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Change in Myoglobin Denaturation Among Three Degrees of Doneness of Three Muscles

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Abstract

The objective of this study was to determine the change in myoglobin denaturation through three degrees of doneness (medium rare, medium, and well-done) within the *longissimus dorsi, gluteus medius,* and *biceps femoris.* Strip loins (n = 12) and top butts (n = 12) were collected and fabricated into 1-in steaks, aged for 28 days, and held frozen at -10°F. Steaks were cooked to the assigned degree of doneness (DOD) and used for either lab assays or cook loss and Warner-Brazler shear force (WBSF). After cooking to the appropriate degree of doneness, the steaks were sliced in half, scanned internally for CIE L* (lightness), a* (redness), b* (yellowness), and powdered for lab assays. Myoglobin denaturation was determined using a modified protocol from the American Meat Science Association Color Measurement Guidelines and calculated as a percentage of deoxymyoglobin denaturation compared to the raw sample. Myoglobin was denatured 29.08%, 48.34%, or 70.17% at each degree of doneness, respectively. As expected, the a* values decreased ($P < 0.05$) with each different degree of doneness; however, the pH was not impacted ($P > 0.05$). Similarly, the cooking loss percentages increased ($P < 0.05$) with each degree of doneness and the WBSF force values were higher ($P < 0.05$) for well-done steaks in comparison to the other treatments. As expected, the myoglobin denaturation percentage increased with increasing DOD and behaved similarly to changes in the a* values. This research gives more insight to the impacts of cooking and the changes that proteins, especially myoglobin, undergo between different DOD.

Introduction

Myoglobin is a complex molecule mostly responsible for meat color. It undergoes a series of changes through the aging and cooking processes, altering the pigment (Hughes et al., 2020). Consumers are heavily motivated by raw meat color, both when evaluating the overall appearance and willingness to purchase; however, the impact and mechanisms of cooked color is less understood (Carpenter et al., 2001; Hughes et al., 2020). Salim et al. (2021) determined myoglobin undergoes post-translational changes through the cooking process, but the basic question of myoglobin denaturation has yet to be answered. Therefore, the objective of this study was to determine the changes in myoglobin denaturation through cooking three different muscles to medium rare, medium, or well-done degrees of doneness.

Experimental Procedures

Beef strip loins (n = 12) [Institutional Meat Purchase Specifications (IMPS) # 180, NAMP, 2010], and top butts (n = 12) (IMPS #184, NAMP, 2010) were collected at a Midwest beef processing plant and brought to Kansas State University for processing. The strip loins (LL) and the top butts were denuded, and the top butts were separated into the *biceps femoris* (BF) and *gluteus medius* (GM). The muscles were sliced into 1-in steaks and assigned to one of the following treatments: raw, medium rare (MR), medium (MED), or well-done (WD). All three muscles were aged for 28 days at 36°F, frozen and held at -10°F.

The steaks assigned for lab assays were cooked to the appropriate degree of doneness (DOD) to peak temperatures of 145°F, 160°F, or 170°F for each DOD. A thermopen (Thermopen Mk4, ThermoWorks, Salt Lake City, UT) was inserted into the geometric center of the steak to record the peak internal temperature. Color readings [CIE L* (lightness), a* (redness), b* (yellowness)] were taken using a Hunter Lab Miniscan (Model 2500L, Hunter Associates Laboratory Inc, Reston, VA), and then samples were powdered immediately for moisture, fat, pH, and myoglobin denaturation. Myoglobin denaturation was determined using a modified protocol provided in the American Meat Science Association Color Measurement Guidelines (AMSA, 2012). The samples were weighed, homogenized in a potassium phosphate buffer, centrifuged, and filtered. A 200 µL sample was plated on a 96-well plate in duplicate and 1% sodium hydrosulfite was added to reduce all forms of myoglobin to deoxymyoglobin. The plates were evaluated with a spectrophotometer at 433 nm and the absorbance was used to calculate the percent myoglobin denaturation of each sample.

Cook loss and Warner-Brazler shear force (WBSF) samples were cooked using the same procedures outlined above. For cook loss, the raw steak was weighed and recorded and then reweighed following cooking. The steaks were allowed to cool for a minimum of 12 hours to a temperature of 36°F before taking six 0.5-in cores parallel to the muscle fiber. Each core was sheared perpendicular to the muscle fiber orientation using an Instron testing machine (Model 5569, Instron Corp., Canton, MA) with a cross-head speed of 9.8 in/min and a load cell of 220 lb. The shear force measurements from all six cores were averaged for each sample and presented as average peak force (lb).

Moisture, fat, and pH were determined using the powdered meat samples. Moisture content was determined using the drying oven method (950.46 and 934.01; AOAC, 1995). The fat content was measured using the Folch method (Folch et al., 1957). The pH was measured by homogenizing a 0.17 oz sample in 1.69 oz of deionized water using a calibrated InLab Solids Pro-ISM probe (Part #51344155; Mettler-Toledo, Schwerzenbach, Switzerland) connected to a Seven Compact pH meter (Mettler-Toledo).

Results and Discussion

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 (Table 1). Similarly, moisture content decreased ($P < 0.05$) with each DOD while the LL resulted in the lowest ($P < 0.05$) moisture content (Table 1). Only the DOD impacted the WBSF values, with MR having the lowest ($P < 0.05$) value, being the most tender (Table 1). The L* values were not impacted ($P > 0.05$) by the different DOD; however, the LL resulted in the highest ($P < 0.05$) L* value followed by the GM and then the BF (Table 2). As expected, the a*

values decreased ($P < 0.05$) with each different DOD. The MR and MED treatments had a higher ($P < 0.05$) b* value in comparison to the WD treatments. Even though color and pH are intimately related, only muscle impacted pH, with the BF having a higher ($P < 0.05$) pH value compared to the other muscles. Similar to the a* values, the myoglobin denaturation increased ($P < 0.05$) for each DOD, but muscle did not ($P > 0.05$) have an impact (Table 1). Myoglobin was denatured 29.08%, 48.34%, or 70.17% respectively at each DOD. Even though myoglobin undergoes post-translational changes through cooking to increase thermal stability as described by Salim et al. (2020), it still significantly denatures with each DOD to correspond to the visual changes associated with each DOD.

Implications

Myoglobin is a complicated molecule that has been shown to undergo post-translational changes in previous research, but the question of how much of myoglobin is denatured at certain DOD was not answered until this research. As expected, the myoglobin denaturation percentage increased with increasing DOD and behaved similarly to changes in the a* values. These changes were accompanied by expected changes in L*, a*, b*, moisture content, WBSF, and cook loss. This research gives more insight to the impacts of cooking and the changes that proteins, especially myoglobin, undergo between different DOD. Lastly, this research can act as a baseline for changes in myoglobin denaturation and cooked color for future research projects with other processing steps such as different aging periods, cooking methods, or freezing methods.

References

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Table 1. Fat, moisture, cook loss, WBSF, and myoglobin denaturation percentages of three degrees of doneness and three muscles

Degree of doneness	Fat, %	Moisture, %	Cook loss,[1] %	WBSF[2]	Myoglobin denaturation,[3] %
Raw	4.41[b]	72.69[a]	---	---	---
Medium rare, 145°F	4.69[ab]	66.52[b]	14.13[c]	7.47[b]	29.08[c]
Medium, 160°F	4.97[ab]	64.61[c]	20.00[b]	9.46[a]	48.34[b]
Well done, 170°F	5.25[a]	62.93[d]	24.71[a]	10.20[a]	70.17[a]
SEM[4]	0.30	0.25	1.46	0.18	2.08
P-value	0.04	<0.01	<0.01	<0.01	<0.01
Muscle[5]					
LL	5.23[a]	65.88[b]	16.64[b]	9.17	48.85
BF	5.32[a]	67.01[a]	21.86[a]	8.71	49.65
GM	3.94[b]	67.18[a]	20.34[a]	9.30	49.08
SEM[4]	0.53	0.41	1.74	0.22	3.17
P-value	0.02	<0.01	<0.01	0.44	0.97

[abcd] Means within the same column without a common superscript differ ($P < 0.05$).
[1]Cooking loss = 1 - (cooked weight / raw weight) × 100.
[2]Warner-Bratzler shear force; lb.
[3] Myoglobin denaturation, % = [1 - (raw - cooked) / raw] × 100.
[4]Standard error (largest) of the least squares means.
[5]LL = *longissimus dorsi*; BF = *biceps femoris*; GM = *gluteus medius*.

Table 2. CIE L*, a*, and b*, and pH of three degrees of doneness and three muscles

Degree of doneness	L*[1]	a*[2]	b*[3]	pH
Medium rare, 145°F	50.04	25.32[a]	20.69[a]	5.82
Medium, 160°F	50.90	21.90[b]	20.68[a]	5.83
Well done, 170°F	50.33	17.68[c]	19.49[b]	5.75
SEM[4]	0.36	0.59	0.34	0.06
P-value	0.06	<0.01	<0.01	0.09
Muscle[5]				
LL	53.03[a]	20.07	20.17	5.74[b]
BF	48.21[c]	21.89	20.40	5.87[a]
GM	50.03[b]	21.60	20.28	5.71[b]
SEM[4]	0.83	0.80	0.40	0.06
P-value	<0.01	0.05	0.84	0.01

[abc] Means within the same column without a common superscript differ ($P < 0.05$).
[1]L*: 0 = black, 100 = white.
[2]a*: -60 = green, 60 = red.
[3]b*: -60 = blue, 60 = yellow.
[4]Standard error (largest) of the least squares means.
[5]LL = *longissimus dorsi*; BF = *biceps femoris*; GM = *gluteus medius*.