DYNAMICS OF MYOGLOBIN LAYER CHANGE DURING DISPLAY OF COLOR-STABLE AND COLOR-LABILE BEEF MUSCLES

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Background

The ability of myoglobin (Mb) to oxygenate or "bloom" to a bright cherry red color of oxymyoglobin (OMb) and to retain its stability during retail display differs among different muscles (Hood, 1980; O'Keeffe and Hood, 1982; Renerre and Labas, 1987). Muscle cell respiration is temperature dependent (Urbin and Wilson, 1958; Bendall and Taylor, 1972) and was found to deteriorate with post mortem age (Bendall and Taylor, 1972; O'Keeffe and Hood, 1982). Lower temperatures enhance oxygen solubility in water, increase Mb oxygenation (Urbin and Wilson, 1958), and reduce enzyme activity (Urbin and Wilson, 1958; Bendall and Taylor, 1972). Bloom color on a muscle surface is influenced by oxygen consumption of muscle cells, oxygen partial pressure (pO_2) on the muscle surface, and the depth of Mb oxygenation (Brooks, 1929; O'Keeffe and Hood, 1982). Research has determined the depth of oxygen penetration into muscles directly by determining pO_2 (Morley, 1971; Feldhusen et al., 1995) or indirectly by observing pigment oxygenation (Brooks, 1929; O'Keeffe and Hood, 1982). We investigated both OMb and metmyoglobin (MMb) layer depths, and their relationship to surface color using novel open-topped clear Plexiglas® containers for continuous observation of pigment changes in muscle cubes and digital image analysis to quantitate the dynamics of pigment layer change.

Objectives

We investigated the combined effects of post mortem time (PT; 3, 10, or 14 d), storage temperature (ST; 0° or 4.4°C), and display temperature (DT; 0° or 3.3°C) on depths of oxymyoglobin (OMb) and MMb layers and instrumental color of beef *Longissimus lumborum* (LL) and *Psoas major* (PM) muscle cubes at 3 h and 1, 3, and 5 d of display.

Materials and methods

For each of 4 replications, sixteen paired muscles of LL and PM from the right side of USDA Select (n=48) and Choice (n=16) carcasses were obtained 48 h post slaughter from a commercial processing facility. Eight paired LL or PM were assigned randomly to 0ºC ST, while another eight pairs were assigned to 4.4ºC ST. At 3, 10, and 14 d PT, tissue from the anterior end of LL or posterior end of PM (same carcass for each ST) was cut into a cube and placed into an open-top, clear Plexiglas container (3.8-cm^3 for LL, 3.2-cm^3 for PM). Immediately after cutting, two adjacent sides of each muscle cube, with muscle fibers running perpendicular to the container base, were placed tightly in one corner of the Plexiglas container to maintain the deoxygenated form of Mb on muscle surfaces next to the Plexiglas. The extra portion of the muscle cube which extended above the top of the Plexiglas container was cut off at the open-top edge by cutting across the muscle fibers, to allow oxygen diffusion along the muscle fibers. The top muscle surface was covered immediately with PVC film (23,250 cc O_2/m^2/24 h at 23ºC and 0%RH) and exposed to ambient air. The LL or PM muscle cubes from each ST were placed in open-top display cases at 0º or 3.3ºC. The display luminance of 1614 lux was provided by the Ultralume™ 30 continuous fluorescent lighting (34 watts, 3000 K, Phillips, Sommerset, NJ, USA).

Due to time demands for muscle cube preparation, surface instrumental color measurement on top surface for each muscle cube was first done at 3 h bloom time. CIE L*, a*, b* (Illuminant A / 10 º observer; 1.27-cm diameter aperture) and reflectance spectral data (400-700 nm, 10-nm increments), were obtained using a HunterLab LabScan 6000 spectrophotometer (Hunter Associates Laboratory, Inc.; Reston, VA, USA). Values for %R 630 nm - %R 580 nm were calculated. The percentages of OMb and MMb on the muscle surfaces were estimated using K/S spectral data (AMSA, 1991). Surface instrumental color measurements and digital image photography of the pigment layers were recorded during 3 h, and 1, 3, and 5 d of display.

Following instrumental color measurement, digital image photography was performed on each muscle cube using a Sony Digital Mavica Still Camera model MVC-FD91 (Sony Corporation, Japan). Extreme care was taken to standardize digital photography. A scale in 1-mm increments, was included in each photograph for unit calibration for pigment layer measurement. Analysis of images was performed on an IBM ThinkPad T20 personal laptop computer model 2647-41U 700 MHz 128.0 MB RAM 12 GB storage capacity (IBM Corp., Armonk, NY, USA). Images were displayed on a high resolution (1024 x 768 pixels) TFT IBM ThinkPad LCD on S3 Inc. Savage/IX with true color (32- bit per pixel) screen setting. Digital images were processed using Adobe Photoshop 5.0 software (Adobe Systems Inc., San Jose, CA, USA). OMb and MMb layer depths were analyzed using the National Institutes of Health (NIH, Bethesda, MD, USA) *Scion Image* software (ScnImage release Beta 3b).

Color processing of images for the OMb layer depth measurement was performed by using the RGB mode TIFF image in Adobe Photoshop, converting it to CYMK color mode (Ringkob, 1997), and adjusting the color balance. The adjustments resulted in a bright orange yellow color of the OMb layer contrasting with a greenish brown color of the deoxymyoglobin layer. To separate the three Mb layers, the CYMK images were adjusted for color balance, hue, and saturation. Image processing and analysis for MMb layer depth measurement was performed on images recorded during 1, 3, and 5 d display. The image analysis for pigment layer depth determination was performed on ScnImage Software by acquiring the color processed RGB image. Stacks of an originally adjusted 24-bit color image were generated from a slice of the red channel (grayscale) with a "Stacks" drop down menu. The analysis of an image could be performed on this converted 24–bit color TIFF image. To calibrate the measurement unit, for each acquired image, a line selection tool was used to drag a 10-mm straight line on the scale presented underneath the muscle cube Plexiglas container. On the "Analyze" and "set scale" menu, the unit was set to mm. The measured distance in pixels was then calibrated with a known 10-mm scale. Five locations were measured for each pigment layer depth and averaged. The experiment was in a strip-split-split plot design. Analysis of Variance was performed utilizing the MIXED procedure of SAS (2000). Least-squares means for all variables and interactions were generated and separated using the DIFF option.

Results and discussion

As expected, muscle type and PT had the major effects on the depths of OMb and MMb layers and surface color. Their interactions also occurred in the data, especially on d 5 of display (Table 1). The effects of DT and ST on the OMb and MMb layers and surface color are presented in Table 2 and 3, respectively. Influences of main effects (muscle, PT, DT, and ST) and interactions will be discussed by each day of display.

At 3 h of bloom, the color-stable LL had a deeper ($P<.05$) OMb layer (3.45 mm) than the color-labile PM (2.02 mm). LL developed greater ($P<.05$) surface OMb, was ($P<.05$) lighter (higher L*), more ($P<.05$) yellow (higher b*), and had higher ($P<.05$) %R630 - %R580 values than PM, but it was not ($P>.05$) redder (a*). Feldhusen et al. (1995) reported no clear relationship between Mb oxygenation and a* value on muscle surface during 5 h of air exposure. PT affected ($P<.05$) surface MMb and bloom color attributes where muscles stored longer had a better bloom color. Surface OMb for 3, 10, and 14 d PT was similar (95.1, 95.1, and 96.4%, respectively). However, muscles 10 and 14 d PT had less ($P<.05$) surface MMb than 3 d PT and were brighter red and more yellow than 3 d PT. The greater surface MMb on muscles 3 d PT was likely due to lower pO_2 on muscle surface (Feldhusen et al., 1995), which likely resulted from higher oxygen consumption in muscles with less PT (O'Keeffe and Hood, 1982). Feldhusen et al. (1995) found that the oxygen penetration measured at 5 h after cutting increased with storage time. However, we found no effect ($P>.05$) of PT on the OMb layer at 3 h of bloom. Compared to 3.3ºC DT, muscles displayed at 0ºC were ($P<.05$) lighter, more yellow, and had higher %R630 - %R580 values, but were similar ($P>.05$) in OMb layer, surface OMb and MMb, and a* values (Table 2). There was no ($P>.05$) influence of ST (Table 3) on 3 h bloom color attributes.

At 1 d of display, the OMb layer of LL increased to 4.55 mm, while that of PM decreased to 1.73 mm. Feldhusen et al. (1995) reported a 4.5-mm deep OMb layer in *Longissimus dorsi* at 5 h of air exposure. They indicated a clear increase in oxygen penetration with time of air exposure during 5 h. The MMb layer of LL was ($P<.05$) thinner (1.18 mm) than PM (1.67 mm-MMb), which resulted in a brighter red and more yellow surface color (Table 1) in LL than PM. As expected, muscles 10 d PT had greater ($P<.05$) surface OMb, were redder and were more yellow than those at 3 d PT (Table 1), but 10 d did not ($P>.05$) differ from 14 d PT. We did not find an influence ($P>.05$) of PT on OMb and MMb layer depths. Colder DT (0°C) promoted a

deeper (P<.05) OMb layer formation (Table 1), likely due to better oxygen solubility in the intracellular water and less enzyme respiration (Urbin and Wilson, 1958). Surface color of muscles displayed at 0°C was better than those displayed at 3.3°C, however, the differences were not significant (P>.05).

At 3 d display, OMb layer (4.60 mm) in LL increased slightly from d 1, while that in PM continued to decrease (1.15 mm). In contrast, MMb layers of both muscles increased (2.24 mm for LL and 2.88 mm for PM). The OMb layer of LL was thicker (P<.05) and MMb layer was thinner (P<.05) than those in PM. As a result, LL had (P<.05) greater surface OMb, less surface MMb, was brighter red and more yellow than PM (Table 1). O'Keeffe and Hood (1982), however, found no differences in depth of OMb layer between these 2 muscles at 2 d PT. Muscles stored 14 d PT developed a thicker (P<.05) MMb layer than 3 d PT, but did not differ (P>.05) from 10 d PT. Storage at 0°C resulted in a thinner (2.34 mm, P<.05) MMb layer than at 4.4°C (2.78 mm, Table 3). Muscles stored and displayed at higher temperatures (4.4°C ST, 3.3°C DT) provided (P<.05) the least surface OMb and lowest value of %R630 - %R580 (data not shown).

After 5 d display, more interactions occurred between muscle and PT (Table 1). The a*, b*, %R630 - %R580, surface OMb and MMb, and OMb layer were influenced (P<.05) by muscle x PT. Among LL from 3 different PT, LL stored 14 d had (P<.05) the lowest values of a*, b*, %R630 - %R580, surface OMb, and OMb layer, but the most surface MMb. The OMb layer of 14 d PT LL, however, was similar (P>.05) to those of PM stored 3 or 10 d. PM from 3 different PT had similar (P>.05) a*, b*, and %R630 - %R580, which were (P<.05) lower than those in all the LL. Although not always significant, PM stored 14 d had the worse overall color, least surface OMb, most surface MMb, and thinnest OMb layer. More degradation of substrates and coenzymes may occur during longer PT of muscles, which likely causes less MMb to be reduced (O'Keeffe and Hood, 1982). Interestingly, no differences (P>.05) in MMb layer were observed. L* was affected by muscle main effect where LL was (P<.05) lighter than PM. ST at 0°C had (P<.05) greater a*, %R630 - %R580, and less surface MMb (Table 3).

Conclusions

This study suggests that longer PT (10 or 14 d) provided better color bloom, but as PT increased, color stability decreased as shown by faster OMb layer thinning and faster MMb layer thickening. The more color stable LL allowed a deeper O_2 penetration during bloom, a thicker OMb layer, and slower developing MMb layer during display. While MMb layer of the 2 muscle types reached similar thickness at 5 d display, the deeper OMb layer had a greater influence on surface color stability. The dynamics of myoglobin layer during bloom, which may be explained by their inherent biochemical traits of oxygen consumption and reducing capacity, were related to surface color bloom attributes and stability during display of the LL and PM muscles.

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Table 1. Effects of muscle and postmortem time (PT) on color attributes, oxymyoglobin (OMb) layer, and metmyoglobin (MMb) layer depths at 3 h, 1, 3, and 5 d of refrigerated display

Attributes	Muscle	3 h display			1 d display			3 d display			5 d display		
		3 d PT	10 d PT	14 d PT	3 d PT	10 d PT	14 d PT	3 d PT	10 d PT	14 d PT	3 d PT	10 d PT	14 d PT
L*	LL	**37.7ay**	**40.5ax**	**41.7ax**	**38.6a**	**41.1a**	**41.2a**	**39.7a**	**40.6a**	**40.9a**	**39.7a**	**39.3a**	**38.4a**
	PM	**36.9by**	**37.5bx**	**39.5bx**	**35.5b**	**35.8b**	**36.9b**	**35.9b**	**35.3b**	**36.1b**	**36.2b**	**35.1b**	**36.2b**
a*	LL	**29.2y**	**32.9x**	**32.8x**	**29.2ay**	**30.8ax**	**30.1axy**	**27.8a**	**29.4a**	**27.6a**	**26.0p**	**25.6p**	**21.7q**
	PM	**28.5y**	**32.7x**	**32.6x**	**22.7by**	**26.1bx**	**23.6bxy**	**18.7b**	**20.8b**	**18.6b**	**17.1r**	**18.1r**	**15.3r**
b*	LL	**24.9ay**	**27.3ax**	**26.9ax**	**24.5ay**	**26.1ax**	**25.4axy**	**23.6ay**	**25.0ax**	**23.4ay**	**22.4p**	**23.0p**	**21.2p**
	PM	**23.7by**	**26.4bx**	**26.9bx**	**20.8by**	**23.0bx**	**21.9bxy**	**19.0by**	**20.1bx**	**19.5by**	**18.5q**	**19.0q**	**18.3q**
%R630-%R580	LL	**23.2ay**	**27.3ax**	**28.3ax**	**21.8ay**	**25.6ax**	**25.1ax**	**21.0a**	**23.1a**	**20.8a**	**19.3p**	**19.0p**	**14.4q**
	PM	**22.3by**	**24.8bx**	**26.2bx**	**12.6by**	**16.6bx**	**14.5bx**	**8.6b**	**10.3b**	**7.9b**	**7.0r**	**8.1r**	**5.8r**
%OMb	LL	**97.1a**	**99.3a**	**98.4a**	**88.2aq**	**92.0ap**	**90.7aq**	**83.4a**	**87.7a**	**82.1a**	**77.5p**	**76.7p**	**62.9q**
	PM	**93.0b**	**91.0b**	**94.4b**	**55.9bq**	**70.5bp**	**60.7bq**	**37.7b**	**47.3b**	**37.7b**	**30.7rs**	**34.7r**	**18.2s**
%MMb	LL	**2.9bp**	**0.7bq**	**1.5bpq**	**11.4s**	**8.1s**	**8.6s**	**16.1b**	**11.2b**	**16.0b**	**21.5s**	**23.0s**	**37.2r**
	PM	**7.0ap**	**2.0aq**	**5.0apq**	**40.3p**	**28.6r**	**39.3pq**	**58.3a**	**49.7a**	**60.8b**	**67.7pq**	**59.7q**	**75.7p**
OMb layer (mm)	LL	**3.2a**	**3.4a**	**3.5a**	**4.1a**	**4.9a**	**4.4a**	**5.2a**	**4.6a**	**3.5a**	**4.8p**	**3.1q**	**1.8r**
	PM	**2.1b**	**2.2b**	**1.9b**	**1.5b**	**2.1b**	**1.6b**	**1.5b**	**1.4b**	**0.8b**	**0.9rs**	**0.7rs**	**0.2s**
MMb layer (mm)	LL	**N/A**	**N/A**	**N/A**	**1.0b**	**1.4b**	**1.2b**	**2.0by**	**2.1bxy**	**2.8bx**	**3.1**	**3.4**	**3.9**
	PM	**N/A**	**N/A**	**N/A**	**1.5a**	**1.6a**	**2.0a**	**2.3ay**	**3.2axy**	**3.3ax**	**3.4**	**3.8**	**3.5**

a,b **LSmeans for each attribute with a different letter within a column on the same display time differ (P<.05)**
p,q,r,s **LSmeans for each attribute with a different letter on the same display date differ (P<.05)**
x,y,z **LSmeans for each attribute with a different letter in the same row on the same display time differ (P<.05)**

Table 2. Effects of display temperature (DT) on color attributes, oxymyoglobin (OMb) layer, and metmyoglobin (MMb) layer depths at 3 h, 1, 3, and 5 d of display

Attributes	3 h display		1 d display		3 d display		5 d display	
	0°C DT	3.3°C DT	0°C DT	3.3°C DT	0°C DT	3.3°C DT	0°C DT	3.3°C DT
L*	**39.4x**	**37.9y**	**38.9**	**37.1**	**38.7**	**37.0**	**38.3**	**36.7**
a*	**31.4**	**31.1**	**27.8**	**26.6**	**24.5**	**23.0**	**22.0**	**20.0**
b*	**26.0x**	**25.4y**	**23.9**	**22.9**	**21.9**	**21.2**	**21.0**	**19.9**
%R630-%R580	**25.5x**	**24.0y**	**20.2**	**17.8**	**16.5**	**13.9**	**13.9**	**10.9**
%OMb	**94.3**	**94.1**	**78.0**	**73.9**	**64.9**	**59.8**	**55.1**	**47.2**
%MMb	**4.0**	**3.4**	**20.8**	**23.8**	**32.9**	**38.3**	**44.0**	**49.7**
OMb layer (mm)	**2.9**	**2.6**	**3.4x**	**2.9y**	**3.1**	**2.7**	**2.3**	**2.0**
MMb layer (mm)	**N/A**	**N/A**	**1.5**	**1.4**	**2.7**	**2.4**	**3.6**	**3.1**

x,y **LSmeans for each attribute with a different letter in the same row on the same display time differ (P<.05)**

Table 3. Effects of storage temperature (ST) on color attributes, oxymyoglobin (OMb) layer, and metmyoglobin (MMb) layer depths at 3 h, 1, 3, and 5 d of refrigerated display

Attributes	3 h display		1 d display		3 d display		5 d display	
	0°C ST	4.4°C ST	0°C ST	4.4°C ST	0°C ST	4.4°C ST	0°C ST	4.4°C ST
L*	**38.2**	**39.1**	**37.7**	**38.3**	**37.6**	**38.1**	**37.1**	**37.9**
A*	**31.3**	**31.2**	**27.1**	**27.2**	**24.1**	**23.4**	**22.0x**	**19.9y**
B*	**25.8**	**25.6**	**23.4**	**23.5**	**21.7**	**21.5**	**20.8**	**20.1**
%R630-%R580	**24.4**	**25.0**	**18.9**	**19.1**	**15.5**	**14.9**	**13.5x**	**11.4y**
%OMb	**93.6**	**94.8**	**76.6**	**75.5**	**64.4**	**60.3**	**55.7**	**46.6**
%MMb	**3.7**	**3.7**	**22.1**	**22.5**	**33.6**	**37.5**	**42.2y**	**51.4x**
OMb layer (mm)	**2.7**	**2.8**	**3.2**	**3.1**	**3.1**	**2.8**	**2.4**	**1.8**
MMb layer (mm)	**N/A**	**N/A**	**1.5**	**1.3**	**2.3y**	**2.8x**	**3.1**	**3.6**

x,y **LSmeans for each attribute with a different letter in the same row on the same display time differ (P<.05)**