The Relationship between Meat Color (CIE L^* and a^*), Myoglobin Content, and Their Influence on Muscle Fiber Characteristics and Pork Quality

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Abstract

This study examined the relationship between meat color and myoglobin content, and evaluated their influence on muscle fiber characteristics and overall pork quality. Four groups of pigs were classified by lightness (CIE L^*) and redness (CIE a^*): HH, high lightness and high redness; HL, high lightness and low redness; LH, low lightness and high redness; LL, low lightness and low redness. Myoglobin content ranged from 1.2 mg/g to 2.1 mg/g, and was highest in the LH group and lowest in the LL group ($p < 0.05$). Myoglobin content correlated significantly with redness (CIE a^*) ($r = 0.45$, $p < 0.01$). Fiber compositions of type I and IIA were closely related to lightness and redness. Pork with higher sizes of type IIA and IIB fibers had lower lightness and redness, respectively, which was tougher than the other pork. Pork having the highest lightness and lowest redness, often considered “pale”, has higher values in drip loss than the other classes of pork tested.

Key words: myoglobin content, meat color, muscle fiber, pork quality

Introduction

Meat color is influenced by many factors, such as the concentration of heme pigments (especially myoglobin), the chemical state of myoglobin and the physical characteristics of the meat (Jeong *et al.*, 2009). Although other heme proteins such as hemoglobin and cytochrome C may also play a role in meat color, myoglobin, the sarcoplasmic heme protein, is the principle protein responsible (Mancini and Hunt, 2005). The functional property of myoglobin is both to reversibly bind molecular oxygen and to facilitate the diffusion of oxygen from the plasma membrane to the mitochondria, where oxygen is consumed (Renner, 1990). Therefore, red muscle fibers, which have predominantly aerobic metabolism and require more oxygen than white muscle fibers, tend to have higher concentrations of myoglobin (Seideman *et al.*, 1984).

Meat quality is influenced by muscle fiber characteristics such as fiber number, fiber composition, cross-sectional area and fiber density. Lightness, one of the important meat quality parameters, is correlated with fiber com-

position of type I and IIB (Ryu and Kim, 2005). Fiber composition of IIB has also negative correlation with both myoglobin content and visually determined color (Whipple *et al.*, 1992).

Instrumental color measures from the color systems such as Hunter and CIE are useful for assessing meat surface color. One or more values of instrumental color are used as indicators of meat quality and a predictor of preferred visual color. Both lightness (CIE L^*) and redness (CIE a^*) were especially suggested as the indicator of PSE (pale, soft, exudative) and/or DFD (dark, firm, dry) pork or visual meat color (Brewer *et al.*, 2001; Hulsege *et al.*, 2001). Therefore, many researchers have been studied myoglobin chemistry (Bekhit *et al.*, 2003; Faustman *et al.* 1999; Mancini *et al.*, 2004), and factors affecting color and color stability of meat (Cornforth, 1994; Faustman and Cassens, 1990; Moelich *et al.*, 2003; Renner, 1990; Seideman *et al.*, 1984). However, there has been paid little attention to the influences of myoglobin content and color of meat on muscle fiber characteristics and overall meat qualities.

Therefore, the aim of the present study was to gain more knowledge about the relationships between instrumental color (CIE L^* and a^*) and myoglobin content, and their influences on muscle fiber characteristics and pork qualities.

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Materials and Methods

Samples

A total of 50 crossbred (Korean native black pig $\times$ Landrace) F₂ pigs (24 gilts and 26 barrows) were selected at a commercial slaughtering house. Animals were slaughtered at 203 ($\pm$ 4) d of age and their carcass weight was 89.2 $\pm$ 3.3 kg. Muscle samples (*longissimus dorsi*) were taken for histochemical analysis within 1 h post-mortem and frozen in isopentane chilled with liquid nitrogen according to the method proposed by Brooke and Kaiser (1970). After 24 h chilling of the carcasses, *longissimus dorsi* (LD) muscles were taken to evaluate the following meat quality traits: pH, meat color (CIE L*, a* and b*), drip loss, Warner-Bratzler shear force (WBSF), moisture content, intramuscular fat content, and sarcomere length.

Histochemical analysis

Transverse serial sections of 10 μ m were cut from entire blocks (1.0 $\times$ 1.0 $\times$ 2.0 cm) on a cryostat microtome (HM525, Microm GmbH, Germany) at -27°C and subsequently incubated for histochemical demonstration of myosin adenosine triphosphatase (mATPase) following alkaline (pH 10.70) and acid (pH 4.70) pre-incubation, using a modification of the Brooke and Kaiser (1970) method. An image analysis system (Image-Pro[®] plus 5.1, Media Cybernetics Inc., USA) was used to examine the stained sections. Then the muscle fibers were divided into fiber types I, IIA and IIB according to the nomenclature of Brooke and Kaiser (1970). About 600 fibers per sample were counted to estimate the distribution and size of fiber types. Total number of fiber, fiber number and area composition, cross-sectional area, fiber diameter and fiber perimeter were determined. Fiber number percentage (%) refers to the ratio of counted fiber numbers of each fiber type to the total counted fiber number. Fiber area percentage (%) was considered to be the ratio of the total cross-sectional area of each fiber type to the total measured fiber area.

Meat quality traits

Myoglobin content (mg/g) was measured by the method of Warriss (1979) with modifications. 4 g of sample was homogenized with phosphate buffer (pH 6.8 and ionic strength of 0.04). After centrifuging the slurry at 5,000 g for 30 min, the supernatant was filtered with Whatman No. 1 filter paper and the extract retained. 1 mL of myoglobin extract was oxidized with 100 μ L of 60 mM K₃F(III)(CN)₆ and treated with 80 mM NaCN to measure

the final volume cyanides to the addition of the cyanides. After the change in color from yellow to red with the addition of potassium cyanide, the absorbance was measured at 540 nm. Molar extinction coefficient of 11300 for cyanmetmyoglobin (CNMMb) was used to determine total molar concentration of myoglobin (Drabkin 1950). Molar concentrations were converted to mg of myoglobin by the following formula: Myoglobin content (mg/g) = $(A_{540}/11300) \times [16800 \times (0.01 + 0.001 + d) \times 1000/\text{sample (g)}]$, where A_{540} = absorbance at 540 nm, 11300 = molar extinction coefficient of CNMMb at 540 nm, 16800 = equivalent weight of the bovine myoglobin, 0.01 = volume of the myoglobin extract, 0.001 = volume of the potassium ferricyanide, and d = volume increase due to the addition of potassium cyanide.

pH was measured on homogenates of 3.0 g muscle in 27 mL of de-ionized water, using a pH-meter (MP230, Mettler toledo, Switzerland). Meat color (CIE L*, a* and b*) was measured on the muscle surface, using a Minolta Chromameter (CR-300, Minolta Co., Japan) that was standardized with a white plate (Y=93.5, X=0.3132, y=0.3198). Drip loss was determined by suspending muscle samples standardized for surface area in an inflated plastic bag for 24 h at 4°C.

Moisture content (%) of the sample was determined using the oven drying method (AOAC, 1995). Intramuscular fat content (%) was extracted from 3 g of the homogenized meat sample using the procedure of Folch *et al.* (1957), with modification: 3 g of sample was combined with 30 mL of folch solution I (chloroform: methanol, 2:1, v/v) and homogenized with a Polytron homogenizer (T25-B, IKA[®]Works Inc., Brasil) for 30 s. The homogenate was filtered with Whatman No.1 filter paper in a 100 mL measuring cylinder after stirring for 2 h at 4°C. The filtered solution was stirred with 0.88% of NaCl (25% volume of the filtered solution), and then allowed to separate into two layers, for 1 h at room temperature. After washing the wall of the measuring cylinder with 10 mL of folch solution II (chloroform:methanol:H₂O = 3:47:50), the final volume of the lower layer was recorded. The upper layer (methanol and water layer) was removed using an aspirator, and 10 mL of the lower layer (chloroform containing lipid extracts) was taken into a dish to dry at 50°C. The weight of the dish was measured before and after drying, and the fat content was calculated using the following equation: Fat content (%) = $\{[(c-b) \times a/10]/\text{grams of sample}\} \times 100$, where a = the final volume of the lower layer, b = weight of the dish before drying, and c = weight of the dish after drying.

Warner-Bratzler shear force (WBSF, kg/cm²) values were determined using an Instron Universal Testing Machine (Model 4400, Instron Corp., USA). The samples were 1.3-cm diameter cores obtained from steaks cooked to 70°C internal temperature for 30 min. Sarcomere length (μm) was determined according to the method of Cross *et al.* (1981): the sample was placed in a vial with solution A (0.1 M KCl, 0.39 M boric acid, and 5 mM ethylenediaminetetraacetic acid in 2.5% glutaraldehyde) for 2 h. The samples were then transferred to fresh vials containing solution B (0.25 M KCl, 0.29 M boric acid, and 5 mM ethylenediaminetetraacetic acid in 2.5% glutaraldehyde) for 17 to 19 h. On the following day, individual fibers were torn into pieces and placed on a microscope slide with a drop of solution B. The slide was then placed horizontally in the path of a vertically oriented laser beam to give an array of diffraction bands on a screen. These bands were perpendicular to the long axis of the fibers as described by Cross *et al.* (1981). Sarcomere length (μm) = $\{ \{ 632.8 \times 103 \times D \times \text{SQRT}[(T/D)^2 + 1] \} / T \} \times 100$, where D equals the distance (mm) from the specimen-holding device to the screen (D = 98 mm) and T equals the separation (mm) between zero and the first maximum band. An average of 10 sarcomere lengths was acquired from each meat sample.

Statistical analysis

The experimental data were analyzed by the analysis of variance procedure of statistical analysis systems (SAS, 2002). The data were classified into two clusters based on lightness (high, n = 28; low, n = 22), and into another clusters according to redness (high, n = 26; low, n = 24). General linear model procedure was used to evaluate the differences ($p < 0.05$) between lightness, redness, and lightness $\times$ redness interaction. The results are presented as least-square means with the standard errors. Pearson correlation coefficients were evaluated to describe the relationship between myoglobin content and meat color (CIE L*, a* and b*).

Results and Discussion

To evaluate the differences in myoglobin content, muscle fiber characteristics and meat qualities (including meat color), four groups were categorized by lightness and redness (Fig. 1). The high lightness and high redness (HH) group and the high lightness and low redness (HL) group had significantly higher lightness than did the low lightness and high redness (LH) group and the low light-

Fig. 1. Lightness (CIE L*) and redness (CIE a*) in different pig groups categorized by lightness and redness of longissimus muscle at 24 h postmortem. Pig groups: HH, high lightness and high redness; HL, high lightness and low redness; LH, low lightness and high redness; LL, low lightness and low redness. Significance ($p < 0.05$) is indicated by differing letters (a and b in lightness; A and B in redness) on the bars which indicates SE.

ness and low redness (LL) group ($p < 0.05$). HH and LH groups had significantly higher redness than HL and LL groups ($p < 0.05$).

Relationship between myoglobin content and meat color

Myoglobin content ranged from 1.2 mg/g to 2.1 mg/g, and was higher in the LH group and lower in the LL group than in the other groups ($p < 0.05$; Fig. 2). In general, high myoglobin results in more red color within skeletal muscle (Bekhit and Faustman, 2005). However, in the present study, myoglobin content was affected not only by redness but also lightness, although there was no

Fig. 2. Myoglobin content (mg/g) of longissimus muscle in different pig groups categorized by lightness and redness. Pig groups: HH, high lightness and high redness; HL, high lightness and low redness; LH, low lightness and high redness; LL, low lightness and low redness. Significance ($p < 0.05$) is indicated by differing letters (a, b, c) on the bars which indicates SE.

Table 1. Correlation coefficients between meat color and myoglobin content

	Lightness (CIE L*)	Redness (CIE a*)	Yellowness (CIE b*)
Myoglobin content	-0.20	0.45**	0.11
Lightness (CIE L*)		-0.26	0.41*
Redness (CIE a*)			0.47*

* $p < 0.05$; ** $p < 0.01$.

significant correlation between myoglobin content and lightness ($p > 0.05$; Table 1). The results in low lightness groups (LH and LL) agreed with those of Bekhit and Faustman (2005), whereas, in high lightness groups (HH and HL), myoglobin content was not affected by redness. That is why we did not consider the chemical states of myoglobin which influence on the variation of lightness (Lindahl *et al.*, 2001; Lundström *et al.*, 1988).

Table 1 shows the correlation coefficients between myoglobin content and meat color (CIE L*, a* and b*). Myoglobin content was significantly correlated with redness (a*) ($r = 0.45$, $p < 0.01$), however there were no significant correlations between myoglobin content and lightness (L*) and yellowness (b*) ($p > 0.05$). Yellowness was positively correlated with lightness ($r = 0.41$) and redness ($r = 0.47$) ($p < 0.05$). However, there were no significant correlations between lightness and redness and between myo-

globin and yellowness ($p > 0.05$).

Muscle fiber characteristics

Muscle fiber types were classified into three fiber types: I, IIA and IIB according to the method of Brooke and Kaiser (1970) (Fig. 3). Total number of fibers was significantly different among the groups ($p < 0.05$) (Fig. 4). In terms of total number of fibers, the HH group had the highest number, and the HL group had the lowest

Fig. 4. Total number of fibers in *longissimus* muscle of different pig groups categorized by lightness and redness. Pig groups: HH, high lightness and high redness; HL, high lightness and low redness; LH, low lightness and high redness; LL, low lightness and low redness. Significance ($p < 0.05$) is indicated by differing letters (a, b and c) on the bars which indicates SE.

Fig. 3. Cross-sections of *longissimus* muscle stained for myosin ATPase, after being pre-incubated in pH 4.70. Magnification of 100× was used. (Bar = 50 μm). I: fiber type I; IIA: fiber type IIA; IIB: fiber type IIB. Pig groups: HH, high lightness and high redness; HL, high lightness and low redness; LH, low lightness and high redness; LL, low lightness and low redness.

number.

Muscle fiber composition (%) and fiber size are presented in Table 2 and Table 3. Fiber number composition of type IIA was affected by a lightness $\times$ redness interaction ($p < 0.05$). The LL group had the highest value of fiber number composition of type IIA among the groups. Fiber area compositions (%) of type I and IIA were related to both lightness and redness, however, that of type IIB was just related to redness. Fiber area compositions of both types I and IIA were higher in the low lightness groups, regardless of redness, while those of type I and IIA were higher in the high redness groups than in the low redness groups, regardless of lightness. Area composition of fiber type IIB was higher in the low redness groups. The results of this study agree with Ryu and

Kim (2006), who reported that lightness was negatively correlated with the amount of type I and IIA fibers, and was positively correlated with type IIB fiber.

Fiber diameter and perimeter of type IIA were affected by lightness ($p < 0.05$), and cross-sectional area, fiber diameter and fiber perimeter of type IIB were related to redness ($p < 0.05$, $p < 0.01$ and $p < 0.05$). Fiber diameter and perimeter of type IIA were higher in low lightness groups than in high lightness groups regardless of redness. All parameters of fiber size for type IIB were higher in low redness groups than in high redness groups, without reference to lightness. In cattle, Ozawa *et al.* (2000) showed that fiber diameter of red fiber types (βR and αR) were positively correlated with the meat color CIE a^* value. In general, oxidative fibers such as fiber type I and IIA have

Table 2. Muscle fiber composition (%) in different pig groups categorized by lightness and redness

Traits	Pig groups ¹⁾				Significance ²⁾		
	HH	HL	LH	LL	L*	a*	L* $\times$ a*
Fiber number composition (%)							
Type I	9.9 (1.3)	11.6 (2.5)	12.3 (2.4)	14.3 (1.9)	ns	ns	ns
Type IIA	13.8 ^{ab} (4.6)	7.0 ^b (0.2)	10.9 ^{ab} (1.5)	15.5 ^a (2.1)	ns	ns	*
Type IIB	76.3 (5.8)	81.4 (2.5)	76.8 (3.7)	72.9 (1.3)	ns	ns	ns
Fiber area composition (%)							
Type I	9.9 (1.7)	6.7 (0.4)	11.5 (0.6)	7.9 (0.9)	*	**	ns
Type IIA	10.9 (1.1)	4.2 (0.3)	12.3 (1.6)	9.2 (0.6)	*	**	ns
Type IIB	79.2 (0.6)	86.7 (3.0)	74.8 (3.6)	82.4 (0.8)	ns	*	ns

Data are least-square means (standard error) of pigs in each lightness $\times$ redness combination.

¹⁾Pig groups: HH, high lightness and high redness; HL, high lightness and low redness; LH, low lightness and high redness; LL, low lightness and low redness.

²⁾Significance: L*, lightness; a*, redness; ns, no significance; * $p < 0.05$; ** $p < 0.01$.

^{a, b}Least-square means with different superscripts in the same row are significantly different in lightness $\times$ redness interaction.

Table 3. Muscle fiber size in different pig groups categorized by lightness and redness

Traits	Pig groups ¹⁾				Significance ²⁾		
	HH	HL	LH	LL	L*	a*	L* $\times$ a*
Cross-sectional area (μm^2)							
Type I	3886.8(1471.6)	3830.0(114.0)	4635.3(309.5)	3118.5(206.6)	ns	ns	ns
Type IIA	3263.2(1100.9)	3084.2(264.0)	4893.8(204.5)	3611.1(149.6)	ns	ns	ns
Type IIB	3790.2(650.8)	5485.9(733.9)	4211.7(159.0)	5894.9(129.7)	ns	*	ns
Fiber diameter (μm)							
Type I	66.2(13.6)	67.3(5.8)	73.7(6.6)	56.4(5.6)	ns	ns	ns
Type IIA	61.0(10.8)	59.5(4.8)	75.2(5.7)	69.0(3.6)	*	ns	ns
Type IIB	64.2(6.5)	76.9(5.3)	66.3(5.8)	80.9(4.7)	ns	**	ns
Fiber perimeter (μm)							
Type I	228.0(41.3)	237.5(31.7)	263.4(28.6)	196.7(10.0)	ns	ns	ns
Type IIA	220.1(36.5)	221.8(28.4)	278.3(15.2)	241.7(16.9)	*	ns	ns
Type IIB	245.4(21.5)	294.5(17.3)	259.7(13.1)	294.2(17.6)	ns	*	ns

Data are least-square means (standard error) of pigs in each lightness $\times$ redness combination.

¹⁾Pig groups: HH, high lightness and high redness; HL, high lightness and low redness; LH, low lightness and high redness; LL, low lightness and low redness.

²⁾Significance: L*, lightness; a*, redness; ns, no significance; * $p < 0.05$; ** $p < 0.01$.

Table 4. Meat quality characteristics in different pig groups categorized by lightness and redness

Traits	Pig groups ¹⁾				Significance ²⁾		
	HH	HL	LH	LL	L*	a*	L* $\times$ a*
pH	5.6 (0.1)	5.6 (0.0)	5.8 (0.1)	5.7 (0.0)	*	ns	ns
Moisture content (%)	73.4 (0.4)	73.7 (0.1)	73.3 (1.0)	75.1 (0.7)	ns	ns	ns
Intramuscular fat content (%)	4.2 (1.6)	1.9 (0.2)	3.7 (1.2)	2.8 (0.6)	ns	ns	ns
Yellowness (b*)	5.1 (0.6)	2.3 (0.5)	2.6 (0.5)	1.2 (0.6)	*	*	ns
Drip loss (%)	2.6 (0.8)	2.7 (0.2)	1.5 (0.6)	0.4 (0.1)	*	ns	ns
Warner-Bratzler shear force (kg/cm ²)	4.3 ^{ab} (0.3)	3.6 ^b (0.2)	2.4 ^c (0.4)	4.8 ^a (0.3)	ns	*	*
Sarcomere length (μ m)	1.9 (0.2)	1.6 (0.1)	1.8 (0.1)	1.5 (0.1)	ns	**	ns

Data are least-square means (standard error) of pigs in each lightness $\times$ redness combination.

¹⁾Pig groups: HH, high lightness and high redness; HL, high lightness and low redness; LH, low lightness and high redness; LL, low lightness and low redness.

²⁾Significance: L*, lightness; a*, redness; ns, no significance; * p <0.05; ** p <0.01.

a, b, c Least-square means with different superscripts in the same row are significantly different in lightness $\times$ redness interaction.

predominantly aerobic metabolism and require more oxygen (Seideman *et al.*, 1984). Thus, red muscle fibers contain more myoglobin than white muscle fibers (Cassens and Cooper, 1971; Whipple *et al.*, 1992). Therefore, our results show that the pork loin (*longissimus* muscle) with a higher composition of oxidative fibers (like type I or IIA) and with lower composition of glycolytic fiber (type IIB) has higher redness. In addition, the pork loin with higher fiber size of type IIA and IIB has lower lightness and redness, respectively.

Meat qualities

Meat quality characteristics in different pig groups categorized by lightness and redness were presented in Table 4. Both the pH value at 24 h post-mortem and drip loss (%) were related to lightness (p <0.05). Low lightness groups (LH, LL) had higher values of pH than did the high lightness groups (HH, HL). Contrary to pH, drip loss was higher in the HH and HL groups than in the LH and LL groups. As presented before (Table 1), yellowness was related to both lightness (p <0.5) and redness (p <0.05). The pig groups which had high value of lightness or redness had higher lightness or higher redness. Warner-Bratzler shear force (kg/cm², WBSF) and sarcomere length (μ) were related to redness (p <0.05 and p <0.01, respectively). WBSF were also affected by L* $\times$ a* interaction (p <0.05). WBSF was the highest value in the LL group, while LH group had the lowest value of WBSF. HH and LH, which were the higher redness groups, had higher sarcomere length regardless of lightness. However, moisture content (%) and intramuscular fat content (%) were not significantly different among the groups (p >0.05).

The pH value, water-holding capacity or meat color are generally used to predict the degree of meat quality (Joo *et al.*, 1999; Fischer, 2007). The pork having higher lightness and lower redness is seen as pale, and having relatively higher values in pH and drip loss compared to the other pork. As well, pork having both low lightness and low redness was tougher than the other pork. Therefore, the results of this study corroborate the previous research, in that water-holding capacity and tenderness can be predicted by the lightness and redness of meat.

According to Lengerken *et al.* (1997), pigs with a higher total number of fibers exhibited higher muscle pH and lower drip loss. However, differences in pH and drip loss between the HH and HL groups which had the highest and the lowest number of total fibers, respectively, were not observed in this study. In pig, muscle with a larger fiber size of type IIB exhibit tougher meat than muscles of smaller fiber size (Karlsson *et al.*, 1993; Maltin *et al.*, 1997). Our results also showed that low redness group with higher size of type IIB had tougher (higher WBSF and low sarcomere length) meat, and thus was of a lower quality.

Although there was no significant correlation between myoglobin and lightness, myoglobin content was related to not only redness but also lightness. Fiber compositions of type I and IIA were related to lightness and redness. The pork with higher sized type IIA and IIB fibers had lower lightness and redness, respectively, and was tougher than the other pork. The pork having higher lightness and lower redness was seen as pale, and had relatively higher values in drip loss compared to the other pork.

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