimals were aged for 28 d in covered cardboard boxes at 2–4°C before being sliced into 2.54-cm thick steaks. Eight steaks were randomly assigned a DOD by numbering the steaks from the anterior end. Based on the randomization, each steak was assigned one of the following DOD: raw, MR, MED, or WD. Two steaks from each DOD were

reserved for Warner-Bratzler shear force (WBSF) or lab assays. Once labeled and vacuum sealed, the steaks were frozen in a commercial freezer at KSU and held at –20°C until time of use. All samples were frozen at the same time under the same conditions, minimizing any confounding effects of freezing.

### Cooking procedures and internal color

Each steak was thawed 24 h prior to cooking at 2–4°C by placing a single layer of steaks on trays. Once thawed, a thermometer (Thermopen mk4, Salt Lake City, UT) was inserted into the thickest part of the steak and added to a clamshell grill (Cuisinart Griddler Deluxe, East Windsor, NJ) set to 175°C. The steaks were removed 5–7°C below the designated DOD, and the peak internal temperatures were recorded. The MR, MED, and WD samples were cooked to an end-point temperature of 62.8°C, 71.1°C, and 76.7°C, respectively. The steaks were removed from the heat source and allowed to rest for 3 min before being sliced in the geometric center from cooked surface to cooked surface. The surface was covered with film and used to evaluate internal color after a 3-min rest period according to the AMSA Color Guidelines (King et al., 2023). Three color readings were taken at different internal locations. An average of 3 readings for CIE  $L^*$ ,  $a^*$ , and  $b^*$  were taken using a calibrated Hunter Lab Spectrophotometer (Illuminant A, 1.27 cm aperture, 10° observer, Hunter Lab Associates Laboratory, Reston, VA). The spectrophotometer was calibrated using the white and black tiles covered in film, and a green tile test was used to ensure proper calibration. After the color readings, the steaks were chopped and frozen in liquid nitrogen and blended (Waring Products, New Hartford, CT) into powder for future assays and held in a –80°C freezer.

### Warner-Bratzler shear force

Before cooking, the raw sample was weighed and recorded for a cook loss measurement. The WBSF samples were cooked using the same protocol as described earlier and followed the approved method from the AMSA Sensory Guidelines (AMSA, 2015). Samples were weighed to determine the percentage of weight lost in cooking before being cooled for at least 12 h at 2–4°C. Six cores were taken parallel to the muscle fiber orientation and sheared with the WBSF blade perpendicular to the muscle fiber orientation using an Instron (Model 5569, Instron Corp., Canton, MA) with a crosshead speed of 250 mm/min and a load cell of 100 kg. All 6 measurements were averaged and represented as kg of force.

## Myoglobin denaturation

Myoglobin denaturation was determined using the protocol in the AMSA Color Guidelines (King et al., 2023). A frozen 0.3 g powdered sample of each steak was weighed into a 2 mL microcentrifuge tube with 6 ceramic beads pre-added. Immediately after weighing, 1.3 mL of ice cold 20 mM potassium phosphate buffer was added to each tube. The samples were homogenized for 30 s using the bead homogenizer and transferred to 1.5 mL microcentrifuge tubes to be centrifuged for 30 min at 10,000 x g. The supernatant was poured into a 50 mL beaker and added to a 5 mL syringe. A filter was added to the end of the syringe, and the supernatant was filtered into a new 1.5 mL centrifuge tube as the final sarcoplasmic extraction. A 200 µL sample was plated in duplicate on a 96-well plate. Sodium hydrosulfite (50 µL) was added to each well to reduce all forms of myoglobin to the deoxymyoglobin form. The absorbance was read at 433 nm and converted to the percentage denatured using the following formula:

$$\% \text{Myoglobin Denaturation} = (\text{Cooked Absorbance (A)}/\text{Raw A}) \times 100.$$

## Metmyoglobin-reducing activity

The MRA was determined using the protocol in the AMSA Color Guidelines (King et al., 2023). The protocol was modified to use a 0.3 g sample instead of 5 g sample. Briefly, 0.3 g of the powdered sample was weighed and added to a bead homogenizer tube with 1.2 mL phosphate buffer. Samples were homogenized and centrifuged at 14,000 rpm for 30 min. The supernatant was added to an additional 1.5 mL centrifuge tube. A 96-well plate was prepared by pipetting 50 µL of 0.75 mM metmyoglobin, 25 µL of 3.0 mM potassium ferrocyanide, 50 µL of deionized water, 25 µL of 5 mM EDTA, and 25 µL 3 mM sodium citrate buffer to each well. Next, 50 µL of each sample was added to the plate in duplicate. After the sample was added to each well, 25 µL of 1 mM NADH was quickly added, before being read at 580 nm every 60 s for a total of 180 s. Beer's law was used to calculate the change in absorbance to represent the change from metmyoglobin to oxymyoglobin (King et al., 2023).

## Surface hydrophobicity

Surface hydrophobicity is a measure of aggregation of the hydrophobic portions of the side chains of proteins caused by denaturation (Dominguez-Hernandez and Ertbjerg, 2021). This assay uses bromophenol blue (BPB) as a chromophore indicator for exposed hydrophobic

groups as described by Dominguez-Hernandez and Ertbjerg (2021) and modified to evaluate the sarcoplasmic protein portion. A 0.3 g sample of meat powder was weighed and added to bead homogenizer tubes with 1.5 mL of sodium phosphate buffer. The mixture was homogenized for 30 s and centrifuged at 4,000 x g for 5 min, and the resulting supernatant was collected as the sarcoplasmic proteins. A 1 mL portion of the protein stock was added to 200 µL of 1 mg/mL concentration of BPB and incubated for 10 min in the dark before being centrifuged an additional time at 12,000 x g for 5 min. The remaining supernatant was diluted to a 1/10 ratio with buffer plated in duplicate on a 96-well plate. A control with no protein was used to calculate the change in absorbency as the bound BPB per mg of protein.

## Lipid oxidation

Lipid oxidation was determined through the thiobarbituric acid reactive substances assay following the procedures described by Ahn et al. (1998) and Dahmer et al. (2022). A standard curve was created with malondialdehyde diethyl acetal to represent 1.56, 3.13, 6.25, 12.5, and 25 µM of malondialdehyde (MDA). Briefly, 0.1 g sample of powdered sample was weighed and added to bead homogenizer tubes with 0.7 mL of thiobarbituric acid and trichloroacetic acid and 0.5 mL of butylated hydroxy toluene. The samples were centrifuged at 2,000 x g for 5 min, and 0.6 mL of the supernatant was transferred into 13 x 100 mm glass tubes. The tubes were vortexed, and then covered in aluminum foil to be incubated in a 70°C water bath for 30 min before being cooled in an ice bath for 5 min. Lastly, 0.2 mL of the supernatant was pipetted into a 96-well plate in duplicate. The plate was run at 532 nm absorbance, and the standard curve was used to determine the concentration of MDA of each sample.

## Proximate analysis

Proximate analysis was performed on all cooked and raw samples, as applicable. The fat content was measured using the Folch method (Folch et al., 1957). A 5 g sample was weighed in duplicate into 50 mL centrifuge tubes. Water was added to each tube before vortexing. Next, chloroform and methanol were added to each tube, and the mixture was shaken by a mechanical shaker for 4 min. The sample was centrifuged for 10 min at 5,000 rpm, and the resulting polar supernatant was removed and decanted using a pipette. Next, a 4 mL sample of the chloroform layer under the hard solid film was added to a pre-dried and weighed glass tube. The samples were evaporated using heating

stones and nitrogen gas. The resulting sample was dried for 24 h at 100°C and reweighed for a fat percentage measurement.

Moisture was determined by the approved AOAC drying oven method (950.46 and 934.01; AOAC, 1995). A 4-g sample was weighed in duplicate into pre-dried and weighed aluminum pans. The samples were dried in a drying oven for 24 h at 100°C and re-weighed to determine moisture loss.

The pH was determined by weighing 5 g of each raw and cooked sample in duplicate with 50 mL of deionized water. The sample was homogenized with a medium head homogenizer for 20 s at a 10,000 x g head speed. The pH of the mixture was read with a calibrated InLab Solids Pro-ISM probe (Part #51344155; Mettler-Toledo, Schwerzenbach, Switzerland) connected to a Seven Compact pH meter (Mettler-Toledo, Columbus, Ohio).

**Statistical analysis.** The statistical analysis was conducted using SAS (Version 9.4; SAS Inst., Inc., Cary, NC) PROC GLIMMIX. Carcass served as the experimental unit. Fixed effects were muscle type and DOD. Data were analyzed as a split-plot design with the whole plot factor as muscle and the subplot factor of DOD. An  $\alpha$  of 0.05 was set for a level of significance. The Kenward-Roger adjustment was used in all analyses.

## Results and Discussion

### Cooked internal color

It is well documented that internal color changes throughout the cooking cycle; however, the relationship between the factors impacting cooked color remain unknown (Hunt et al., 1999; Prill et al., 2019; Salim et al., 2020; Schwartz et al., 2022). This study aimed to evaluate all factors impacting cooked color, especially myoglobin. Consumers expect DOD to appear a certain way when cooked or ordered at a restaurant, and if they do not, their eating experience could be altered (Cox et al., 1994; Prill et al., 2019). While consumer acceptance and expectations of each DOD have been established, the mechanisms behind the color change have remained unexplored (Prill et al., 2019; Schwartz et al., 2022).

Color acceptability is mostly linked to the  $a^*$  and  $L^*$  values, with  $a^*$  having the largest changes throughout shelf life and during the cooking cycle (Mancini and Hunt, 2005; Dikeman et al., 2015; Prill et al., 2019). As expected, in the current study, DOD

impacted the  $a^*$  values, but surprisingly  $L^*$  and  $b^*$  values were not affected. The  $L^*$ ,  $a^*$ , and  $b^*$  values are shown in Table 1.

$L^*$  values were not impacted ( $P > 0.05$ ) by the different DOD. Dikeman et al. (2013) found the same relationship for  $L^*$  values between the MR and MED DOD for the *longissimus lumborum* (LL). However, Prill et al. (2019) found a time and DOD interaction where the MR samples lightened over time and WD samples darkened. While  $L^*$  and  $a^*$  values impact raw meat color, these studies indicate  $L^*$  values might have less of an impact on cooked meat color compared to raw meat color (Dikeman et al., 2013; Prill et al., 2019).

Additionally, the LL resulted in the highest ( $P < 0.05$ )  $L^*$  value followed by the *gluteus medius* (GM), with the *biceps femoris* (BF) having the lowest ( $P < 0.05$ )  $L^*$  value. Lightness is highly dependent on the muscle fiber type, muscle metabolism, moisture content, pH, and color stability, with differences commonly found among raw muscles (Mancini and Hunt, 2005; Suman et al., 2014; Park et al., 2018). Our results would indicate such muscle-to-muscle differences in lightness are present not only in raw muscles but in the cooked form as well. Overall, the current study illustrates  $L^*$  values have the potential to represent other intrinsic differences between various cooked muscles, while not being impacted by different DOD.

**Table 1.** Objective cooked color readings of 3 degrees of doneness and 3 beef whole muscles

	$L^*1$	$a^*2$	$b^*3$
Degree of doneness			
Medium rare, 62.8°C	50.04	25.32 <sup>a</sup>	20.69 <sup>a</sup>
Medium, 71.1°C	50.90	21.90 <sup>b</sup>	20.68 <sup>a</sup>
Well-done, 76.7°C	50.33	17.68 <sup>c</sup>	19.49 <sup>b</sup>
SEM <sup>4</sup>	0.36	0.59	0.34
<i>P</i> Value	0.06	<0.01	<0.01
Muscle			
LL	53.03 <sup>a</sup>	20.07	20.17
BF	48.21 <sup>c</sup>	21.89	20.40
GM	50.03 <sup>b</sup>	21.60	20.28
SEM <sup>4</sup>	0.83	0.80	0.40
<i>P</i> Value	<0.01	0.05	0.84

<sup>a-c</sup>Means within the same column without a common superscript differ ( $P < 0.05$ ).

<sup>1</sup> $L^*$ : 0 = black, 100 = white.

<sup>2</sup> $a^*$ : -60 = green, 60 = red.

<sup>3</sup> $b^*$ : -60 = blue, 60 = yellow.

<sup>4</sup>SEM (largest) of the least-squares means.

BF = *Biceps femoris*; GM = *Gluteus medius*; LL = *Longissimus lumborum*; SEM = standard error of the means.

As expected, the  $a^*$  values decreased ( $P < 0.05$ ) with each increase in DOD. The current study and others provide evidence that the  $a^*$  value is the best representation of the DOD (Prill et al., 2019; Salim et al., 2021). However,  $a^*$  values were not different ( $P > 0.05$ ) among muscles. These similarities are surprising as  $a^*$  values are also linked to muscle metabolism and functions in the body and therefore color stability (Mancini and Hunt, 2005; Suman et al., 2014; Park et al., 2018), though such differences were not observed in the current study in cooked samples.

Previous studies support the idea that consumers associate certain internal color patterns with each DOD, and these changes can be measured by changes in  $a^*$  values (Yancey et al., 2011, 2016; Prill et al., 2019; Salim et al., 2020). Two studies done by Yancey et al. (2011, 2016) found similar relationships between DOD and  $a^*$  values. Regardless of the dry heat cookery method, Yancey et al. (2011, 2016) found the  $a^*$  values to decrease from a MR, MED, and WD DOD. However, Yancey et al. (2016) observed a muscle effect where this relationship was not expressed within the *infraspinatus* muscle, but it was within the *Longissimus thoracis* and *Semimembranosus* muscles (Yancey et al., 2011, 2016). While the current study did not find differences among muscles for  $a^*$  values, the *infraspinatus* and other muscles evaluated by Yancey et al. (2016) were not included. Taken together, these results indicate additional muscles may produce differences in  $a^*$  values that were not observed within the muscles of the current work. This potential interaction between muscle and DOD could potentially be explained by inherent differences in intrinsic factors such as pH, moisture content, and oxidation levels of different muscles at the time of cooking.

Dikeman et al. (2013) found a similar trend of DOD and  $a^*$  values within the LL; however, their study found an interaction between DOD and aging method of wet versus dry-aging. The dry-aged steaks did not have a significant difference in  $a^*$  values between the MR and MED treatments, but the wet-aged steaks produced similar results as those found in the current study. While not explored in that paper, this may provide evidence that differences in oxidation from dry-aging or other processes could be impacting cooked color or cooked color stability of whole muscles and warrants further investigation. The current study and the previously cited studies provide support for a strong relationship between  $a^*$  values and DOD while highlighting some unknown factors between the relationship between  $a^*$  values and different cooked muscles and aging methods.

## Myoglobin denaturation

Myoglobin denaturation has been briefly researched within ground beef but remains unexplored for cooked whole muscles before the current study. In general, myoglobin begins to denature at 55°C and continues to become fully denatured at 80°C (Hunt et al., 1999; King and Whyte, 2006). Within a ground product, the level of myoglobin denaturation is dependent on the oxidation state of myoglobin at the time of cooking and the rate and method of cooking (Hunt et al., 1999; Ryan et al., 2006). Ground beef also has a much higher recommended end-point temperature (71.1°C) to ensure food safety compared to whole muscle cuts (62.8°C) (Mancini and Hunt, 2005; USDA, 2016). This elevated and required end-point temperature for food safety prevents the same variation within internal color and myoglobin denaturation for ground products in comparison to whole muscle products. Hunt et al. (1999) determined myoglobin denaturation of ground beef with varying percentages of deoxymyoglobin, oxymyoglobin, and metmyoglobin. The authors determined the relationship between end-point temperature and myoglobin denaturation was highly dependent on the relative percentages of each myoglobin form at the time of cooking (Hunt et al., 1999). However, a study from Ryan et al. (2006) determined the time and temperature parameters of cooking were also extremely important for the final pigment in addition to oxidation. Cooked color research is expansive for ground beef due to the prevalence of premature browning (Hunt et al., 1999). Because of the inherent differences of a ground product versus a whole muscle cut, these denaturation percentages and the relationship with temperature could be different between ground beef and whole muscles.

Myoglobin denaturation, shown in Table 2, was hypothesized to increase as  $a^*$  values decreased, potentially providing an explanation to the changes in  $a^*$  values across DOD. As expected, myoglobin denaturation increased ( $P < 0.05$ ) for each DOD, similar to the  $a^*$  values. The linear regression model including all 3 DOD between  $a^*$  and myoglobin denaturation is shown in Figure 1 and show a significant relationship ( $P < 0.01$ ) with an  $R^2$  value of 0.59. Myoglobin was denatured 29.08%, 48.34%, and 70.17% at MR, MED, and WD DOD, respectively. However, muscle did not ( $P > 0.05$ ) have an impact on myoglobin denaturation percentage. Therefore, myoglobin denaturation percentages help explain the lack of differences found across muscles for the  $a^*$  values, with each muscle evaluated having a similar amount of myoglobin denatured at each DOD. These results show that regardless

**Table 2.** Myoglobin denaturation, metmyoglobin-reducing activity, and sarcoplasmic surface hydrophobicity of 3 degrees of doneness and 3 beef whole muscles

	Myoglobin denaturation <sup>1</sup> , %	MRA <sup>2</sup>	Surface hydrophobicity <sup>3</sup>
Degree of doneness			
Raw		3.03 <sup>a</sup>	
Medium rare, 62.8°C	29.08 <sup>c</sup>	2.49 <sup>b</sup>	30.91 <sup>c</sup>
Medium, 71.1°C	48.34 <sup>b</sup>	1.76 <sup>c</sup>	41.06 <sup>b</sup>
Well-done, 76.7°C	70.17 <sup>a</sup>	0.85 <sup>d</sup>	49.49 <sup>a</sup>
SEM <sup>4</sup>	2.08	0.18	4.9
<i>P</i> Value	<0.01	<0.01	<0.01
Muscle			
LL	48.85	1.58 <sup>b</sup>	42.5
BF	49.65	2.17 <sup>a</sup>	37.0
GM	49.08	2.34 <sup>a</sup>	39.4
SEM <sup>4</sup>	3.17	0.18	4.7
<i>P</i> Value	0.97	<0.01	0.05

<sup>a-c</sup>Means within the same column without a common superscript differ ( $P < 0.05$ ).

<sup>1</sup>Myoglobin denaturation, % =  $(1 - [\text{Raw} - \text{Cooked}]/\text{Raw}) \times 100$ .

<sup>2</sup>Metmyoglobin-reducing activity (MRA); nmol/min/g of muscle.

<sup>3</sup>Sarcoplasmic surface hydrophobicity: bromophenol blue (BPB)/mg of protein.

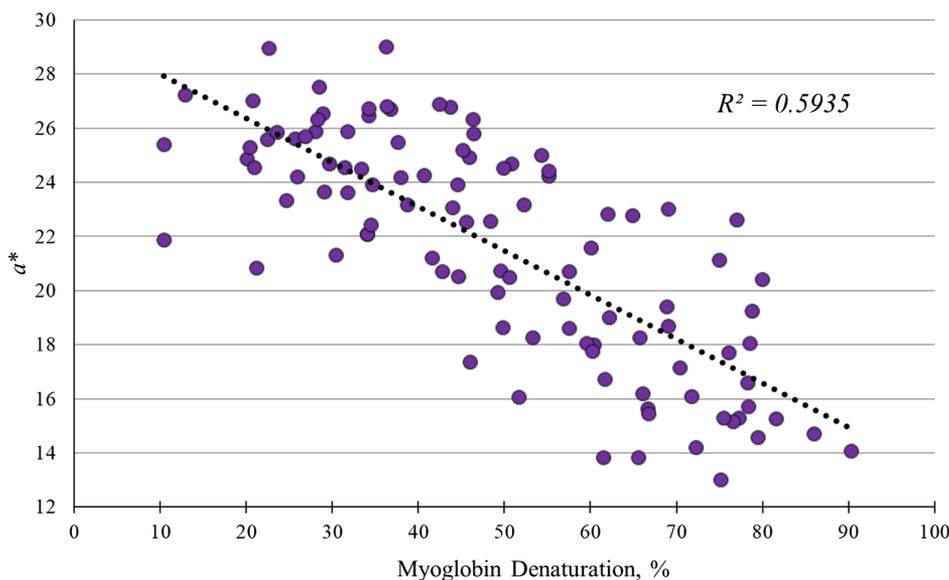
<sup>4</sup>SEM (largest) of the least-squares means.

BF = *Biceps femoris*; GM = *Gluteus medius*; LL = *Longissimus lumborum*; SEM = standard error of the means.

of muscle, the DOD and myoglobin denaturation relationship is constant. While muscle source played a role in other differences found in the study, it did not dictate the thermal stability of myoglobin.

This same trend of increased myoglobin denaturation at increased DOD has been shown in ground beef products as well (Hunt et al., 1999; Mancini and Hunt, 2005; Ryan et al., 2006). Ryan et al. (2006) suggested an 80% denaturation must be reached to achieve a WD DOD appearance for ground beef, while the current study suggests only a 70% denaturation is required for whole muscles. This difference could be due to the inherent differences of a ground meat product compared to a whole muscle cut. This key difference provides further evidence that the previous work in ground beef cannot be fully translated into whole muscle cuts. While ground beef and whole muscles are similar, understanding the differences between the two products and how myoglobin changes through cooking in each product is pertinent to the overall understanding of cooked meat color development.

Understanding the baseline myoglobin denaturation level for certain DOD, as was established in the current study, can be used for future studies evaluating differences in cooked color stability while looking at other factors such as aging, processing techniques, pH, or cookery methods. Even though myoglobin undergoes post-translational changes through cooking to increase thermal stability as described by Salim et al. (2020), it still significantly denatures at each DOD and directly corresponds to the visual changes associated with each DOD. Myoglobin denaturation helps explain most of the color differences between differing DOD, but cooked color stability can help explain further color differences after cooking and slicing.

**Figure 1.** Relationship between  $a^*$  values and myoglobin denaturation percentages.

### **Metmyoglobin-reducing activity**

MRA is shown in [Table 2](#) and illustrates a component of color stability. While MRA is normally associated with raw meat color stability, it can also impact the amount a cooked sample can bloom after cooking, potentially playing a role in consumer acceptability. The MRA assay was used in the current work to determine the ability of the cooked pigment to bloom after cooking, or as a measure of cooked color stability ([Bekhit and Faustman, 2005](#)). MRA decreased ( $P < 0.05$ ) with increasing DOD. The raw treatment resulted in the highest MRA with  $3.03 \text{ nmol} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$  of sample. The WD sample resulted in minimal activity. Muscle type also impacted MRA. The BF and GM resulted in a greater ( $P < 0.05$ ) MRA than the LL. While all 3 muscles are typically considered moderate to stable for color stability of raw product, cooked color stability has not been previously investigated for the same muscles ([Suman et al., 2014](#)).

Cooked color stability is imperative to understand as it impacts the last pigment seen before consumption. It explains differences in the internal appearance of a steak a few minutes after slicing compared to the initial appearance. This phenomenon was illustrated by Prill et al. (2019), who took internal color readings at 0 min up to 12 min after slicing. Prill et al. (2019) illustrated the relationship between DOD and the ability of the  $a^*$  to recover after cooking. Their research supports the findings of the present study as the MR DOD resulted in a greater  $a^*$  change compared to the MED or WD DOD ([Prill et al., 2019](#)). The current study found MRA to gradually decline with increasing DOD, but even at the WD DOD, there was a marginal level of MRA remaining. These results indicate the ability for myoglobin to be reduced even after an extensive cooking process, further indicating an intimate link between MRA and DOD up to a certain point. Prill et al. (2019) found all DOD to recover some  $a^*$  values, besides the very well-done DOD, indicating the irreversible damage done to myoglobin is between the WD (77°C) and very well-done (82°C) DOD for whole muscles. This was supported by Moriyama and Takeda (2010), who determined for cardiac muscle, myoglobin was irreversibly denatured at 75°C. While Prill et al. (2019) did not directly measure color stability, they did measure the visual impact of color stability, further emphasizing the results found in the current study. Our study serves as the first to investigate MRA of cooked whole muscles and may help explain differences in cooked color and the associated color stability of cooked beef within future studies.

While other studies have not looked at the MRA of cooked whole muscles, some studies have determined differences in the thermal stability of cooked proteins, which is intimately linked to color stability ([Suman and Joseph, 2013](#); [Suman et al., 2014](#)). Previous differences in the cooked color of ground beef have been associated with the thermal stability of the primary form of myoglobin at the time of cooking ([Hunt et al., 1999](#); [Suman and Joseph, 2013](#)). It is understood that the relative ratio of the myoglobin pigments at the time of cooking heavily drives the thermal and color stability of ground beef, and thus potentially whole muscles ([Hunt et al., 1999](#)). This link between the relative ratio of the myoglobin pigments at the time of cooking to the elicited color stability previously evaluated in ground product needs to be similarly established for whole muscles.

The present study found muscle to impact MRA, with the GM and BF having a higher ( $P < 0.05$ ) MRA compared to the LL. Conversely, the raw color stabilities of the 3 muscles have been determined in previous works, with the LL having the highest color stability in raw product ([Suman et al., 2014](#)). Therefore, our study indicates the raw color stability does not completely translate to cooked color stability. This could be due to differences in thermal stability or the relative ratios of myoglobin at the time of cooking but needs to be investigated further.

While the MRA among the different DOD was expected, the difference between muscles was surprising. Since it is well known that this visual component of the cooked steak is crucial to satisfy the eating experience for the consumer, and the final internal appearance is dictated by color stability, understanding cooked color stability warrants further investigation ([Bekhit and Faustman, 2005](#); [Prill et al., 2019](#)). MRA could be used to help explain differences in cooked color stability; therefore, future studies should include other factors impacting cooked color such as the oxidation state of myoglobin, aging method, differing muscles, display period, freezing techniques, and aging periods.

### **Surface hydrophobicity**

Surface hydrophobicity measures the amount of hydrophobic regions exposed on a protein, thus measuring the level of denaturation and aggregation. As expected, the surface hydrophobicity of the sarcoplasmic proteins matched the myoglobin denaturation and  $a^*$  values for the different DOD as shown in [Table 2](#). As DOD increased, surface hydrophobicity increased ( $P < 0.05$ ); however, muscle did not impact ( $P > 0.05$ )

the level of aggregation. Yang et al. (2022) found similar results using sarcoplasmic proteins extracted from pale, soft, and exudative (PSE) pork after heating to 25°C, 40°C, or 55°C, but they found more bound BPB due to using PSE pork. Additionally, Yang et al. (2022) determined a pH and surface hydrophobicity interaction as a higher pH lessened the level of denaturation. However, the current study did not find differences in pH between the different DOD. Most studies have used this assay with the myofibrillar portion to help explain differences in tenderness and textural properties. Regardless, surface hydrophobicity and aggregation have been shown to increase with an increasing internal cooking temperature (Dominguez-Hernandez and Ertbjerg, 2021).

## pH

The pH data are shown in Table 3. Even though color and pH are related, only muscle impacted pH, with the BF having a higher ( $P < 0.05$ ) pH value compared to the other muscles. Typically, pH and  $a^*$  values have a significant correlation in raw meat, but the relationship overall has been shown to be weaker for cooked products (Mahmood et al., 2017). While pH did not align with the  $a^*$  differences or myoglobin denaturation in the current study, it did provide some

support for the differences found among muscles for the other color attributes tested. Potentially, pH could help explain differences in color stability and cooked  $L^*$  values among muscles.

Cooking concentrates solutes in meat, typically increasing the pH (Fletcher et al., 2000), but the impact of cooking on pH has not been widely studied. While the current study aimed to look at the impact of DOD and muscle on pH, other studies have evaluated the cooked color stability differences within set ranges of raw meat pH (Cox et al., 1994; Fletcher et al., 2000). As pH increased, the color stability increased, allowing a steak to be overcooked for a specific DOD but still have the appropriate  $a^*$  value (Cox et al., 1994). This relationship with color stability and pH is long-standing for ground beef (Schoenbeck et al., 2000). A higher pH within the raw materials has been linked to persistent pinking and ground beef with a higher ratio of deoxymyoglobin compared to the lower pH groups (Schoenbeck et al., 2000). Our study only found pH differences among muscles, but the observed difference did not elicit any color differences within the  $a^*$  values as hypothesized. However, these results did align with the differences found for cooked color stability for the different muscles, as the BF had a higher pH, MRA, and  $L^*$  value in comparison to the LL, potentially supporting a theory of pH impacting myoglobin oxidation and therefore color stability and warrants further investigation.

**Table 3.** Fat, moisture, pH cook loss, and WBSF of 3 degrees of doneness and 3 beef whole muscles

	Fat, %	Moisture, %	pH	Cook loss <sup>1</sup> , %	WBSF, kg
Degree of doneness					
Raw	4.41 <sup>b</sup>	72.69 <sup>a</sup>	5.68		
Medium rare, 62.8°C	4.69 <sup>ab</sup>	66.52 <sup>b</sup>	5.82	14.13 <sup>c</sup>	3.39 <sup>b</sup>
Medium, 71.1°C	4.97 <sup>ab</sup>	64.61 <sup>c</sup>	5.83	20.00 <sup>b</sup>	4.29 <sup>a</sup>
Well-done, 76.7°C	5.25 <sup>a</sup>	62.93 <sup>d</sup>	5.75	24.71 <sup>a</sup>	4.63 <sup>a</sup>
SEM <sup>2</sup>	0.30	0.25	0.06	1.46	0.18
<i>P</i> Value	0.04	<0.01	0.09	<0.01	<0.01
Muscle					
LL	5.23 <sup>a</sup>	65.88 <sup>b</sup>	5.74 <sup>b</sup>	16.64 <sup>b</sup>	4.16
BF	5.32 <sup>a</sup>	67.01 <sup>a</sup>	5.87 <sup>a</sup>	21.86 <sup>a</sup>	3.95
GM	3.94 <sup>b</sup>	67.18 <sup>a</sup>	5.71 <sup>b</sup>	20.34 <sup>a</sup>	4.22
SEM <sup>2</sup>	0.53	0.41	0.06	1.74	0.22
<i>P</i> Value	0.02	<0.01	0.01	<0.01	0.44

<sup>a-d</sup>Means within the same column without a common superscript differ ( $P < 0.05$ ).

<sup>1</sup>Cooking loss =  $1 - (\text{cooked weight/raw weight}) \times 100$ .

<sup>2</sup>SEM (largest) of the least-squares means.

BF = *Biceps femoris*; GM = *Gluteus medius*; LL = *Longissimus lumborum*; SEM = standard error of the means; WBSF = Warner-Bratzler shear force.

## Proximate analysis and cooking characteristics

Cooked proximate analysis results are shown in Table 3. The attributes evaluated for proximate analysis can provide further insight into the physical changes that occur throughout cooking between different DOD. The differences found within the proximate analysis data match previous studies evaluating different DOD (Smith et al., 2011; Yancey et al., 2011; Lucherker et al., 2016; Drey et al., 2019). The elicited impact of heat on meat products occurs through a few basic mechanisms such as structural changes, denaturation, and aggregation of myofibrillar and sarcoplasmic protein and expulsion of water molecules previously bound to the proteins (Schwartz et al., 2022). Additionally, these factors could help explain differences seen among muscles for the objective color measurements.

Cook loss and moisture content are inherently linked and are a predictor of water-holding capacity and perceived juiciness during sensory analysis

(Yancey et al., 2011). While moisture content has been used as an indicator of water-holding capacity, it could have an additional relationship with color attributes such as  $L^*$  values (Yancey et al., 2011). As expected, cook loss increased ( $P < 0.05$ ) with each DOD, while the LL had the lowest ( $P < 0.05$ ) cook loss compared to the other muscles. Similarly, moisture content decreased ( $P < 0.05$ ) with each DOD, while the BF and GM resulted in a higher ( $P < 0.05$ ) moisture content compared to the LL. As cooking loss increases, inherently, the moisture content and perceived juiciness decreases as shown by our study and others (Smith et al., 2011; Yancey et al., 2011; Drey et al., 2019; Schwartz et al., 2022). However, Drey et al. (2019) did not find the same relationship between cooked moisture content and DOD as found in our study. USDA Select and Choice graded steaks did not decrease in moisture content with increasing DOD (Drey et al., 2019). Additionally, Drey et al. (2019) found an interaction between DOD and quality grade (QG), illustrating the moisture content change was driven more by QG than it was by DOD. However, because only one QG was used in the present study, the moisture content decreased and cook loss increased with each DOD.

Interestingly, moisture content is an intrinsic factor that could help explain the results previously seen among muscles. The BF and GM had a higher moisture content than the LL, following the trend established by the pH and the objective color attributes. Potentially, a higher pH can lead to more bound water leading to a higher moisture content, and more light reflectance before and after cooking (Dikeman et al., 2013).

On the other hand, the fat content had an inverse relationship to moisture content, as demonstrated by previous studies (Smith et al., 2011; Yancey et al., 2011; Drey et al., 2019). The WD samples had a higher ( $P < 0.05$ ) percentage of fat compared to the raw samples due to the expulsion of moisture during cooking; however, the WD and raw treatments were similar ( $P > 0.05$ ) to MR and MED for fat content. Additionally, the LL and BF had a greater ( $P < 0.05$ ) fat content than the GM. The change in fat content seen in the current study and others is the result of the decrease in moisture content (Drey et al., 2019). During cooking, it is well documented that cook loss and fat percentages increase while moisture content decreases, with an increase in cook time, temperature, or DOD (Smith et al., 2011; Yancey et al., 2011; Drey et al., 2019).

While pulverized cooked steaks is less common, it did highlight the differences in proximate analysis between the different DOD and cooked muscles, serving as a better indicator of the factors potentially

impacting cooked color while helping to highlight known changes in the physical properties of meat.

### Objective tenderness

Tenderness has a well-documented relationship with DOD in which DOD negatively impacts tenderness (Smith et al., 2011; Yancey et al., 2011; Lucherker et al., 2016). In the current study, only the DOD impacted the WBSF values, with MR having the lowest ( $P < 0.05$ ) value, being the most tender, while the MED and WD samples were similar ( $P > 0.05$ , Table 3). Surprisingly, there were no differences ( $P > 0.05$ ) among muscles for WBSF, which is in contradiction to previous literature classifying the *longissimus dorsi* as intermediate and the GM and BF as tough for tenderness classes (Calkins and Sullivan, 2007). These discrepancies could be due to the anterior BF being used and that all DOD were pooled due to a lack of interaction. For DOD, the current study further supported the findings from previous research (Smith et al., 2011; Yancey et al., 2011; Lucherker et al., 2016). As the end-point temperature increases, WBSF values and subjective tenderness ratings are negatively impacted through protein oxidation, denaturation, and aggregation (Smith et al., 2011; Yancey et al., 2011; Lucherker et al., 2016). Further, there are many mechanisms that play a role in impacting tenderness changes with varying DOD, including the expulsion of moisture, the aggregation of the hydrophobic regions of protein side chains due to denaturation, and the formation of disulfide bonds (Bertola et al., 1994). The loss of water-holding capacity as illustrated through the changes of cook loss help explain the decrease in tenderness due to the expulsion of water occurring with increasing DOD. There is a potential connection with protein oxidation, aggregation, and cooked meat color in understanding the mechanism of cooked meat tenderness.

### Cooked lipid oxidation

While typically associated with off odors and flavors of meat, lipid oxidation has a complex relationship with meat flavor (Kerth and Miller, 2015). Heat is a known oxidizer and contributes positively to the development of flavor (Kerth and Miller, 2015). Additionally, different muscles can have different levels of lipid oxidation based on fat content and antioxidant capacity (Kerth and Miller, 2015). Cooked lipid oxidation could positively correlate with flavor development or lead to potential off-flavors such as warmed-over flavor (Kerth and Miller, 2015). While cooked lipid oxidation simultaneously impacts flavor, the current study

**Table 4.** The two-way interaction of lipid oxidation of 3 beef whole muscles within raw, medium rare, medium, and well-done degrees of doneness

Degree of doneness	Muscle	Lipid oxidation <sup>1</sup>
Raw	BF	1.70 <sup>gh</sup>
	GM	1.35 <sup>h</sup>
	LL	1.17 <sup>h</sup>
Medium rare	BF	3.42 <sup>ef</sup>
	GM	3.11 <sup>efg</sup>
	LL	2.12 <sup>fgh</sup>
Medium	BF	6.87 <sup>b</sup>
	GM	5.11 <sup>cd</sup>
	LL	3.91 <sup>de</sup>
Well-done	BF	8.73 <sup>a</sup>
	GM	6.54 <sup>bc</sup>
	LL	4.12 <sup>de</sup>
SEM <sup>2</sup>		0.81
P Value		<0.01

<sup>a-h</sup>Means within the same column without a common superscript differ ( $P < 0.05$ ).

<sup>1</sup>Lipid oxidation; MDA/kg protein.

<sup>2</sup>SEM (largest) of the least-squares means.

BF = *Biceps femoris*; GM = *Gluteus medius*; LL = *Longissimus lumborum*; MDA = malondialdehyde; SEM = standard error of the means.

aimed to determine if cooked lipid oxidation had any connection with myoglobin denaturation, color stability, or the other factors impacting cooked color. As previously discussed, oxidation of myoglobin can play a role in cooked color; therefore, there could be a connection with lipid oxidation and cooked color as well.

Lipid oxidation is shown in Table 4. There was an interaction ( $P < 0.05$ ) for lipid oxidation between muscle and DOD. Within each DOD, there were differences between the 3 muscles. There were no oxidation differences ( $P > 0.05$ ) within the raw treatment between the 3 muscles. However, for both the MR and WD treatments, the BF had greater ( $P < 0.05$ ) lipid oxidation compared to the LL. Within the MED DOD, the BF resulted in the greatest ( $P < 0.05$ ) MDA concentration. The WD BF sample resulted in the highest ( $P < 0.05$ ) lipid oxidation amount compared to all other treatments, while the raw LL and raw GM resulted in the lowest ( $P < 0.05$ ) oxidation compared to all treatments besides the raw BF. Overall, within each DOD, the BF resulted in more ( $P < 0.05$ ) lipid oxidation than the LL, while the GM resulted in intermediate oxidation. These results mirror differences found within pH,  $L^*$

values, and MRA, highlighting a potential relationship between pH, lipid oxidation, and cooked color stability.

## Conclusions

Even though changes in cooked color are widely accepted and expected, few studies have taken a comprehensive look at the factors impacting these changes. A baseline is needed to further investigate the changes that are observed within the cooked color of whole muscles. Overall, the data in the current work supported the hypothesis that an increase in DOD would decrease cook yield, moisture content,  $a^*$  values, and color stability and would increase perceived toughness, lipid oxidation, and myoglobin denaturation. However, an impact of muscle type on cooked color was not expected. The differences observed between the 3 muscles outlined the potential relationships between the intrinsic properties of meat and cooked meat color and color stability. The physiochemical changes that alter the final pigment of beef whole muscles are complex and warrant future studies for better understanding.

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