

# The Influence of Myoglobin on the Color of Beef During the Storage

Tonggang Zhang<sup>1</sup>, Ling Fan<sup>2</sup>, Yongchang Yu<sup>1\*</sup>

<sup>1</sup>College of Mechanical and Architectural Engineering, Taishan University, Tai'an 271000, China

<sup>2</sup>Shandong University of Engineering and Vocational Technology, Jinan 271000, China

\*Corresponding Author

**ABSTRACT.** *The objectives of present study were to investigate the influence of pigments and myoglobin on the color of fresh beef during the storage. The effect of the relative amounts of myoglobin, and the total and chromatic absorbance, on the lightness, redness and yellowness of beef longissimus lumborum, were evaluated. The results showed that the changes in color parameters of 0 and 7d depended primarily on the relative content of the three chemical forms of myoglobin. The value of the L\* depended mainly on variation A525. The value of the a\* depended on the forms of the myoglobin, while the value of the b\* depended on the proportions of the deoxymyoglobin (Mb), oxymyoglobin (MbO<sub>2</sub>) and metmyoglobin (MetMb).*

**KEYWORDS:** *beef, color, deoxymyoglobin, oxymyoglobin, metmyoglobin*

## 1. Introduction

The color of meat plays an important role in determining consumers' purchasing decisions because color is usually used as an indicator of freshness[1-2]. Color of meats mainly depends on the content and status of myoglobin—as well as on the meat's tissue structure. Myoglobin has three chemical forms, the oxygenated form (oxymyoglobin), the oxidised form (metmyoglobin) and the reduced form (deoxymyoglobin). The relative content of these chemical forms have remarkable influence on the meat color. This is reasoned largely by the amounts of myoglobin chemical compositions and by the level of the post mortem pH reduction that influences on both the myofilament lattice spacing [3]and denaturation of muscle proteins[4].

Feldhusen reported that low correlation coefficients between the parameters of color and the amount of pigment in pork, particularly a\*, show the significant role of elements other than pigments[5]. One of those elements is the change in the

structure of meats, and this is significant when the naturally low amounts of pigments in meats. The structure of meats has an important impact on the penetration depth of oxygen and light, the chromatic absorbance and color. Chromatic absorbance also depends on the amount of pigment reached by the penetrated light and on the content of myoglobin in the meat[6-10].

The ways used to measure the amounts of pigments in the meats, containing the extraction and colourimetric assays, but these methods do not take into account the effect of tissue structure. Therefore, they can not accurately measure the impact of pigment and the relative content of myoglobin on meats color parameters[11-12]. Consequently, the more effective method is needed. Spectrophotometric methods could be used to access the impact of pigment on the color parameters of meats, due to measuring the isobestic point of the three status of myoglobin (the chromatic absorbance at a wavelength of 525 nm).  $A_{525p}$  is proportional to the quantity of pigment reached by the light. That is to say, the quantity of pigment involved in the formation of color. Krzywicki reported that when the path length of light in two samples is the same, then the value of  $A_{525p}$  is proportional to the pigment content in the samples[13-14].

Therefore, determinations of the reflectance absorbance are more useful than the measurement of the amount of pigment of the meat. This methods were applied largely to the samples with low pigment levels and color parameters which are mainly influenced by the sample structure.

The objectives of this paper were to get more information about the impacts of total pigment, achromatic absorbance and the relative amounts of the three chemical status of myoglobin on the parameters of color measured in the beef by applying the CIELAB and CIELCh ratings.

## **2. Materials and Methods**

### ***2.1. Sample preparation***

The longissimus lumborum (LL, 12th rib to the last lumbar vertebrae) from 8 Holstein-Friesian young bulls (around 18 months old; average carcass weight  $239.5 \pm 36.2$  Kg) was obtained from the College of Agronomy, Ningxia University at 24 h post mortem . The meat was cut into slices (8 mm thickness) using a scalpel. All meat samples were individually vacuum packed and stored at 4°C in the dark. The color parameters of meat samples was first measured using a Minolta CM-2500d spectrophotometer. The relative content of myoglobin were obtained using a spectrophotometer.

### ***2.2. Color measurements***

The color of the beef was measured by a Minolta CM-2500d spectrophotometer (Konica Minolta Sensing Inc., Japan) with a measuring port diameter of 8mm, D65

illuminant, 10° standard observer and color scale recommended for meat color measurements [15]. The instrument was calibrated using a white color standard plate ( $L^*=97.28 \pm 0.02$ ,  $a^*=-0.51 \pm 0.10$ ,  $b^*=-3.92 \pm 0.10$ ). The average value of five measurements on the meat surface was used.

### 2.3. Measurement of the relative content of myoglobin

According to the Krzywicki method, 5 g of each sample was mixed with 25 ml phosphate buffer (40 mM, pH=6.8) and homogenized with an ultra-fine homogenizer (FLUKO F6/10 Germany) (10,000 rpm, 25 s). After standing for 60 min at 4 °C in the dark, mixture was centrifuged at 4,500×g for 20 min at 4 °C. The supernatant was filtered through filter paper and the absorbance was measured at 525, 545, 565 and 572 nm separately with a spectrophotometer. Each sample was measured three times, and the average value was taken for the further statistical data analysis.

The following formulas:

$$P1 = (0.369R_1 + 1.140R_2 - 0.941R_3 + 0.015) \times 100 \quad (1)$$

$$P2 = (0.882R_1 - 1.267R_2 + 0.809R_3 - 0.361) \times 100 \quad (2)$$

$$P3 = (-2.514R_1 + 0.777R_2 + 0.800R_3 + 1.098) \times 100 \quad (3)$$

where P1, P2, and P3 are the mass fractions of Mb, MbO<sub>2</sub>, and MetMb, respectively.

$$R_1 = A_{572}/A_{525}, R_2 = A_{565}/A_{525}, R_3 = A_{545}/A_{525}.$$

### 2.4. Statistical analysis

The results of the aforementioned measurements and computations analyses were completed with the aid of chemometric software Statistical Analysis System (Version 9.2; SAS Inst. Inc., Cary, NC). Simple and partial correlation coefficients and coefficients of determination were determined, as was the significance at  $P \leq 0.01$  and  $P \leq 0.001$ .

## 3. Results and discussion

### 3.1. The relative content of the myoglobin in the beef

The absorbance value indicates achromatic light absorption at 730 nm in meat. The value was subtracted as a correction for the absorbance of pigments at 525 nm and the relative content of the myoglobin in the beef. A problem when applying the Krzywicki (1979) is the range of available spectrophotometers, which only cover the range 400-700 nm; so they could not measure the absorbance/reflectance of light at 730 nm. Therefore someones measured pigments at some wavelengths (which the

impact of pigments is very poor) to correct of achromatic absorption values. The impact of pigments decreased at 650-660 nm. The absorbance values in the section of the spectrum beyond 650-660 nm display little discriminations, concerning sample with low pigment content and thus with poorly developed reflectance spectra. In this study, the correction of achromatic absorption was absorbance at 700 nm, which perhaps had no great effect for the last results.

### 3.2. Relationship between the characteristics of the meat

Table 1 showed the average and standard deviation of L\*, a\*, b\*; A525, A525<sub>p</sub> and A700; the relative content of Mb, MbO<sub>2</sub> and MetMb, and physicochemical characteristics of the fresh beef.

Table 1 Characteristics of the meat (mean and SD)

Feature	Mean±SD		
	0d	7d	21d
L*	32.39±3.49	30.51±2.84	27.27±4.97
a*	14.06±2.12	13.50±1.76	12.39±2.01
b*	5.63±1.93	5.09±1.83	3.86±1.90
A <sub>525</sub>	0.492±0.062	0.405±0.055	0.431±0.072
A <sub>525p</sub>	0.296±0.038	0.211±0.041	0.256±0.058
A <sub>700</sub>	0.212±0.045	0.263±0.032	0.238±0.041
Mb(%)	26.7±0.21	20.15±0.19	4.11±0.22
MbO <sub>2</sub> (%)	61.3±0.16	40.75±0.28	10.32±0.20
MetMb(%)	14.5±0.12	45.26±0.19	70.33±0.16
Crude protein(%)	26.41±0.48	24.12±0.59	22.47±0.25
Fat(%)	3.16±0.86	3.02±0.75	2.69±0.93
Water holding capacity(%)	38.80±0.73	34.36±0.71	31.93±0.96
pH	5.50±0.24	5.86±0.38	6.28±0.27

Table 2 showed the simple correlation coefficients between physicochemical characteristics and the parameters of color and A525 and A525<sub>p</sub>, A700 and the relative content of Mb, MbO<sub>2</sub> and MetMb in the meat. On 0d, there were no significant correlation between the relative content of crude protein and other characteristics. There was a significant correlation between the relative content of fat and water holding capacity, the values of color parameters (a\*, b\*), A525<sub>p</sub>. The value of pH had a significant correlation with the basic chemical components of the meat, except with A525<sub>p</sub>. On 7d and 21d, there were no significant coefficients of simple correlation between the relative content of crude protein and fat in the meat, any of the studied characteristics. There was a significant correlation between the water holding capacity and the values of color parameters (a\*, b\*), A525<sub>p</sub>, A700, MetMb. The value of pH and the basic chemical components of the meat had a significant correlation, except with A525<sub>p</sub> and MbO<sub>2</sub>. This result is consistent with the previous study.

Table 2 Simple correlation coefficient for the basic chemical components and pH of the meat.

	Feature	Crude protein(%)	Fat(%)	Water holding capacity(%)	pH
0d	L*	0.093	0.072	0.205	-0.866 <sup>b</sup>
	a*	-0.089	0.331 <sup>a</sup>	0.386 <sup>a</sup>	-0.362 <sup>a</sup>
	b*	0.074	0.376 <sup>b</sup>	0.383 <sup>b</sup>	-0.850 <sup>b</sup>
	A <sub>525</sub>	-0.082	-0.051	-0.168	0.834 <sup>b</sup>
	A <sub>525p</sub>	-0.069	0.437 <sup>a</sup>	0.317 <sup>a</sup>	0.121
	A <sub>700</sub>	0.036	0.353	-0.433 <sup>a</sup>	0.895 <sup>b</sup>
	Mb(%)	-0.104	-0.317	-0.464 <sup>b</sup>	0.817 <sup>b</sup>
	MbO <sub>2</sub> (%)	0.064	0.374	0.399 <sup>a</sup>	-0.864 <sup>b</sup>
	MetMb(%)	0.136	0.349	0.458 <sup>b</sup>	-0.649 <sup>b</sup>
7d	L*	0.112	0.093	0.257	-0.830 <sup>b</sup>
	a*	-0.093	0.163	0.506 <sup>a</sup>	-0.401 <sup>a</sup>
	b*	0.059	0.098	0.433 <sup>b</sup>	-0.829 <sup>b</sup>
	A <sub>525</sub>	-0.075	-0.068	-0.128	0.737 <sup>b</sup>
	A <sub>525p</sub>	-0.093	0.106	0.397 <sup>a</sup>	0.163
	A <sub>700</sub>	0.051	0.395	-0.419 <sup>a</sup>	0.835 <sup>b</sup>
	Mb(%)	-0.904	-0.139	-0.124	0.429 <sup>a</sup>
	MbO <sub>2</sub> (%)	0.103	0.382	0.081	-0.183
	MetMb(%)	0.117	0.125	0.862 <sup>b</sup>	-0.796 <sup>b</sup>
21d	L*	0.087	0.081	0.103	-0.803 <sup>b</sup>
	a*	-0.088	0.126	0.462 <sup>a</sup>	-0.527 <sup>a</sup>
	b*	0.093	-0.076	0.537 <sup>b</sup>	-0.550 <sup>a</sup>
	A <sub>525</sub>	-0.068	0.051	-0.125	0.816 <sup>b</sup>
	A <sub>525p</sub>	-0.081	-0.116	0.319 <sup>a</sup>	0.099
	A <sub>700</sub>	0.052	0.239	-0.439 <sup>a</sup>	0.665 <sup>b</sup>
	Mb(%)	-0.094	-0.175	-0.219	0.419 <sup>a</sup>
	MbO <sub>2</sub> (%)	0.103	-0.094	0.289	-0.093
	MetMb(%)	0.087	0.398	0.495 <sup>b</sup>	-0.559 <sup>a</sup>

a Significant at  $P \leq 0.01$ . b Significant at  $P \leq 0.001$ .

The value of pH is decreased due to a reduction in the depth of invasion by light, and the reflectance increases, which causes an increase in L\* and a decrease in the relative content of Mb. According to Krzywicki (1979), a decrease in pH is accompanied with a reduction in the depth of penetration by light, and the increase of reflectance, leading to an increase in L\* and a decrease in the relative content of the Mb. A higher pH indicated a low susceptibility of muscle pigments to oxidation and oxygenation, and hence, a low content of MbO<sub>2</sub> and MetMb. However, that usually appears in minced meat because of the metmyoglobin reducing system is destroyed by mincing[16]. There was no significant correlations between the value of pH and the value of the A525<sub>p</sub>. It shows that the discrepancies in chromatic parameters between the samples with different pH were due mainly to the discrepancies in the relative amount of myoglobin forms rather than discrepancies in the quantity of pigments reached by the light. On 0d, the highest simple correlation coefficients appeared between pH values and L\*( $r=-0.866^{**}$ ), b\*( $r=-0.850^{**}$ ), A525( $r=0.834^{**}$ ), A700( $r=0.895^{**}$ ), the relative contents of Mb ( $r =0.817^{**}$ ), MbO<sub>2</sub>

( $r=-0.864^{**}$ ); On 7d, the highest simple correlation coefficients appeared between pH values and  $L^*$  ( $r=-0.866^{**}$ ),  $b^*$  ( $r=-0.850^{**}$ ), A525 ( $r=0.834^{**}$ ), A700 ( $r=0.895^{**}$ ), the relative content of MetMb ( $r=0.862^{**}$ ); On 21d, the simple correlation coefficients were found between pH values and  $L^*$  ( $r=-0.803^{**}$ ), A525 ( $r=0.816^{**}$ ), A700 ( $r=0.665^{**}$ ) (Table 2).

### 3.3 Effect of the status of myoglobin and amount of pigments on the color $L^*$

The effect of the myoglobin on color of meat had been widely studied. However, The effect of the relative content myoglobin on meat color parameters, the effect of the relative content myoglobin on the stable of meat color, and the relationship between the meat color and shelf life have not clearly explained. As a kind of achromatic color element,  $L^*$  depends on the light reflected from the surface of meat. The effect of achromatic and chromatic absorption on the  $L^*$  depending on the content and chemical forms of the pigments in the samples. The relative content of myoglobin in the meat could influence the  $L^*$ . Fernández-López J and Aranda-Catalá V, who took samples from the carcasses of eight-month-old gilts some of which contained 100% of MbO<sub>2</sub>, some 100% of MetMb and some 100% of Mb in the samples. And the result showed that the meats with only MetMb had the highest  $L^*$  [17-18]. There was lowest  $L^*$  in muscle samples with only MbO<sub>2</sub>. Therefore, meat containing a high MetMb content should be expected to have a high value of  $L^*$ . That was clearly seen in the pork. But it is not known whether an increase in  $L^*$  was caused by the increase in Mb or by changes of the structure of myoglobin in the meat.

Lindahl found that an increase in MbO<sub>2</sub> and a reduction in Mb led to an increase in  $L^*$  in meat. The content of pigmentation reached by the light has a significant affect not only on  $L^*$ , but also on  $a^*$ . The authors found that no significant differences in  $a^*$  between the different groups of muscles with 100% of each of the status of myoglobin. In spite of, it is known that each of the forms of myoglobin is characterized with different values of  $a^*$ . It is not known yet, what the influence on  $a^*$  was of the quantity of pigment penetrated by the light in each of the different groups of muscles. In this study, the coefficient of linear correlation between A525 and  $L^*$  were  $r_{0d}=-0.879^{**}$ ,  $r_{7d}=-0.917^{**}$  and  $r_{21d}=0.492^*$  (Table 3). The Table 4 showed that the variability of  $L^*$  in the muscle samples can be almost totally explained by the change of the A252. The sum of chromatic absorbance and achromatic, which are proportional to the content of pigment reached by the light. The coefficient of linear correlation among the total absorbance at the isobestic point of the three chemical status of myoglobin—i.e.  $L^*$  and the value of A525 was maximum and equated  $r=-1.00$ , or very close to -1.00 (400–700 nm).

Changes in  $L^*$  mainly based on oscillations in absorbance at 700 nm ( $R^2_{0d}=0.6583^{**}$ ,  $R^2_{7d}=0.6112^{**}$  and  $R^2_{21d}=0.3863^{**}$ ), and clearly to a lesser degree, on changes in chromatic absorbance at 525 nm ( $R^2_{0d}=0.3850^{**}$ ,  $R^2_{7d}=0.4381^{**}$  and  $R^2_{21d}=0.5537^{**}$ ). The sum of effect of total absorbance at 525 nm and the relative content of the forms of myoglobin (Mb+ MbO<sub>2</sub>+MetMb+A525) on the  $L^*$  of the meat color was up to 89.76% ( $R^2=0.8976^{**}$ ). To conclusion, the relative amounts of

the chemical forms of myoglobin had less impact on the L\* of the meat, although there was a clearly correlation between them ( $R^2_{0d}= 0.4388^{**}$ ,  $R^2_{7d}=0.4089^{**}$  and  $R^2_{21d}=0.3915^{**}$ ). This effect resulted mainly from the relationship between the relative content of the status of myoglobin (Mb, MbO<sub>2</sub> and MetMb) and achromatic absorption, which had the greatest effect on the L\* of the meat color.

**3.4. Effect of the status of myoglobin and amount of pigments (A525p) on the color chromatic parameters**

The relative contents of myoglobin had a clearly effect on the values of the chromatic parameters (a\* and b\*). Lindahl reported that an increase of MbO<sub>2</sub> in meat resulted in an increase of a\* and b\*. MetMb is the lightest red compared with other chemical forms of myoglobin. An increase in MetMb content contributes to a decrease in a\* [19].

Table 3 Simple correlation coefficients

		a*	b*	A <sub>525</sub>	A <sub>525p</sub>	A <sub>700</sub>	Mb	MbO <sub>2</sub>	MetMb
0d	L*	-0.096	0.633 <sup>b</sup>	-0.879 <sup>b</sup>	-0.621 <sup>b</sup>	-0.796 <sup>b</sup>	-0.868 <sup>b</sup>	0.690 <sup>b</sup>	0.802 <sup>b</sup>
	a*	1	0.571 <sup>b</sup>	0.329	0.683 <sup>b</sup>	-0.420 <sup>b</sup>	-0.667 <sup>b</sup>	-0.395	-0.196
	b*		1	-0.589 <sup>b</sup>	0.109	-0.866 <sup>b</sup>	-0.549 <sup>b</sup>	0.758 <sup>b</sup>	0.493 <sup>b</sup>
	A <sub>525</sub>			1	0.718 <sup>b</sup>	0.755 <sup>b</sup>	0.851 <sup>b</sup>	-0.816 <sup>b</sup>	-0.741 <sup>b</sup>
	A <sub>525p</sub>				1	0.095	-0.538 <sup>b</sup>	-0.091	0.349
	A <sub>700</sub>					1	-0.831 <sup>b</sup>	-0.690 <sup>b</sup>	-0.753 <sup>b</sup>
	Mb						1	-0.803 <sup>b</sup>	-0.619 <sup>b</sup>
	MbO <sub>2</sub>							1	-0.923 <sup>b</sup>
MetMb								1	
7d	L*	-0.102	0.562 <sup>b</sup>	-0.917 <sup>b</sup>	-0.510 <sup>b</sup>	-0.864 <sup>b</sup>	-0.767 <sup>b</sup>	0.850 <sup>b</sup>	0.628 <sup>b</sup>
	a*	1	0.721 <sup>b</sup>	0.292	0.605 <sup>b</sup>	-0.392 <sup>b</sup>	-0.689 <sup>b</sup>	-0.351	-0.097
	b*		1	-0.893 <sup>b</sup>	0.097	-0.606 <sup>b</sup>	-0.797 <sup>b</sup>	0.568 <sup>b</sup>	0.391 <sup>b</sup>
	A <sub>525</sub>			1	0.688 <sup>b</sup>	0.915 <sup>b</sup>	0.691 <sup>b</sup>	-0.763 <sup>b</sup>	-0.795 <sup>b</sup>
	A <sub>525p</sub>				1	0.105	-0.836 <sup>b</sup>	-0.087	0.692
	A <sub>700</sub>					1	-0.517 <sup>b</sup>	-0.809 <sup>b</sup>	-0.737 <sup>b</sup>
	Mb						1	-0.941 <sup>b</sup>	-0.599 <sup>b</sup>
	MbO <sub>2</sub>							1	-0.763 <sup>b</sup>
MetMb								1	
21d	L*	-0.116	-0.710 <sup>b</sup>	-0.492 <sup>a</sup>	0.719 <sup>b</sup>	0.516 <sup>b</sup>	-0.708 <sup>b</sup>	0.813 <sup>b</sup>	0.655 <sup>b</sup>
	a*	1	0.115	-0.092	-0.438	0.638 <sup>b</sup>	-0.417 <sup>b</sup>	-0.135	-0.116
	b*		1	0.139	0.109	0.175	-0.150	0.582 <sup>b</sup>	-0.336 <sup>b</sup>
	A <sub>525</sub>			1	0.079 <sup>b</sup>	-0.497 <sup>b</sup>	-0.783 <sup>b</sup>	-0.411 <sup>b</sup>	0.591 <sup>b</sup>
	A <sub>525p</sub>				1	0.103	-0.469 <sup>b</sup>	-0.093	-0.486
	A <sub>700</sub>					1	-0.136	0.406 <sup>b</sup>	0.852 <sup>b</sup>
	Mb						1	-0.090	0.739 <sup>b</sup>
	MbO <sub>2</sub>							1	0.598 <sup>b</sup>
MetMb								1	

a Significant at  $P \leq 0.01$ . b Significant at  $P \leq 0.001$ .

In this study, it was shown that an increase in b\*, which was followed by a decrease in pH. This could be due to an increase in the content of the MbO<sub>2</sub> and MetMb with the expense of the Mb. These results was in accordance with previous study. It was shown that b\* was more highly affected by MbO<sub>2</sub> than Mb and

MetMb, which was confirmed by simple correlation coefficients (Table 3) and partial correlation coefficients (Table 5) between them.

In addition, an increase in the relative content of MetMb, which had a less effect on the increase in  $b^*$  than decrease the  $a^*$ . Furthermore, Fernández-López J and Aranda-Catalá V reported that the greatest value of the  $b^*$  was related with the relative of content of MbO<sub>2</sub>, while the value of  $b^*$  was lower in relation to MetMb and the lowest in relation to Mb. Meat had an increase in the value of  $a^*$  should be expected to have an increase in the relative content of MetMb lead to an increase in the amounts of pigments, resulting in changing relationship between the relative content of the myoglobin and achromatic absorption effect on color of meat.

Table 4 The coefficients of determination R<sup>2</sup>(corrected) for color parameters.

Feature	0d			7d			21d		
	L*	a*	b*	L*	a*	b*	L*	a*	b*
A <sub>525</sub>	0.7427 <sup>a</sup>	0.1431	0.3980 <sup>a</sup>	0.8640 <sup>a</sup>	0.1523	0.7396 <sup>a</sup>	0.6491a	0.2825	0.7680a
A <sub>525p</sub>	0.3850 <sup>a</sup>	0.3877 <sup>a</sup>	0.1793	0.4381 <sup>a</sup>	0.4820 <sup>a</sup>	0.1038	0.5537a	0.3639a	0.3293
A <sub>700</sub>	0.6583 <sup>a</sup>	0.2295	0.5546 <sup>a</sup>	0.6112 <sup>a</sup>	0.4915	0.4745 <sup>a</sup>	0.3863a	0.2995	0.5741a
MbO <sub>2</sub>	0.6023 <sup>a</sup>	0.2326	0.6002	0.8860 <sup>a</sup>	0.5026	0.7512	0.4627a	0.2126	0.7902
MetMb	0.2145 <sup>a</sup>	0.1874 <sup>a</sup>	0.8374 <sup>a</sup>	0.2986 <sup>a</sup>	0.1046 <sup>a</sup>	0.3574 <sup>a</sup>	0.5823a	0.3427a	0.6368a
Mb	0.3637 <sup>a</sup>	0.1022	0.4487	0.3189 <sup>a</sup>	0.2138	0.8873	0.7450a	0.1943	0.8223
Mb+MbO <sub>2</sub> +MetMb	0.4388 <sup>a</sup>	0.4836 <sup>a</sup>	0.6773 <sup>a</sup>	0.4089 <sup>a</sup>	0.5862 <sup>a</sup>	0.4761 <sup>a</sup>	0.3915a	0.4346a	0.5398a
A <sub>525p</sub> +MbO <sub>2</sub>	0.6653 <sup>a</sup>	0.5654	0.8972 <sup>a</sup>	0.6553 <sup>a</sup>	0.5349	0.7977 <sup>a</sup>	0.5613a	0.5657	0.6321a
A <sub>525p</sub> +MetMb	0.7835 <sup>a</sup>	0.8760 <sup>a</sup>	0.6771 <sup>a</sup>	0.8635 <sup>a</sup>	0.7390 <sup>a</sup>	0.6038 <sup>a</sup>	0.7565a	0.3411a	0.6356a
A <sub>525p</sub> +Mb	0.5437 <sup>a</sup>	0.3282	0.6759 <sup>a</sup>	0.7407 <sup>a</sup>	0.3512	0.8936 <sup>a</sup>	0.7657a	0.5802	0.3953a
A <sub>525p</sub> +Mb+MbO <sub>2</sub> +MetMb	0.4870 <sup>a</sup>	0.9024 <sup>a</sup>	0.9073 <sup>a</sup>	0.6709 <sup>a</sup>	0.7928 <sup>a</sup>	0.7793 <sup>a</sup>	0.4671a	0.7594a	0.7438a
A <sub>525</sub> +Mb+MbO <sub>2</sub> +MetMb	0.8976 <sup>a</sup>	0.7995 <sup>a</sup>	0.8033 <sup>a</sup>	0.7756 <sup>a</sup>	0.6857 <sup>a</sup>	0.8327 <sup>a</sup>	0.7651a	0.6742a	0.6710a

a Significant at  $P \leq 0.001$ .

Lindahl et al. (2001) reported that an increase in  $a^*$  was accompanied by an increase in content of MetMb, a decrease in amount of Mb and an increase in the amount of pigment in the samples. They also reported that the value of  $b^*$  depended primarily on oscillations in the relative amount of the three chemical forms of myoglobin, but they found no effect of MetMb on  $b^*$ , likely due to the small content of this kind of chemical form. Table 4 showed that the variability of  $a^*$  was effected both on the relative amounts of the three chemical forms of myoglobin and the content of pigment reached by the light (A525p). In Table 5, each chemical form of myoglobin is characterized by a difference in L\*,  $a^*$  and  $b^*$  on 0 and 7d. However, it is only characterized by a difference in L\* on 21d.



*Table 5 Partial correlation coefficients for parameters of color.*

		Mb,MbO <sub>2</sub> ,Mb (constant)	A <sub>525p</sub> and Mb (constant)	A <sub>525p</sub> and MbO <sub>2</sub> (constant)	A <sub>525p</sub> and MetMb (constant)
		A <sub>525p</sub>	MbO <sub>2</sub> /MetMb	MetMb/Mb	MbO <sub>2</sub> /Mb
0d	L*	-0.946a	0.025	0.624a	0.637a
	a*	0.858a	0.417a	-0.799a	0.759a
	b*	-0.038	0.539a	0.590a	0.409a
7d	L*	-0.757a	0.622	0.681a	0.720a
	a*	0.759a	0.875a	-0.573a	0.399a
	b*	-0.053	0.195	0.709a	0.621a
21d	L*	-0.406a	0.375a	0.416a	0.349a
	a*	0.053	0.039	0.025	0.103
	b*	0.143	0.285	0.192	0.107

a Significant at  $P \leq 0.001$ .

MbO<sub>2</sub> has the highest level of the value of a\*, while MetMb has the lowest. Different chemical forms of Mb have different effects on the b\* value in the order MbO<sub>2</sub> > MetMb > Mb. Accordingly, an increase in Mb significantly conduces to decreases in b\*, while an increase in MbO<sub>2</sub> significantly conduces to an increases in a\* and b\*, and an increase in MetMb significantly conduces to decreases in a\*. Our results were in accordance with other studies (Lindahl et al. (2001), i.e. the uncertainty of b\* depended primarily on changes in the relative amount of the three chemical status of myoglobin. In addition, for 0 and 7d, the changes in a\* was due to both oscillations in the relative amount of the three chemical forms of myoglobin and in the content of pigment reached by the light (A525p). However, there were no significant coefficients of simple correlation between the relative amount of the three chemical forms of myoglobin and a\*, b\*. This is consistent with the previous studies that hemoglobin is depolymerization and denaturation during storage 34 days at room temperature.

The changes in color parameters of beef depended primarily on the relative content of the three chemical forms of myoglobin on 0 and 7d, while changes in color parameters depended primarily on the content of pigment (A525p) on 21d. In analysing the patterns in the values of color parameters, it should be noted that some of the characteristics that significantly correlate are not directly caused by one another. For instance, the significant correlations between achromatic absorbance and b\* are due to elements such as protein denaturation, fat oxidation. Thus, the significant correlations between the relative content of the three chemical forms of myoglobin and the L\* were dependent mainly on variations in the values of pH. The effect of the relative amount of MbO<sub>2</sub> on L\* is small in fresh beef. However, the regression coefficients or correlation values obtained may cause the conclusion that the relative content of myoglobin is responsible for L\*. The greatest relative content of MbO<sub>2</sub> can be measured in fresh beef with low pH values, which is characterized by high L\*, primarily due to the low absorption of light.

#### 4. Conclusion

Variations of  $L^*$  in fresh beef can be almost totally interpreted by variations in the total absorption of light at 525 nm, which is made up of chromatic and achromatic absorbance depending on the content of pigment penetrated by light in the fresh beef. Changes in the relative content of the three chemical forms of myoglobin had small effect on  $L^*$  in the fresh beef. However, the myoglobin had a significant effect on all the chromatic parameters values ( $L^*$ ,  $a^*$  and  $b^*$ ). The influence of the three chemical forms of myoglobin on  $a^*$  depended primarily on the ratio of the content of MbO<sub>2</sub> and MetMb, while their effect on  $b^*$  depended primarily on the ratio of the contents of Mb, MbO<sub>2</sub> and MetMb. A greater influence on the value of  $b^*$  was exerted by undulations in the relative content of MbO<sub>2</sub> than of MetMb. The changes in color parameters of beef depended primarily on the relative content of the three chemical forms of myoglobin on 0 and 7d, while changes in color parameters depended primarily on the content of pigment reached by light on 21d.

#### References

- [1] Mancini, R. A., & Hunt, M. (2005). Current research in meat color. *Meat science*, 71(1), 100-121.
- [2] McAfee, A. J., McSorley, E. M., Cuskelly, G. J., Moss, B. W., Wallace, J. M., Bonham, M. P., & Fearon, A. M. (2010). Red meat consumption: An overview of the risks and benefits. *Meat science*, 84(1), 1-13.
- [3] Goodson, J., Beckstead, R. B., Payne, J., Singh, R. K., & Mohan, A. (2015). Amino acid sequence of Japanese quail (*Coturnix japonica*) and northern bobwhite (*Colinus virginianus*) myoglobin. *Food chemistry*, 181, 256-262.
- [4] Suman, S. P., & Joseph, P. (2013). Myoglobin chemistry and meat color. *Annual review of food science and technology*, 4, 79-99.
- [5] Feldhusen, J. F. (2005). Giftedness, talent, expertise, and creative achievement. *Conceptions of giftedness*, 2, 64-79.
- [6] Barros-Velázquez, J., Carreira, L., Franco, C., Vázquez, B. I., Fente, C., & Cepeda, A. (2003). Microbiological and physicochemical properties of fresh retail cuts of beef pack-aged under an advanced vacuum skin system and stored at 4°C. *Journal of Food Protection*, 66, 2085–2092.
- [7] Bekhit, A. E. D., & Faustman, C. (2005). Metmyoglobin reducing activity. *Meat Science*, 71, 407–439.
- [8] Brewer, M. S., Zhu, L. G., Bidner, B., Meisinger, D. J., & McKeith, F. K. (2001). Measuring pork color: Effects of bloom time, muscle, pH and relationship to instrumental parameters. *Meat Science*, 57, 169–176.
- [9] Karamucki, T., Jakubowska, M., Rybarczyk, A., & Gardzielewska, J. (2013). The influence of myoglobin on the colour of minced pork loin. *Meat science*, 94(2), 234-238.
- [10] Díaz, P., Linares, M. B., Egea, M., Auqui, S. M., & Garrido, M. D. (2014). TBARS distillation method: Revision to minimize the interference from yellow pigments in meat products. *Meat science*, 98(4), 569-573.

- [11] Lindahl, G., Lundström, K., & Tornberg, E. (2001). Contribution of pigment content, myoglobin forms and internal reflectance to the colour of pork loin and ham from pure breed pigs. *Meat Science*, 59(2), 141-151.
- [12] Lavieri, N., & Williams, S. K. (2014). Effects of packaging systems and fat concentrations on microbiology, sensory and physical properties of ground beef stored at 4±1 C for 25days. *Meat science*, 97(4), 534-541.
- [13] Krzywicki, K. (1979). Assessment of relative content of myoglobin, oxymyoglobin and metmyoglobin at the surface of beef. *Meat Science*, 3(1), 1-10.
- [14] Krzywicki, K. (1982). The determination of haem pigments in meat. *Meat Science*, 7(1), 29-36.
- [15] Honikel, K. O. (1998). Reference methods for the assessment of physical characteristics of meat. *Meat science*, 49(4), 447-457.
- [16] Ledward, D. A., Smith, C. G., Clarke, H. M., & Nicholson, M. (1977). Relative role of catalysts and reductants in the formation of metmyoglobin in aerobically stored beef. *Meat science*, 1(2), 149-156.
- [17] Fernández-López, J., & Aranda-Catalá, V. (2000). Effect of mincing degree on colour properties in pork meat. *Color Research & Application*, 25(5), 376-380.
- [18] Škrlep, M., & ČANDEK-POTOKAR, M. A. R. J. E. T. A. (2007). Pork color measurement as affected by bloom time and measurement location. *Journal of muscle foods*, 18(1), 78-87.
- [19] Brewer, S. (2004). Irradiation effects on meat color—a review. *Meat Science*, 68(1), 1-17.