CIELAB color coordinates versus relative proportions of myoglobin redox forms in the description of fresh meat appearance

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Abstract This work examined changes in color that can be explained solely on the basis of the total myoglobin content and the relative proportions of deoxymyoglobin, oxymyoglobin (OMb) and metmyoglobin (MMb) during storage of meat. Meat color was evaluated for L^* , a^* , b^* , C^* and h_{ab} . Total pigment content was measured from the reflex attenuance at 525 nm (A_{525}). The relative proportions of each pigment was determined using two different methods: the Krzywicki method based on the reflex attenuance values at 473, 525, 572 and 700 nm and the KS method based on K/S ratios of the absorption and scattering coefficients $(K/S)_{474} \div (K/S)_{525}$, $(K/S)_{572} \div (K/S)_{525}$ and $(K/S)_{610} \div (K/S)_{525}$. The study was performed on beef (*Longissimus lumborum*) samples measured after 1, 4 and 7 days of exposure to air. Result revealed that L^* values can be fully explained by A_{525} alone. C^* and a^* were well explained by those parameters related to OMb content. The other color parameters depended on pigment forms. The KS method gave better results. The parameters related to MMb content were relevant to classify samples according to the time of exposure to air. In any case, information provided by color and pigment parameters were complementary to each other.

Keywords Color · CIELAB · Myoglobin · Oxymyoglobin · Meat · Beef

Introduction

Food color and general appearance are at the roots of consumer's acceptance criteria and purchasing decisions. In the development of new treatments and techniques to improve nutritional quality or to extend product shelf-life for instance, success cannot be achieved if color or other appearance attributes are negatively affected. Color in particular is strongly associated with the expected meat quality. Myoglobin content and its oxidation state on the surface are the main factors that determine meat color. Myoglobin is a water-soluble protein that contains iron in its structure. In fresh meat it is principally found in three basic states: deoxymyoglobin (DMb), oxymyoglobin (OMb) and metmyoglobin (MMb), (Mancini and Hunt 2005). The characteristic colors of these three myoglobin forms are a consequence of their distinctive reflectance spectra. On the meat surface these three forms are in a dynamic equilibrium driven by the oxygen availability. In the absence of oxygen deoxymyoglobin is the dominant form, imparting a purplish-red color typically associated with vacuum packaged meat or with fresh pieces immediately after cutting. When meat is exposed to oxygen the pigment oxygenated form oxymyoglobin is formed, accompanied by the development of a bright cherry red color. Color deterioration on the meat surface is due to the appearance of metmyoglobin, the myoglobin oxidized state. As the proportion of metmyoglobin increases meat color turns to brown. This induces a negative response in consumers and when the proportion of MMb reaches 20% it has been found that the product is rejected by half of the potential consumers (Hood and Riordan 1973; Renerre and Mazuel 1985).

The distinctive spectral features of each myoglobin form permits the estimation of their respective proportions in a

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given sample through parameters obtained from the sample spectral reflectance (R) at specific wavelengths. In fact, there are two different possible choices of parameters, two different methods, to estimate these proportions; they are described in the publication of the American Meat Science Association “Meat Color Measurement Guidelines” (AMSA 2012). One method uses the reflex attenuance $A = \log(1/R)$ (Krzywicki 1979). The other method uses the K/S ratio (K the absorbance coefficient and S the scattering coefficient) that is obtained using the equation $K/S = (1 - R)^2/2R$. In both cases R is the reflectance at a particular wavelength, expressed in the $[0, 1]$ range.

With respect to meat color, it can be objectively described by a set of color coordinates that can be obtained from non destructive spectrophotometric measurements on the food sample. The CIELAB color space is the most frequently used system to specify food colors (Arana 2012). It is a three dimensional Cartesian space with three mutually perpendicular color coordinates: L^* , the correlate of perceptual lightness; a^* that represents the red ($a^* > 0$) green ($a^* < 0$) axis and b^* that represents the yellow ($b^* > 0$) blue ($b^* < 0$) axis. In the a^*b^* plane polar coordinates, better correlated with perceptual color attributes, are also defined: the chroma $C^* = (a^{*2} + b^{*2})^{1/2}$ that refers to the vividness or dullness of a color (the greater the chroma the more vivid the color) and the hue angle $h_{ab} = \arctan(b^*/a^*)$, the angle with the a^* axis, that correlates with the color description in common language (red, yellow, green, blue, etc.) (Wyszecky and Stiles 2000). Meat colors are located in the first quadrant of the a^*b^* plane ($a^*, b^* > 0$, $0 \leq h_{ab} \leq 90^\circ$) and in this case larger values of the hue angle indicate less red colors.

The most frequently used instruments in the objective measurement of meat color are spectrophotometers and spectrophotometers (Arana 2012). They measure the spectral composition of the light reflected by the sample, and from it they compute the color coordinates in the CIELAB color space or in any other color representation system. The spectral reflectance of the sample provided by these devices can be also used to calculate the relative proportion of each pigment form. There is however a simpler color measurement device, the tristimulus colorimeter, which consists in an instrument that uses three filters to simulate the spectral sensitivity of the human visual system. This kind of devices provides only color coordinates. They do not provide spectral data and therefore cannot be used to determine pigment content.

Color coordinates and other parameters obtained from spectral measurements are frequently used to evaluate the impact of a particular treatment on the product appearance, to characterize breeds or products or in shelf-life studies. They are compared and related with the relevant variables to the specific application or study (Insausti

et al. 1999; Li et al. 2012; Lindahl et al. 2006; Luciano et al. 2011; Strydom and Hope-Jones 2014). Color changes in meat products affect all color coordinate values and, although they are frequently correlated, effects on just one parameter may not tell the complete color story (AMSA 2012).

Comparatively the underlying relationship between color and spectral parameters has deserved little attention. Moreover the scarce literature refers to meat products other than beef. Karamucki et al. (2013) investigated the influence of total myoglobin content and of the relative content of MMb, OMb and DMb, calculated with the Krzywicki method, on the CIELAB color coordinates of minced pork loin, measured after 20 min of oxygenation. With the same method Lindahl et al. (2001) using partial least squares (PLS) found a significant relationship between total pigment content and the proportion of each myoglobin form with the color of loin and ham muscles of pork, 3 days after slaughter.

This work reported the relationship between CIELAB color coordinates and the two sets of parameters used for quantifying myoglobin redox forms in meat: KS and Krzywicki sets. The study was performed in beef during 7 days of exposure to air. The first aim was to determine if either color coordinates or pigment parameters were related changes in meat during oxygenation. The second was to investigate the performance of each pigment parameter set to predict CIELAB color coordinate values, and to what extent pigment parameters and color coordinates provide equivalent or complementary information.

Materials and methods

Meat samples

Meat samples were obtained in a local slaughterhouse from the beef *Longissimus lumborum* muscle of 15 different animals. All animals were male and slaughtered when they were approximately 12 months old. They had an average carcass weight of 264 ± 19 kg and pH values 24 h post mortem between 5.6 and 5.7.

The *Longissimus lumborum* muscle was removed 24 h post mortem. Meat samples were sliced into steaks about 2 cm thick. The steaks were individually placed in plastic foam trays overwrapped with oxygen permeable polyvinyl chloride (PVC) film (OTR $1760 \text{ cm}^3 \text{ cm}^{-2} \text{ day}^{-1}$) and stored at 4°C in the dark.

Reflectance and color measurements

A Minolta CM-2002 Spectrophotometer with an integrating sphere, d/8 (diffuse illumination/ 8° viewing angle)

geometry, and 8 mm diameter circular aperture, was used to record the meat spectra from 400 to 700 nm, at 10 nm intervals and to obtain the CIELAB color coordinates L^* , a^* , b^* , chroma C^* and hue angle h_{ab} for the D65 illuminant and a 10° standard observer. In order to avoid pillowing effects and to prevent dirtying the instrument's internal integrating sphere all measurements were made through a protecting glass cover supplied with the spectrophotometer (cover set CM-A40). Measurements were made directly on the meat surface without the film. Calibration was performed with the glass, using a white calibration plate (CM-A21) and a light trap (Zero Calibration Box CM-A32). Each measurement was the average of five different readings in five non-overlapping zones of each steak, changing the instrument orientation each time to reduce the effect of possible inhomogeneities like fat and blood. Measurements were taken after 1, 4 and 7 days of exposure to air, thus allowing the time evolution of myoglobin forms and providing a representative set of pigment concentrations. Consequently there were equal, 3 day intervals between consecutive measurements.

Determination of parameters related with myoglobin content

Two methods are recommended by AMSA (2012) to compute the total myoglobin content and the relative proportions of each myoglobin forms. Both methods are known to give pigment proportions that differ in absolute value but they are nonetheless highly correlated (Hernandez et al. 2015).

Total myoglobin content

Total myoglobin content was obtained from the reflex attenuance A at 525 nm, A_{525} (Karamucki et al. 2013). The reflex attenuance is defined as $A = \log(1/R)$, with R the reflectance in the [0,1].

Krzywicki parameter set

The first set of parameters to compute the relative proportions of each myoglobin form is calculated from the reflex attenuance at 473, 525, 572 and 700 nm according to Krzywicki (1979):

$$A_D = \left[2.375 \times \left(1 - \frac{A_{473} - A_{700}}{A_{525} - A_{700}} \right) \right] \times 100$$

$$A_M = \left[1.395 - \left(\frac{A_{572} - A_{700}}{A_{525} - A_{700}} \right) \right] \times 100$$

$$A_O = 100 - (A_D + A_M)$$

with A_D , A_M and A_O the proportions, in %, of DMb, MMb and OMb respectively. Here the absorbance at 700 nm has been used instead of the absorbance at 730 nm as it is recommended when 730 nm is beyond the spectral range of the measuring instrument (AMSA 2012). Reflectance values at wavelengths not given by the instrument (473, 525 and 572) were calculated using linear interpolation.

The set of parameters A_{525} , A_D , A_O and A_M can be then used to assess the total pigment content and the relative proportion of each pigment. This set will be referred as Krzywicki set.

KS parameter set

The second set of parameters uses the ratio K/S of the absorption coefficient K and the scattering coefficient S at the wavelengths 525 nm (isobestic wavelength of three myoglobin forms), 572 nm (isobestic wavelength for OMb and DMb), 610 nm (isobestic wavelength for MMb and DMb) and 474 nm (isobestic wavelength for MMb and OMb). The K/S ratio is calculated as $K/S = (1 - R)^2/2R$, with R the reflectance in the range [0–1]. These K/S ratios can be then used to compute the parameters defined as:

$$KS_D = - \frac{(K/S)_{474}}{(K/S)_{525}}$$

$$KS_O = - \frac{(K/S)_{610}}{(K/S)_{525}}$$

$$KS_M = - \frac{(K/S)_{572}}{(K/S)_{525}}$$

with KS_D , KS_O and KS_M proportional to the proportions of DMb, OMb and MMb respectively. These ratios can be also used to obtain the absolute values of pigment proportions if reference values of samples having 0 and 100% of each pigment are available. These reference samples are usually obtained following the AMSA (1991, 2012) guidelines. We will use only the ratios so that they can be obtained from each sample reflectance spectra without further modification. The minus sign has been included for easier comparison with Krzywicki parameters. So defined these ratios give negative numbers, but are positively correlated with pigment content (Lindahl et al. 2006; Mancini et al. 2003).

The set of parameters KS_D , KS_O and KS_M can be then used to assess the relative proportion of each pigment. These parameters, together with A_{525} to estimate the of total pigment content, will be referred as KS set.

Statistical analysis

The effect of exposure to air in the measured parameters has been individually evaluated for each color coordinate and pigment parameter in the Krzywicki and KS sets comparing mean values at different measurement times using *t* Student tests for paired samples ($n = 15$). Discriminant analysis (DA) has been used to determine the performance of each set of CIELAB color coordinates, Krzywicki parameters, and KS parameters to classify samples according to the time of exposure to air and to evaluate the relative importance of each individual coordinate or parameter within each set.

The simple correlation coefficients between all studied variables together with their statistical significance have been used to study relationships between variables. Due to the existing collinearity, partial least squares (PLS) has been used to the study the relationship between color coordinates and pigment parameters, to evaluate the effectiveness of each parameter set as predictor of color variables and to assess the relative importance of each parameter within the set.

Statistical analysis has been performed with Minitab (Minitab Inc.) and SPSS (IBM Corporation) statistical software.

Results and discussion

Time evolution of CIELAB color and pigment parameters after 1, 4 and 7 days of exposure to air and reported in Table 1.

For each variable, statistical significant differences between measurement days has been evaluated by *t* Student tests for paired samples ($n = 15$). Pigment proportions and color coordinates were expected to change during

oxygenation, and these changes were faster in the first hour of exposure to air. Our first measurement corresponds to day 1 (24 h after cutting) when evolution of these parameters is assumed to have reached a slower pace. Comparison of their mean values in days 1, 4 and 7 showed that not all variables exhibited the same evolution pattern.

Color coordinates showed a tendency to change, but none of them exhibited a marked, distinctive evolution through measured days. In particular, b^* remained constant through days 1–7 since differences between mean values were not significant. L^* and a^* , varied significantly in the first 3 days, from day 1–4, but not from day 4–7. This was similar to results reported by Li et al. (2012) for *Longissimus lumborum* of young bulls where changes in L^* were significant between days 1 and 5.

The decrease in C^* was more subtle and differences can be found between days 1 and 7, this change in beef color corresponded to a shift to brown color. Significant differences in hue angle h_{ab} , L^* and a^* , exist only between days 1 and 4 although this variable was considered a coordinate related with proportion of MMb and was reported to increase, *i.e.* less redness with oxidation (AMSA 2012; Luciano et al. 2011).

The pigment parameters were related with MMb content, A_M and KS_M , have a marked evolution with different values ($p < 0.05$) at each measurement day. This is in agreement with the importance of this parameter to assess the state of conservation of meat. Parameters related with OMb have less pronounced evolution, A_O and KS_O decrease with time but differences become significant only between days 4 and 7. During the studied period the proportion of MMb was assumed to increase, but it was always less than the proportion of OMb (Hood and Riordan 1973; Renerre and Mazuel 1985). The slow decrease in the A_O and KS_O , correlated with OMb content, the pigment that gave vivid red color to meat, agreed with the slow decay of

Table 1 Mean values and standard error of CIELAB color coordinates, total pigment content A_{525} , Krzywicki parameters A_D , A_O and A_M and KS parameters KS_D , KS_O and KS_M in beef *Longissimus lumborum* in the three studied days

Parameter	Day 1	Day 4	Day 7
L^*	37.61 ± 0.31^a	39.39 ± 0.44^b	39.89 ± 0.60^b
a^*	14.42 ± 0.25^a	13.75 ± 0.19^b	13.04 ± 0.34^b
b^*	8.78 ± 0.45^a	8.96 ± 0.29^a	8.22 ± 0.44^a
C^*	16.93 ± 0.40^a	$16.43 \pm 0.25^{a,b}$	15.48 ± 0.39^b
h_{ab}	31.1 ± 1.2^a	33.02 ± 0.81^b	$32.1 \pm 1.5^{a,b}$
A_{525}	1.076 ± 0.008^a	1.030 ± 0.011^b	1.016 ± 0.016^b
A_D	38.5 ± 1.2^a	37.1 ± 1.6^a	32.7 ± 1.0^b
A_O	50.9 ± 1.6^a	$49.3 \pm 1.9^{a,b}$	47.5 ± 1.4^b
A_M	10.6 ± 1.1^a	13.6 ± 1.2^b	19.8 ± 1.0^c
KS_D	-0.8022 ± 0.0039^a	-0.8120 ± 0.0069^a	-0.8298 ± 0.0058^b
KS_O	-0.3281 ± 0.0067^a	-0.3346 ± 0.0043^a	-0.3543 ± 0.0080^b
KS_M	-1.434 ± 0.018^a	-1.373 ± 0.016^b	-1.280 ± 0.017^c

For each parameter, means with different letters are statistically different at $p < 0.05$

a^* and C^* . A similar pattern was observed in DMb parameters A_D and KS_D , also decreased during the studied period but differences become significant only at day 7. The contribution of DMb to the spectral reflectance was expected to be small, since it disappeared from the surface as oxygenation proceeds, being only found at some depth beneath the exposed surface (Sáenz et al. 2008). A_{525} , a correlate indicator of total pigment varied significantly only between days 1 and 4, similar to L^* , with which it had high correlation ($r = -0.994$, $p < 0.001$).

Given the individual evolution of each color coordinate and pigment parameter, a question arises about what set, either color coordinates, Krzywicki parameters or KS parameters performs better in describing the evolution of meat samples during oxygenation. To answer this question discriminant analysis (DA) has been used to determine the relative importance of each of these sets to classify samples according to the time of exposure to air. The results are summarized in Table 2. There are three different measurement days and therefore only two discriminant functions. The table includes the relative weight of each parameter in each function. DA results show that classifying samples into measurement days using color coordinates succeeds in 64.4% of the cases and L^* , a^* and C^* are the most important predictors, in agreement with the tendencies in Table 1. Better classification results were obtained with the Krzywicki or KS sets, scoring 75.6 and 77.8% of correctly classified samples respectively. In both cases MMB parameters (A_M and KS_M) were the most important predictors in the discriminant functions. This agreed with the marked evolution of these parameters and confirmed the importance of MMB content in describing the time evolution of this product. The decrease of C^* and the increase of MMB content indicated browning of the meat. This was also found in the exhibition of meat in a retail display during 3 days by Strydom and Hope-Jones (2014). This has a measurable effect on the perceived meat color. In a study of the influence of different types of packaging and breeds on meat quality, Insausti et al. (1999) compared instrumental color and pigment content obtained from K/S ratios with perceived color assessed by a trained sensory panel. Panel scores were better the higher the value of the coordinates and a^* and C^* and smaller the proportion of MMB.

Relationship between pigment parameters and color coordinates

Meat color was related with the actual myoglobin content and the relative proportions of each redox form. Quite naturally a question arises about the possibility of obtaining the color coordinates values from either set of pigment parameters. To address this question, correlation

Table 2 Canonical structure matrix derived from discriminant analysis taking the measurement day as clustering variable

Predictors	Function 1	Function 2
CIELAB (64.4%) ^b	(87.0%) ^c	(13.0%)
L^*	0.723 ^a	0.526
a^*	-0.755 ^a	0.105
b^*	-0.197	0.522 ^a
C^*	-0.596 ^a	0.342
h_{ab}	0.131	0.517 ^a
Krzywicki (75.6%)	(95.0%)	(5.0%)
A_{525}	0.440	-0.820 ^a
A_D	0.439 ^a	0.358
A_O	0.203 ^a	-0.055
A_M	-0.813 ^a	-0.338
KS (77.8%)	(93.4%)	(6.6%)
A_{525}	-0.422	-0.537 ^a
KS_D	-0.434 ^a	0.237
KS_O	-0.360	0.367 ^a
KS_M	0.819 ^a	-0.297

^a Higher absolute correlation between each variable and any of the discriminant function

^b For each set of predictors, percentage of correctly classified samples

^c For each discriminant function, percentage of explained variance

coefficients between variables different were computed. Simple correlation analysis showed an uneven and mixed contribution of pigment parameters in color coordinate values. Since correlations between pigment parameters within Krzywicki and KS sets were more partial least squares (PLS) have been used to assess the reliability of each parameter set in explaining the observed values of each color coordinate. PLS results for each color coordinate are summarized in Figs. 1, 2, 3 and 4. In each figure the results obtained for both parameter sets are shown. The detailed results for each color coordinate are evaluated in the following sub-sections.

Lightness L^*

CIELAB L^* is the color coordinate that correlates with lightness, the perception by which white objects are distinguished gray and light colored objects from dark colored objects (Hunter and Harold 1987). Regardless the particular oxidation state, larger total pigment content implied greater absorbance and less total reflectance, and thus a darker sample and lower lightness (L^*). Total pigment content is known to be related with reflex attenuance at 525 nm, A_{525} . Wavelength 525 nm is an isobestic point of the three myoglobin forms, meaning that the three forms have the same reflectance, and therefore the same reflex attenuance, at this particular wavelength. Consequently

Fig. 1 Coefficients of the PLS fit of a^* to the Krzywicki parameters (black bars) and K/S parameters (gray bars). Pigment correlate A₅₂₅ refers to reflex attenuation at 525 nm. DMb, OMb and MMb correlates refer to the corresponding parameters A_D, A_O and A_M of the Krzywicki set and K_S_D, K_S_O and K_S_M of the KS set respectively

Fig. 2 Coefficients of the PLS fit of b^* to the Krzywicki parameters (black bars) and K/S parameters (gray bars). Pigment correlate A₅₂₅ refers to reflex attenuation at 525 nm. DMb, OMb and MMb correlates refer to the corresponding parameters A_D, A_O and A_M of the Krzywicki set and K_S_D, K_S_O and K_S_M of the KS set respectively

both parameters are expected to be highly and negatively correlated, as it has been actually found in our analysis ($r = -0.994$, $p < 0.001$).

Neither L^* nor A₅₂₅ had comparable correlation coefficients with other variables, suggesting a quite direct relationship between total pigment content and sample lightness. This was corroborated to the PLS results, confirming that L^* was well explained by either parameter set, since both incorporate A₅₂₅. The Krzywicki set explained 99.8% of the observed variance in L^* values. KS set showed this value as 99.0%. The reason was the perfect correlation with A₅₂₅ as expected from the correlation results. The other

Fig. 3 Coefficients of the PLS fit of C^* to the Krzywicki parameters (black bars) and K/S parameters (gray bars). Pigment correlate A₅₂₅ refers to reflex attenuation at 525 nm. DMb, OMb and MMb correlates refer to the corresponding parameters A_D, A_O and A_M of the Krzywicki set and K_S_D, K_S_O and K_S_M of the KS set respectively

Fig. 4 Coefficients of the PLS fit of h_{ab} to the Krzywicki parameters (black bars) and K/S parameters (gray bars). Pigment correlate A₅₂₅ refers to reflex attenuation at 525 nm. DMb, OMb and MMb correlates refer to the corresponding parameters A_D, A_O and A_M of the Krzywicki set and K_S_D, K_S_O and K_S_M of the KS set respectively

parameters contribute only marginally. For this color coordinate both parameter sets are equivalent.

Logically one should expect lower L^* values, *i.e.* darker meat, in samples with higher pigment content. Our beef samples have L^* values in the range 37–40, and A₅₂₅ values in the range 1.28–1.43 depending on the measurement day. Pork meat has been found to have larger average L^* values (54.52 after 20 min) and lower average A₅₂₅ values (0.686) (Karamucki et al. 2013). In the same work these authors reported that pigment content was very low and that color was influenced by meat structure as well. Despite this, they found that lightness variations among samples were almost entirely explained by total absorption

of light at 525 nm. It has been reported that in addition total pigment, the relative proportions of each myoglobin form also affects L^* values in pork loin (Lindahl et al. 2001).

a^* and b^*

Oxymyoglobin (OMb) is known to impart the bright red color to meat. It is visually and colorimetrically redder than DMb and MMb and thus the presence of OMb is expected to influence a^* , the CIELAB coordinate whose positive values represent red colors. This influence should manifest itself as an important relationship between a^* and pigment parameters related to OMb. Considering the relationship between total pigment content and A_{525} , a relationship between a^* and A_{525} is also expected.

In the KS set OMb content is given by the KS_O ratio, which is highly correlated with a^* ($r = 0.901$, $p < 0.001$). PLS analysis (Fig. 1) confirms that KS_O is the most important factor in explaining the observed variance in a^* , while the role of the other parameters, including A_{525} , is relatively small. As a whole, the observed variance in a^* is well explained by the KS set (91.7%).

A different situation is found in the Krzywicki set. The OMb measure A_O is only weakly correlated with a^* ($r = 0.337$, $p < 0.05$), a coordinate that is actually better correlated with A_{525} , although to somewhat modest value ($r = 0.624$, $p < 0.001$). PLS results indicate that a^* values are not so well explained by the Krzywicki set. Compared to the KS set, only 54.4% of the observed variance is explained by the Krzywicki set. Furthermore A_O is only second in importance after A_{525} . Parameters related with DMb and MMb have little influence in the PLS results, regardless of the significant correlation between KS_M and a^* ($r = 0.590$, $p < 0.001$).

Despite the differences in explaining observed a^* values, both parameter sets show positive correlations and PLS coefficients with a^* in A_{525} and OMb related parameters A_O and KS_O . The more total pigment content and the more pigment in OMb form, the redder the meat. The same has been found in other works like Li et al. (2012) who also reported that a decrease in a^* was accompanied by an increase in MMb. This trend is also seen in the A_M parameter and similarly in the A_D parameter. The corresponding KS parameters (KS_M and KS_D) show the opposite trend in the PLS analysis. Nonetheless, since the PLS coefficients of all these parameters are very small, the importance of this difference does not seem to be far reaching. However, some authors have found that in pork meat it is the total pigment content and the proportion of MMb the main factors responsible of the variation of a^* and that the influence of OMb in that case is very small (Lindahl et al. 2001).

CIELAB b^* represents the yellow ($b^* > 0$) blue ($b^* < 0$) axis and some authors affirm that it is better than a^* to interpret meat color because of the marked redness of this product (Mancini and Hunt 2005). According to regression and PLS results OMb content is the most significant factor in describing differences in b^* among the samples. Within Krzywicki parameters, coordinate b^* has the highest correlations with A_O ($r = 0.805$, $p < 0.001$). Similarly within the KS set, b^* has its maximum correlation with KS_O ($r = 0.668$, $p < 0.001$). For each parameter set, larger PLS coefficients also correspond to A_O and KS_O (Fig. 2). On the other hand, b^* is not significantly correlated with A_{525} . This variable has the smallest PLS parameter for both Krzywicki and KS. Finally PLS coefficients associated with DMb and MMb pigments are negative and their absolute values are not small. The positive dependence of b^* on OMb content and the negative dependence of b^* on DMb and MMb content has been already reported in other works (Karamucki et al. 2013; Lindahl et al. 2001) although in one of them MMb was found to have a negligible influence (Lindahl et al. 2001). Although OMb seems to bear the fundamental role, the overall fit of b^* values requires the contribution of all pigments. Compared to a^* , a lower performance of PLS models is obtained but again the KS set better explains the observed variance (78.5%) than the Krzywicki set (66.2%).

Chroma C^* and hue h_{ab}

Polar coordinates C^* and h_{ab} are better correlated with perceptual color attributes than a^* and b^* (Arana 2012). CIELAB C^* is directly related with the psychophysical perception vividness or dullness of a color. For this color coordinate, and concerning the KS set, PLS results (Fig. 3) are similar to those obtained in case of a^* , both respect to the explained variance (96.1%) and to the relative importance of each predictor in the model. According to the PLS coefficients, C^* values are basically determined by the OMb content through the KS_O parameter. This is in agreement with the definition chroma as $C^* = (a^{*2} + b^{*2})^{1/2}$ and the fact that KS_O was also the main factor in the PLS models for a^* and b^* and in both cases with positive correlation coefficients. On the other hand the contribution of A_{525} in C^* is vanishingly small and the correlation between them is weak ($r = 0.323$, $p < 0.05$). The influence of the DMb and MMb parameters is also very small and C^* is mainly explained by the OMb related parameter KS_O . Increasing the proportion of OMb in meat increases the vividness of its red color, positively affecting consumer's preference, as it has been reported in a study of the influence of different types of packaging and breeds in beef color (Insausti et al. 1999). These authors found that visual scores of a trained sensory panel were

positively correlated with a^* and C^* and negatively correlated with the MMb content, a proportion that they obtained from the KS parameter.

With respect to the Krzywicki set, the case of C^* is also analogous to the case of a^* . The PLS model performance is similar and 59.3% of the variance in C^* is explained by the model. A_{525} and A_O have larger coefficients but the contribution of A_D and A_M is not much smaller. Although the relative values of each PLS coefficient resemble the situation found in a^* , the influence of A_{525} is smaller in C^* . Lindahl et al. (2001) found that these parameters explained 80% of the observed variance in case of pork meat. They also reported a positive correlation between C^* and MMb, *i.e.* more vivid color in more oxidized meat, which is the opposite of what it has been found in our beef samples.

The perceptual color attribute related to the hue angle h_{ab} is the color name itself, red in this case. For this coordinate, the overall performance of Krzywicki and KS sets is similar and the PLS models (Fig. 4) attain 63.4 and 63.5% of explained variance respectively. This is similar but slightly worse than the case of b^* . All parameters have comparable coefficients in the model. Parameters related with OMb content A_O and KS_O are the only ones positively correlated with hue, but they are not as relevant as they were in case of b^* . Although no parameter stands out from the others, the general behavior of hue respect to pigment content can be deduced from the sign of their coefficients. Greater total pigment content and greater oxygenation provides more reddish hues. On the contrary, color turns to less reddish hues as MMb and DMb proportions increase, as expected.

In general hue angle h_{ab} is considered a color coordinate strongly related with the proportion of MMb (AMSA 2012; Luciano et al. 2011). In a study of color stability in *Longissimus dorsi* from lambs under controlled diet, Luciano et al. (2011) used the Krzywicki parameter A_M and the KS parameter KS_M to assess the proportion of MMb. These authors found a high correlation between both parameters ($r = 0.991$; $p < 0.005$) very similar to the correlation found in this work ($r = 0.969$; $p < 0.001$). They also reported a strong correlation between hue and MMb content ($r = 0.931$; $p < 0.0005$). This is not the case in our work ($r = -0.296$; $p < 0.05$ between h_{ab} and A_M and -0.252 ; not significant at $p < 0.05$ level, between h_{ab} and KS_M) that better agrees with the lack of such a relationship reported in pork loin (Karamucki et al. 2013).

Conclusion

Success in explaining color coordinate values from pigment parameters depend both on the chosen color coordinate and on the parameter set. CIELAB L^* can be fully

explained by the reflex attenuation at 525 nm alone, a result that agrees with the idea that both L^* and A_{525} mainly depend on the total pigment content. For the other color coordinates the KS set performs better than the Krzywicki set. This is particularly true in case of a^* and C^* , where the KS set clearly outperforms Krzywicki set. For these two coordinates the KS_O parameter, the measure of OMb content, is the main factor explaining the color coordinate values. On the other hand b^* and h_{ab} values are not so well explained and all parameters enter the model with similar coefficients. KS set still gives clearly better results for b^* than Krzywicki set. The h_{ab} was similar for both sets.

According to the values at 1, 4 and 7 days of exposure to air, sample evolution produces only subtle changes in color coordinate values. These changes are more noticeable between days 1 and 4 and mainly in L^* , a^* and C^* , while b^* and h_{ab} remain almost unchanged. Respect to pigment forms the MMb parameters A_M and KS_M are the only ones that exhibit a clear evolution, giving statistically different values at the three measurement days. Interestingly, A_M and KS_M are not particularly strongly correlated with any color coordinate, possibly with the exception of KS_M and C^* . According to this, the deterioration of meat color is not related with a change of hue but with a loss of color vividness. In any case the classification of samples into measurement days, *i.e.* time of exposure to air, is better achieved by pigment related parameters than by CIELAB color coordinate values. In this classification either parameter set, KS or Krzywicki, shows equivalent performance.

The results also show that although color coordinates and pigment parameters are related, they are not equivalent. The information they provide is complementary and not just redundant. Color parameters better inform about the product's appearance while pigment parameters are better related with the time evolution of the sample. Since all these parameters can be easily obtained from spectrophotometric measurements it is advisable to provide all of them for completeness. If only colorimetric data is available, the variable better suited to reflect how a particular treatment affects the color or the condition of a product is the chroma C^* .

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