Myoglobin layer depth, surface myoglobin and visual colour-life of beef *Longissimus* and *Psoas major* steaks from various postmortem times

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Abstract

Relative changes of oxymyoglobin layer depth (OLD) and metmyoglobin layer depth (MLD) of muscle (M) cubes as related to surface pigment and visual colour-life of beef *Longissimus lumborum* (LL) and *Psoas major* (PM) steaks from various postmortem times (PT; 3, 5, 10, or 14 d at 0 or 4.4°C in vacuum) were investigated during 120 h of display (0 or 3.3°C). Muscle cubes were placed into open-top, clear Plexiglas® containers to facilitate digital image photography and analysis of myoglobin (Mb) layers at 3, 24, 72, and 120 h. Steaks surface pigment and visual colour were initially evaluated at 1 and 2 h, respectively and at 24, 72, and 120 h. Based on panelists acceptability, PM steaks had slightly better bloom colour during 2 h, but had a shorter colour-life of 31 h with estimated surface metmyoglobin (MMb) of 24%, and OLD and MLD of 1.68 and 2.00 mm, respectively. LL were more colour-stable with estimated colour-life of 91 h, surface MMb of 23%, and OLD and MLD of 4.24 and 2.49 mm, respectively. PT*M affected colour stability at 120 h, where the 3 and 5-d PT LL steaks had greater colour stability with a deeper OLD than MLD.

Keywords: Myoglobin layer, visual colour-life, digital image analysis, beef *Longissimus, Psoas major*
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Introduction

Bloom colour on a muscle surface is influenced by oxygen (O_2) consumption of muscle cells, oxygen partial pressure (PO_2) on the muscle surface, O_2 diffusion into the muscle cells, and the depth of Mb oxygenation (Brooks, 1929; MacDougall & Taylor, 1975; O'Keeffe and Hood, 1982; Lanari & Cassens, 1991; Feldhusen et al., 1995). Muscle cell respiration and enzymic activities are temperature dependent (Urbin and Wilson, 1961; Bendall and Taylor, 1972) and deteriorated with postmortem age (Bendall and Taylor, 1972; O'Keeffe and Hood, 1982; Lanari & Cassens, 1991). Deeper OLD delays the visible appearance of the underlying MMb layer to where it is perceived as discolouration on the muscle surface (MacDougall & Taylor, 1975; Ledward, 1984). Research has determined the depth of O_2 penetration into muscles directly by determining PO_2 (Morley, 1971; Feldhusen et al., 1995) or indirectly by observing pigment oxygenation (Brooks, 1929; MacDougall & Taylor, 1975; O'Keeffe and Hood, 1982; McMillin et al., 1994) and oxidation (Brooks, 1929). We previously investigated both OLD and MLD as related to surface colour using novel open-topped clear Plexiglas containers and image analysis for continuous observation of pigment changes in LL and PM muscle cubes (Limsupavanich et al., 2004). In the present study, we investigated the effects of PT (3, 5, 10, or 14 d in vacuum at 0 or 4.4°C) on relative changes of OLD and MLD and visual colour and surface pigment of beef LL and PM steaks during 120 h of display at 0 or 3.3°C.

Materials and Methods

For each of 4 replications, eight paired LL or PM, obtained 2 d post slaughter, were assigned randomly to 0ºC storage temperature (ST), while another eight pairs were assigned to 4.4ºC. At 3, 5, 10, and 14 d PT, each of two pieces of LL (posterior portion) or PM (anterior portion), matched for carcass, from each ST was cut into five 2.54-cm thick steaks, placed on styrofoam trays, and overwrapped with PVC film (23,250 cc O_2/m^2/24 h at 23ºC and 0%RH) for simulated retail display (0ºC or 3.3ºC). Steaks surface pigment and visual colour evaluations were initially performed approximately 1 and 2 h, respectively, after cutting and before display lighting began (1624-lux Ultralume™ 30 continous fluorescent lights, 34 watts, 3000 K; Phillips, Sommerset, NJ). The fourth steak from anterior end of each LL and the second steak from posterior end of each PM were assigned for colour evaluations initially and at 24, 72, and 120 h. Spectral data (400-700 nm, 10 nm increments) were obtained using

a MiniScan™ XE spectrophotometer (Hunter Associates Laboratory, Inc., Reston, VA). Muscle surface MMb (%) were estimated based on K/S 572 ÷ K/S 525 nm (AMSA, 1991). Seven trained panelists visually evaluated steak colour using a five-point colour scale (AMSA, 1991), where 3.5 was considered as borderline acceptability.

For Mb layer dept determination, at each 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. 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 air. The LL or PM muscle cubes from each ST were displayed at 0º or 3.3ºC under the same lighting condition as the steaks. Digital image photography of the pigment layers was first recorded on each muscle cube during 3 h bloom time due to time demands for muscle cube preparation and at 24, 72, and 120 h of display. Colour image processing and analysis of pigment layer depts (OLD and MLD) were performed as described previously (Limsupavanich et al., 2004). The experiment was in a strip-split-split plot design. Analysis of Variance was performed utilizing the MIXED procedure of SAS (2000).

Results

Visual colour evaluation

Surprisingly, initial visual bloom colour of PM steaks was slightly more desirable ($P<0.05$, Table 1) for panelists than that of LL. However, at 24, 72, and 120 h of display, surface visual colour of LL steaks was appreciably brighter, thus more stable ($P<0.05$) than that of PM steaks. PM muscles had high O_2 consumption rate and bloomed efficiently when exposed to air, but were very susceptible to oxidation (O'Keeffe & Hood, 1982). The interaction of PT*M ($P<0.05$) on visual colour evaluation were noted at 120 h of display. Except for LL steaks from 3 d PT (colour score of 3.5), all steaks were visually unacceptable by 120 h (colour score of 3.6 to 4.7). To estimate the display time (h) when visual colour score reached 3.5, a borderline acceptability for the trained colour panels, prediction regression equations were calculated based on Lsmeans colour scores (Table 1) at 2, 24, 72, and 120 h of display for LL ($r^2 = 0.99$) and PM ($r^2 = 0.97$) steaks. This determined approximately 91 h of display for LL steaks and 31 h for PM steaks, when visual colour of the two muscle types was at panelist borderline acceptability.

Surface metmyoglobin (MMb) concentration of LL and PM steaks

At 1 h of bloom, there was no difference ($P>0.05$, Table 1) in surface MMb of LL and PM steaks. But the two muscles differed ($P<0.05$) for surface MMb formation at 24, 72, and 120 h of display, where surfaces of PM steaks had greater ($P<0.05$) MMb than the LL steaks. Surface MMb accumulation increased with longer display. By 120 h, the PT*M interaction ($P<0.05$) on surface MMb was found . Among all, LL steaks from 3 d (20.8%) and 5 d PT (22.7%) presented the least ($P<0.05$) surface MMb. Compared to PM, all LL steaks had less ($P<0.05$) surface MMb, while no difference ($P>0.05$) was found on surface MMb of PM steaks from different PT.

To estimate the surface MMb concentration point for LL and PM steaks where panelists would be uneasy about colour acceptability (visual colour score = 3.5), prediction regression equations, based on the surface MMb Lsmeans at each display period for LL ($r^2 = 0.92$) and PM ($r^2 = 0.96$) steaks, were determined. At 91 h of display when colour of LL steaks reached the "borderline of panelist acceptability", surface MMb accumulation was approximately 22.7% (S.E.=3.38). For PM steaks, at 31 h, the approximate display time at which panelists acceptability reached the borderline, surface MMb was approximately 23.5% (S.E.=4.37). Hood & Riordan (1973) reported a linear relationship between surface MMb (in the range of 5 to 33% MMb) and the proportion of total sales of discoloured meat. They indicated that a proportion of total sales of 1:2 for discoloured to bright red beef was reached when surface MMb was about 20%.

Calculated oxymyoglobin layer depth (OLD) and metmyoglobin layer depth (MLD)

To calculate the OLD for LL steaks at 91 h and PM steaks at 31 h of display, the time when panelists visually evaluated surface colour score of the steaks as 3.5, regression equations were obtained based on the OLD Lsmeans (Table 1) for muscle main effect at 3, 24, 72, and 120 h of display. For PM ($r^2 = 0.99$), the OLD at 31 h of display was approximately 1.68 mm (S.E.=0.02). For LL, it appeared that the linear regression based on the 4 display periods gave a poor prediction for OLD. A better model could be developed from additional data with more display periods. However, it could be noted that the thickness of OLD for LL at 72 and 120 h decreased from 4.62 to 3.64 mm (Table 1). A linear equation was, therefore, developed based on the OLD Lsmeans at these two display periods to estimate the thickness of OLD at 91 h, which was approximately 4.24 mm. For MLD, the linear regression equations were obtained based on the MLD Lsmeans for the muscle main effect at 24, 72, and 120 h of display (Table 1) for LL ($r^2 = 0.99$) and PM

(r^2 = 0.87). At approximately 91h, the MLD of LL reached approximately 2.49 mm (S.E.=0.03), whereas the predicted MLD for PM at 31 h was approximately 2.00 mm (S.E.=0.40).

Conclusions

Colour stability advantage of LL over PM steaks was clearly demonstrated by visual colour and surface pigment evaluation. However, the initial bloom colour during 1-3 h of both muscles was similar. At approximately 91 h of display for LL and 31 h for PM, when the steak surface colour reached panelist borderline acceptability score as estimated by regression modeling, surface MMb concentration for both muscles was approximately 23-24%. At 91 h, the estimated OLD and MLD of LL muscles were 4.24 and 2.49 mm, respectively. At 31 h, the OLD and MLD of PM muscles reached approximately 1.68 and 2.00 mm, respectively. The interactions of postmortem time and muscle type had great influences on colour stability at 120 h of display. Longer PT muscles provided a more desirable bloom colour, but offered a shorter colour display life, especially for PM. By 120 h of display, LL steaks with shorter PT had deeper OLD than the MLD, which contributed to a better display colour stability.

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Table 1 Least squares means (Lsmeans) of visual colour scores[i], % surface metmyoglobin (%MMb)[j], oxymyoglobin layer depth (OLD, mm)[k], and metmyoglobin layer depth (MLD, mm)[k] for *Longissimus lumborum* (LL) and *Psoas major* (PM) steaks from various postmortem times (PT; 3, 5, 10, 14 d in vacuum at 0 or 4.4°C) during 120 h of display at 0 or 3.3°C

	Colour score[i]		%MMb[j]		OLD (mm)[k]		MLD (mm)[k]	
Display period	**LL**	**PM**	**LL**	**PM**	**LL**	**PM**	**LL**	**PM**
Initial	**2.4 a**	**2.3 b**	0.5	0.0	**3.45 a**	**2.03 b**	N/A	N/A
24 h	**2.7 b**	**3.1 a**	**2.2 b**	**22.3 a**	**4.56 a**	**1.72 b**	**0.86 b**	**1.68 a**
72 h	**3.2 b**	**4.1 a**	**11.9 b**	**56.1 a**	**4.62 a**	**1.23 b**	**2.07 b**	**2.80 a**
120 h	**3.9 b**	**4.6 a**	**34.6 b**	**72.6 a**	**3.64 a**	**0.63 b**	3.16	3.47

[a, b] Lsmeans for each trait (colour score, %MMb, OLD, or MLD) at each display period without a common letter differ (P<0.05).

[i] Visual colour was initially evaluated at 2 h, where 1=very bright cherry red, 2=bright cherry red, 3=slightly dark red to tannish red, 4 = moderately grayish/tan to brown, and 5 = tan to brown; Scores were rated in increments of 0.5, where 3.5 was considered as borderline acceptability. [j] %MMb was determined initially at 1 h. [k] OLD and MLD were determined initially at 3 h.

N/A No metmyoglobin layer was observed.