ACTA SCIENTIARUM POLONORUM
Acta Sci. Pol. Technol. Aliment. 18(4) 2019, 399–411
www.food.actapol.net pISSN 1644-0730 eISSN 1898-9594 http://dx.doi.org/10.17306/J.AFS.2019.0712

ORIGINAL PAPER

Received: 6.09.2019
Accepted: 2.10.2019

COMPARISON OF 2° AND 10° STANDARD OBSERVERS USED WITH C AND D65 ILLUMINANTS IN MEASUREMENTS OF COLOUR IN RAW MINCED PORK LOIN

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ABSTRACT

Background. Colour measurement plays a key role in assessing the quality of pork meat. However, relatively little research has been conducted on the standard observer, one of the several factors influencing colour parameters. Therefore, in this study we examine the usefulness of 2° and 10° standard observers in combination with C and D65 illuminants for pork colour measurement, based on CIELAB and CIELCh systems.

Materials and methods. The study involved 180 samples of the *longissimus* muscle collected from 180 carcasses from pigs slaughtered on an industrial line, with the average carcass weighing 91.3 ±5.7 kg. Total water, total protein, total fat, and pH_{48} of the meat were determined. Measurements of colour (including colour changes) of the raw pork meat were carried out using CIELAB and CIELCh scales, and the following illuminant/observer combinations: C/2°, C/10°, D65/2° and D65/10°. Chromatic absorbance at 525 nm (A_{525p}) and the relative content of Mb, MbO_2 and MetMb were calculated according to the method proposed by Krzywicki (1979).

Results. Pork colour parameters (L^*, a^*, b^*, C* and $h°$) and changes in these parameters (ΔL^*, Δa^*, Δb^*, ΔC^*, $\Delta h°$) during illumination were weak, and often statistically insignificant, correlating with total water, total protein and fat levels. Higher and statistically significant correlation coefficients were found for pH_{48}. The 2° standard observer provided higher correlation coefficients than the 10° observer between pH_{48} and hue ($h°$), pH_{48} and changes in redness (Δa^*), and pH_{48} and changes in hue ($\Delta h°$).

Conclusion. The use of a 2° standard observer compared to a 10° observer for pork meat colour measurements increased the effect of the relative amount of myoglobin chemical forms on hue ($h°$), which increases the correlation coefficients between hue ($h°$) and pH_{48}. In addition, the effect is greater with a higher proportion of MetMb and a lower proportion of MbO_2. When the 2° standard observer is used, higher correlation coefficients between pH_{48} and redness (Δa^*) and between pH_{48} and hue ($\Delta h°$) are found in the colour change measurements. These results indicate that, in assessing the quality of raw pork meat, the 2° standard observer may be more useful for colour measurement, especially with regard to colour stability.

Keywords: colour, CIELAB, CIELCh, illuminant, standard observer, myoglobin, pork quality

INTRODUCTION

Colour measurement plays an important role in assessing the quality of pork. Colour parameters depend on the type of spectrophotometer and its aperture, the illuminant used (Brewer et al., 2001; Yancey and Kropf, 2008), and the standard observer, i.e. the viewing angle, which accounts for the visual field of the

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average human eye when detecting colour (Hunter…, 2008a; 2008b). However, there has been relatively little research carried out on the influence of the viewing angle on the results of colour measurements (Garcia-Esteban et al., 2003; Tapp et al., 2011). Both the 2° standard observer and the 10° standard observer are commonly used in meat colour measurements. The 2° observer is often used in combination with the C illuminant (mean daylight), while the 10° observer is used with the D65 illuminant (daylight).

HunterLab suggests using the 10° observer (developed in 1964) over the 2° observer (1931; Hunter…, 2008b). Similarly, Honikel (1998) recommends the use of the D65/10° illuminant/observer system for meat colour measurements. The D65 illuminant is very similar to the C illuminant in terms of the share of individual wavelengths of light. However, the choice of the illuminant/observer system (C/2° or D65/10°) to measure the colour of pork is important because of the related differences in correlation coefficients between colour parameters and quality characteristics such as pH_{48} and water holding capacity (WHC) (Karamucki et al., 2006). It is not yet known to what extent these differences are caused by the influence of the illuminant used (C or D65) or the choice of the standard observer (2° or 10°).

Therefore, the aim of this study was to examine the usefulness of the standard 2° and 10° observers in pork colour measurement in combination with C (C/2°, C/10°) and D65 (D65/2° and D65/10°) illuminants, using CIELAB and CIELCh systems.

MATERIALS AND METHODS

Materials

The study material consisted of 180 *longissimus lumborum* muscle samples taken from 180 pork carcasses with an average weight of 91.3 ±5.7 kg (of which there was an equal proportion of S, E, and U conformation classes), obtained from 180, six-month old porkers that had been slaughtered on an industrial processing line (60 porkers which were crosses of Deutsche Landschwein and Deutsche Edelschwein sows and Pietrain boars, 60 porkers which were crosses of Danbred sows and PIC boars, and 60 porkers which were crosses of Polish Large White and Polish Landrace sows and Duroc and Pietrain boars). After a two-step cooling of the half-carcasses (cooled for 60 min at –20°C and cold stored for 24 hours at 4°C), ~1 kg samples of meat (meat with bone) were taken during cutting from the segment between the 1st and 4th lumbar vertebrae on the right half-carcass. The samples were packed in foil and transported in a thermos to the laboratory where they were stored at 4°C until the following day.

Methods

Physicochemical assessment of the meat. At about 48 hours after slaughter, each meat sample was separated from the bone, the external fat and perimysium were removed, and the meat was ground twice in a mincer using a 4mm mesh, to be used for further determinations, namely: water content, total protein, total fat, pH_{48}, and colour measurements.

Proximate analysis. The following chemical analyses were performed according to AOAC (2003): moisture content by oven drying of 2 g test samples at 102°C to a constant weight (950.46B, see p. 39.1.02); crude protein content by the classical macro-Kjeldahl method (981.10 see p. 39.1.19); and crude fat content by petroleum ether extraction using a Soxlet apparatus (960.39 (a), see 39.1.05).

pH measurement. Meat pH measurements were carried out after 1 hour of extraction in distilled water with a 1:1 meat to water ratio. A CyberScan 10 pH-meter equipped with a glass composite electrode ERH-12-6 (HYDROMET S.C.) was used for measurement. The electrode was immersed in the extract. Calibration was performed with buffers at pH = 4.0 and pH = 7.0.

Colour measurements. Meat colour was measured using a HunterLab MiniScan XE Plus 45/0 apparatus with a measuring port diameter of 31.8 mm, adapted for measuring the colour of minced meat. After mincing, the meat was placed in measuring vessels and stored for 20 minutes in a refrigerator at 4°C to enable myoglobin oxygenation on the surface layer of the meat (Krzywicki, 1979). Standardization of the apparatus was carried out with reference to standard black glass and a white colour tile with the following coordinates: $X = 78.5$, $Y = 83.3$ and $Z = 87.8$ (for D65 illuminant and 10° observer). For each meat sample, colour parameters were measured using CIELAB

and CIELCh scales (CIE, 1976; 1978) for illuminant/observer combinations: C/2°, C/10°, D65/2° and D65/10° – recommended for meat colour measurements (Honikel, 1998), as well as reflectance in the range 400 to 700 nm at 10 nm intervals. Next, the relative content of MbO_2, MetMb, and Mb in the surface layer of the meat and chromatic absorbance at 525 nm (A_{525p}) were determined according to Krzywicki (1979), using 700 nm, the highest wavelength of the instrument, instead of 730 nm (AMSA, 2012). Reflectance at 473, 525 and 572 nm wavelengths (required to determine the relative amount of chemical forms of myoglobin) was calculated using linear interpolation. Reflectance was converted into absorbance according to the formula: $A = 2 - \log 10R$, where A is absorbance and R is reflectance. Duplicate standards were used, which made it possible to determine reflectance and all colour parameters for each illuminant/observer system in a single measurement. Changes in colour were induced according to the Kortz (1966) method, by 4-hour illumination of samples with a fluorescent tube light at an intensity of 1250 lux in a closed container in an atmosphere at room temperature (22–24°C) saturated with water vapour. After illumination, colour and reflectance measurements of each sample were carried out again. Based on the results of the measurements obtained before and after illumination, differences in colour parameters (ΔL^*, Δa^*, Δb^*, ΔC^*, $\Delta h°$), chromatic absorption at 525nm (ΔA_{525p}) and the relative amounts of chemical forms of myoglobin (ΔMbO_2, ΔMetMb, ΔMb) were calculated.

Statistical analysis. Statistical analyses were carried out using STATISTICA v 12 software. The mean and standard deviations were calculated, as well as simple correlation coefficients (Pearson's r) and coefficients of determination (R^2), whose significance was estimated at probability levels of $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$.

RESULTS AND DISCUSSION

Table 1 presents means and standard deviations of water, total protein, total fat, and pH_{48} of the meat samples, and the following parameters measured before and after illumination: chromatic absorbance at 525 nm (A_{525p}), relative oxymyoglobin (MbO_2), metmyoglobin (MetMb), deoxymyoglobin (Mb), colour parameters (L^*, a^*, b^*, C^*, $h°$) and changes induced by illumination: ΔA_{525p}, ΔMbO_2, ΔMetMb, ΔMb, ΔL^*, Δa^*, Δb^*, ΔC^* and $\Delta h°$.

The mean total water content in the samples was 74.76%, total protein was 22.49%, total fat 1.72%, and mean pH_{48} hours after slaughter was 5.57. Chromatic absorbance at 525 nm (A_{525p}), directly proportional to the amount of pigments reached by light during measurement, was 0.347. Prior to illumination, oxymyoglobin (MbO_2) predominated in the surface layer of the meat – 49.1% (0.491). The amount of deoxymyoglobin (Mb) was 35.0% (0.350) and metmyoglobin (MetMb) was 15.9% (0.159).

After illumination, the mean A_{525p} (0.344) almost did not change. The relative amount of MbO_2 decreased during illumination by 16.7 percentage points (32.4% versus 49.1%), accompanied by an increase in MetMb (by 11.0 percentage points) and Mb (by 5.7 percentage points). As a result, after illumination, Mb dominated the surface layer of the meat samples (40.7%), followed by MbO_2 (32.4%) and MetMb (26.9%).

The results presented in Table 1 show that the colour parameters were clearly influenced by the choice of standard observer (2° or 10°), with the influence of the illuminant (C, D65) being low. The choice of standard observer (2° or 10°), both before and after illumination, influenced three chromatic parameters: redness (a^*), yellowness (b^*) and hue ($h°$). The use of the 2° standard observer, regardless of the illuminant used, resulted in an elevated a^*, and lower b^* and $h°$. After illumination, a^*, b^* and C^* decreased and $h°$ increased. The levels of a^* increased and b^* and $h°$ decreased in the following descending order of illuminant/observer combinations: C/10°, D65/10°, C/2° and D65/2°. The ΔL^*, Δa^*, ΔC^* and $\Delta h°$ were greater and the Δb^* smaller when the 2° standard observer was used, with the largest change for hue ($\Delta h°$) and the least for lightness (ΔL^*) – Table 1.

Table 2 presents simple correlation coefficients (r) between the percentage content of total water, total protein, total fat and pH_{48}, and the colour parameters measured before and after illumination of the samples, as well as correlations between these changes measured with each of the aforementioned illuminant/observer combinations.

Table 1. Means and standard deviations of feature and parameters of colour (n = 180)

Feature	Mean	SD	Parameters of colour	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Moisture content, %	74.76	0.93		C/2°		C/10°		D65/2°		D65/10°	
Total protein, %	22.49	0.63	L^* – lightness	54.92	2.66	54.95	2.63	54.89	2.66	54.93	2.64
Fat, %	1.72	0.78	a^* – redness	10.62	1.22	7.07	1.14	11.50	1.24	7.85	1.15
pH_{48}	5.57	0.15	b^* – yellowness	14.44	1.03	16.34	0.98	13.80	1.03	15.81	0.98
A_{525p}	0.347	0.029	C^* – chroma	17.96	1.18	17.83	1.11	17.99	1.00	17.68	1.14
MbO_2	0.491	0.074	$h°$ – hue angle	53.71	3.46	66.64	3.24	50.25	3.38	63.66	3.22
MetMb	0.159	0.032	L^* – after illumination	55.07	2.80	55.04	2.75	55.03	2.80	55.02	2.75
Mb	0.350	0.094	a^* – after illumination	8.93	1.06	5.99	0.86	9.76	1.06	6.72	0.86
A_{525p} – after illumination	0.344	0.028	b^* – after illumination	13.45	0.98	15.13	0.89	12.85	0.99	14.65	0.90
MbO_2 – after illumination	0.324	0.058	C^* – after illumination	16.19	0.83	16.30	1.11	16.18	1.00	16.14	1.14
MetMb – after illumination	0.269	0.070	$h°$ – after illumination	56.40	4.17	68.38	3.16	52.79	4.15	65.34	3.19
Mb – after illumination	0.407	0.109	ΔL^*	0.15	0.63	0.09	0.63	0.14	0.63	0.09	0.63
ΔA_{525p}	–0.003	0.008	Δa^*	–1.69	0.98	–1.08	0.76	–1.74	1.00	–1.13	0.77
ΔMbO_2	–0.167	0.053	Δb^*	–0.99	0.50	–1.21	0.51	–0.95	0.50	–1.16	0.50
ΔMetMb	0.110	0.045	ΔC^*	–1.77	0.85	–1.53	0.73	–1.81	0.88	–1.54	0.76
ΔMb	0.057	0.060	$\Delta h°$	2.69	2.09	1.74	1.63	2.54	2.06	1.68	1.58

ΔA_{525p}, ΔMbO_2, ΔMetMb, ΔMb, ΔL^*, Δa^*, Δb^*, ΔC^*, and Δh were calculated by subtracting the value of a given parameter before illumination from the value after illumination.

Table 2. Simple correlation coefficients (r) for the basic chemical components and pH_{48} of the meat

Feature	Illuminant/ observer	L^*	a^*	b^*	C^*	$h°$
1	2	3	4	5	6	7
			Before illumination			
Moisture content, %	C/2°	–0.103	–0.153*	–0.239**	–0.258***	0.003
	C/10°	–0.096	–0.245***	–0.192*	–0.251***	0.177*
	D65/2°	–0.102	–0.158*	–0.245**	–0.258***	0.011
	D65/10°	–0.096	–0.245***	–0.198**	–0.260***	0.163*

Table 2 – cont.

1	2	3	4	5	6	7
Crude protein, %	C/2°	0.150*	–0.030	0.240**	0.149*	0.164*
	C/10°	0.145	0.055	0.209**	0.190*	0.028
	D65/2°	0.149*	–0.025	0.245***	0.142	0.011
	D65/10°	0.145	0.055	0.215**	0.189*	–0.163*
Intramuscular fat, %	C/2°	–0.009	0.201**	0.075	0.172*	–0.141
	C/10°	–0.013	0.236**	0.046	0.130	–0.228**
	D65/2°	–0.010	0.202**	0.078	0.178*	–0.134
	D65/10°	–0.013	0.235**	0.049	0.140	–0.224**
pH_{48}	C/2°	–0.719***	0.082	–0.740***	–0.467***	–0.502***
	C/10°	–0.714***	–0.046	–0.739***	–0.615***	–0.257***
	D65/2°	–0.718***	0.061	–0.740***	–0.438***	–0.523***
	D65/10°	–0.714***	–0.056	–0.741***	–0.596***	–0.286***
			After illumination			
Moisture content, %	C/2°	–0.089	0.068	–0.150*	–0.101	–0.107
	C/10°	–0.079	–0.166*	–0.044	–0.099	0.135
	D65/2°	–0.088	0.066	–0.165*	–0.106	–0.124
	D65/10°	–0.078	–0.160*	–0.064	–0.123	0.117
Crude protein, %	C/2°	0.145	–0.231**	0.229*	0.072	0.270***
	C/10°	0.137	–0.057	0.164*	0.135	0.107
	D65/2°	0.144	–0.229**	0.239**	0.059	0.278***
	D65/10°	0.136	–0.060	0.178*	0.141	0.123
Intramuscular fat, %	C/2°	–0.025	0.123	–0.022	0.059	–0.135
	C/10°	–0.030	0.239**	–0.085	0.002	–0.245***
	D65/2°	–0.026	0.124	–0.014	0.076	–0.105
	D65/10°	–0.030	0.236**	–0.075	0.023	–0.245***
pH_{48}	C/2°	–0.745***	0.478***	–0.736***	–0.349***	–0.691***
	C/10°	–0.739***	0.217**	–0.699***	–0.593***	–0.454***
	D65/2°	–0.744***	0.460***	–0.740***	–0.349***	–0.700***
	D65/10°	–0.738***	0.209**	–0.711***	–0.581***	–0.483***
		ΔL^*	Δa^*	Δb^*	ΔC^*	$\Delta h°$
Moisture content, %	C/2°	–0.037	–0.265***	–0.198**	–0.261***	–0.230**
	C/10°	–0.056	–0.180*	–0.290***	–0.266***	–0.090
	D65/2°	–0.039	–0.266***	–0.178*	–0.256***	–0.231**
	D65/10°	–0.062	–0.185*	–0.272***	–0.252***	–0.095

Table 2 – cont.

1	2	3	4	5	6	7
Crude protein, %	C/2°	–0.011	0.213**	0.042	0.138	0.267***
	C/10°	0.006	0.147*	0.114	0.131	0.153*
	D65/2°	–0.009	0.212**	0.030	0.139	0.271***
	D65/10°	0.009	0.150*	0.100	0.125	0.163*
Intramuscular fat, %	C/2°	0.071	0.117	0.200**	0.182*	0.025
	C/10°	0.077	0.084	0.236**	0.195**	–0.022
	D65/2°	0.071	0.119	0.190*	0.175*	0.022
	D65/10°	0.080	0.087	0.229**	0.185*	–0.026
pH_{48}	C/2°	0.272***	–0.417***	–0.076	–0.266***	–0.547***
	C/10°	0.245***	–0.315***	–0.194**	–0.242**	–0.370***
	D65/2°	0.270***	–0.414***	–0.055	–0.272***	–0.553***
	D65/10°	0.242**	–0.317***	–0.174*	–0.236**	–0.391***

*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$.

The correlation coefficients before and after illumination only proved to be statistically significant in some cases and were low, indicating that the influence of these meat constituents on colour parameters was small. Higher and significant correlation coefficients, both before and after illumination, were found between pH_{48} and lightness (L^*), yellowness (b^*), chroma (C^*) and hue ($h°$), while for lightness (L^*) and yellowness (b^*), correlation coefficients were high ($r > 0.7$). The four parameters mentioned above significantly decreased with an increase in pH_{48}. No significant correlation coefficients between pH_{48} and redness (a^*) were observed before illumination, whereas after illumination, redness (a^*) significantly increased with an increase in pH_{48}. The use of the 2° standard observer resulted in higher correlation coefficients before and after illumination for hue ($h°$) and after illumination for redness (a^*), yellowness (b^*) and hue ($h°$) – Table 2.

Changes in redness (Δa^*), yellowness (Δb^*), chroma (ΔC^*) and hue ($\Delta h°$) decreased significantly with an increase in the percentage of water content in the meat; in the case of $\Delta h°$ this concerned only the differences measured with the 2° standard observer. The differences in redness (Δa^*) and hue ($\Delta h°$) increased significantly with an increase in the percentage of total protein in the meat, while the differences in yellowness (Δb^*) and chroma (ΔC^*) increased significantly with an increase in total fat content. Correlation coefficients between the percentage content of total water, total protein and total fat in the meat, and changes (Δ) in colour parameters, were low and in many cases statistically insignificant. Higher correlation coefficients were observed for pH_{48}. Changes (Δ) in the colour parameters increased significantly with a decrease in pH_{48}, while the correlation coefficients between ΔL^*, Δa^*, ΔC^* and $\Delta h°$ and pH_{48} were higher when the 2° standard observer was used, with the highest r for hue ($\Delta h°$) and redness (Δa^*). However, significant correlation coefficients between Δb^* and pH_{48} were observed only in the case of the 10° standard observer and were the lowest (Table 2).

Table 3 presents determination coefficients (R^2) of pigment absorbance (A_{525p}) and the relative amount of chemical forms of myoglobin on particular colour parameters for four illuminant/observer combinations.

The effect of the amount of pigments (A_{525p}), the effect of the relative amount of myoglobin chemical forms (MbO_2 + MetMb + Mb) and the total effect of the amount of pigments and myoglobin chemical

Table 3. The coefficients of determination R^2 (corrected) for colour parameters (n = 180)

Feature	Illuminant/ observer	L^*	a^*	b^*	C^*	$h°$
				R^2		
1	2	3	4	5	6	7
Before illumination						
A_{525p}	C/2°	0.3592***	0.8054***	0.0000	0.2565***	0.7334***
	C/10°	0.3680***	0.7516***	0.0015	0.0702***	0.8818***
	D65/2°	0.3611***	0.7919***	0.0000	0.2944***	0.7084***
	D65/10°	0.3686***	0.7404***	0.0008	0.0939***	0.8769***
MbO_2 + MetMb + Mb	C/2°	0.3902***	0.0722***	0.8069***	0.6110***	0.0960***
	C/10°	0.3800***	0.2065***	0.7461***	0.7488***	0.0549***
	D65/2°	0.3892***	0.0835***	0.8190***	0.5816***	0.1162***
	D65/10°	0.3796***	0.2126***	0.7610***	0.7430***	0.0500***
A_{525p} + MbO_2 + MetMb + Mb	C/2°	0.7997***	0.9058***	0.8151***	0.8433***	0.9000***
	C/10°	0.7955***	0.9163***	0.7584***	0.8053***	0.9087***
	D65/2°	0.8002***	0.9013***	0.8269***	0.8515***	0.8981***
	D65/10°	0.7956***	0.9120***	0.7728***	0.8196***	0.9037***
After illumination						
A_{525p}	C/2°	0.3822***	0.7030***	0.0151	0.2012***	0.4968***
	C/10°	0.3954***	0.8838***	0.0346**	0.0164*	0.8361***
	D65/2°	0.3844***	0.7085***	0.0141	0.2583***	0.4713***
	D65/10°	0.3974***	0.8780***	0.0337**	0.0337**	0.8202***
MbO_2 + MetMb + Mb	C/2°	0.4330***	0.1264***	0.7738***	0.4783***	0.3873***
	C/10°	0.4160***	0.0037	0.6637***	0.6596***	0.0608**
	D65/2°	0.4311***	0.1137***	0.7936***	0.4332***	0.4160***
	D65/10°	0.4148***	0.0027	0.6886***	0.6647***	0.0756***
A_{525p} + MbO_2 + MetMb + Mb	C/2°	0.7883***	0.9508***	0.7773***	0.8086***	0.9121***
	C/10°	0.7810***	0.9349***	0.6698***	0.7291***	0.8648***
	D65/2°	0.7881***	0.9506***	0.7975***	0.8331***	0.9135***
	D65/10°	0.7813***	0.9369***	0.6956***	0.7598***	0.8649***
		ΔL^*	Δa^*	Δb^*	ΔC^*	$\Delta h°$
ΔA_{525p}	C/2°	0.3050***	0.4901***	0.7387***	0.2174***	0.2110***
	C/10°	0.3144***	0.6379***	0.7144***	0.1953***	0.5397***
	D65/2°	0.3048***	0.4997***	0.7297***	0.2201***	0.1802***
	D65/10°	0.3171***	0.6420***	0.7341***	0.2075***	0.5160***

Table 3 – cont.

1	2	3	4	5	6	7
ΔMbO_2 + ΔMetMb + ΔMb	C/2°	0.2275***	0.7261***	0.7187***	0.7575***	0.7147***
	C/10°	0.2119***	0.6582***	0.6988***	0.7532***	0.5518***
	D65/2°	0.2235***	0.7272***	0.7340***	0.7570***	0.7211***
	D65/10°	0.2085***	0.6617***	0.7053***	0.7536***	0.5404***
ΔA_{525p} + ΔMbO_2 + ΔMetMb + ΔMb	C/2°	0.3454***	0.8147***	0.8449***	0.9001***	0.7508***
	C/10°	0.3314***	0.7674***	0.8282***	0.8918***	0.6407***
	D65/2°	0.3420***	0.8186***	0.8544***	0.8976***	0.7532***
	D65/10°	0.3313***	0.7743***	0.8363***	0.8938***	0.6280***

*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$.

forms (A_{525p} + MbO_2 + MetMb + Mb) on the lightness (L*) were very similar for both standard observers (2° and 10°), both before and after illumination. Moreover, before illumination, no significant effect of pigment quantity (A_{525p}) on yellowness (b*) was observed. However, after illumination, this effect turned out to be significant in the case of the 10° observer, where it was small, at 3.46% (R^2 = 0.0346**) and 3.37% (R^2 = 0.0337**) for C/10° and D65/10° respectively. The variability of yellowness (b*) depended mainly on the effect of the relative amount of myoglobin forms, which is consistent with the results of previous studies (Karamucki et al., 2013; Lindahl et al., 2001). In the case of the 2° observer, this effect was greater both before (about 6% – C and about 5% – D65) and after illumination (about 11% – C and about 10% – D65).

Also, the variation of chroma (C*) depended more on the observer (2° or 10°) than the illuminant (C or D65). The effect of the amount of pigment (A_{525p}) on the chroma (C*) was greater in the case of the 2° observer both before and after illumination (by about 19% – C and 5% – D65, and after illumination about 18% – C and 22% – D65). The effect of the relative amount of myoglobin chemical forms on chroma (C*) was lower in the case of the 2° observer both before (by ca. 14% – C and 16% – D65) and after illumination (by ca. 18% – C and 23% – D65).

Changes in redness (a*) and hue (h°) of the colour depended mainly on the effect of the amount of pigments achieved by the light during the measurement (A_{525p}). In the case of redness (a*), this effect before illumination was about 5% higher when using the 2° observer than the 10° observer (R^2 = 0.8054*** for C/2° vs R^2 = 0.7516*** for C/10° and R^2 = 0.7919*** for D65/2° vs R^2 = 0.7404*** for D65/10°), and after illumination, greater by about 17% (C) and 18% (D65) with the standard 10° observer. In the case of hue (h°), the effect of pigment absorbance (A_{525p}) was greater when a 10° observer was used both before and after illumination, by approximately: 15% (C) and 17% (D65), and by 34% (C) and 35% (D65), respectively.

The effect of the relative amount of chemical forms of myoglobin on redness (a*) before illumination for the standard 2° observer was about 7% (C) and 8% (D65), and for the 10° observer about 21% (C and D65), while after illumination it turned out to be statistically significant only for the 2° observer by about 11% (C) and about 13% (D65). The effect of the relative amount of myoglobin chemical forms on the hue (h°) was greater in the case of the 2° observer both before and after illumination by about 4% – C and 7% – D65, as well as after illumination by about 33% – C and 34% – D65 (Table 3).

The total effect of chromatic absorbance at 525 nm and the relative amount of myoglobin chemical forms (A_{525p} + MbO_2 + MetMb + Mb) on the variability of the chromatic parameters of the pre-illumination colour was about 90–91% for parameters a* and h°, about 76–83% for b* and 81–85% for C*, with b* and C* more affected by the 2° observer and a* and h° slightly more affected by the 10° observer. After illumination,

the combined effect of chromatic absorbance at 525 nm and the relative amount of myoglobin chemical forms (A_{525p} + MbO_2 + MetMb + Mb) on the variability of parameters a^*, b^*, C^* and $h°$ was about: 93.5–95%, 67–80%, 73–83% and 86.5–91% respectively, and was higher when the 2° standard observer was used (Table 3).

The results obtained show that in the case of both standard 2° and 10° observers, the variation in hue ($h°$) was more dependent on the effect of the amount of pigments (A_{525p}) achieved by light than on the effect of myoglobin chemical forms. However, the effect of myoglobin forms on the hue ($h°$) was significantly greater in the case of the 2° observer, especially after illumination at a higher amount of MetMb (Table 3), which resulted in higher correlation coefficients (r) between the hue ($h°$) and pH_{48} for the C/2° and D65/2° combinations (Table 2).

The effect of differences in the amount of pigments (ΔA_{525p}) on Δa^* and $\Delta h°$ was greater when using the 10° standard observer, while the total effect of differences in the relative amount of chemical forms of myoglobin (ΔMbO_2 + ΔMetMb + ΔMb) on Δa^* and $\Delta h°$ was greater when using the 2° observer, with determination coefficients (R^2) being the highest for $\Delta h°$ (R^2 = 0.7147 and 0.7211 for C/2° and D65/2°, respectively), and the illuminant did not have a big influence on them. The total effect of differences in chromatic absorbance of pigments and in the relative amount of myoglobin chemical forms (ΔA_{525p} + ΔMbO_2 + ΔMetMb + ΔMb) on changes in all colour parameters (ΔL^*, Δa^*, Δb^*, ΔC^* and $\Delta h°$) was greater for the 2° standard observer, and mainly in hue ($\Delta h°$) and redness (Δa^*) – Table 3.

Low pigment content and high pH differentiation in pork *longissimus* muscle have a significant influence on its colour. The dynamics of pH decrease in the first hours post-mortem (pH_1), and final value of pH, significantly affect the formation of meat structure (Boler et al., 2010; Chmiel et al., 2014; Swatland, 2004), the intensity of oxidation, and the oxidation and reduction of myoglobin (Bekhit and Faustman, 2005). At a low pH, myoglobin is more susceptible to both oxidation and oxygenation (Zhu and Brewer, 1998), therefore, most MbO_2 is found in the surface layer of fresh muscles with a pH < 5.4. Increasing the amount of MbO_2 causes an increase in parameters a^*, b^* and C^* (Karamucki et al., 2013; Lindahl et al., 2001). However, MbO_2 is easily deoxidized to Mb and oxidized to MetMb, the increase of which causes undesirable changes in colour – a decrease in redness (a^*) and deterioration of hue ($h°$) (Luciano et al., 2011). Therefore, the colour of pork with a pH < 5.4 is less stable than that of normal meat with a pH of 5.4–5.7 and meat with a pH > 5.7, with the smallest changes in colour being observed in pork with a pH > 6.0 (Lindahl, 2005).

Changes in the proportions of myoglobin chemical forms mainly affect chromatic parameters. The highest redness (a^*), yellowness (b^*) and chroma (C^*) are characterized by oxymyoglobin (MbO_2), the lowest yellowness (b^*) deoxymyoglobin (Mb), and the lowest redness (a^*) metmyoglobin (MetMb) (Karamucki et al., 2013). Changes in pork colour are also caused by differences in the structure of the meat layer penetrated by light and oxygen, which affects the proportions of chemical forms of myoglobin and the amount of pigments reached by light during measurement (Krzywicki, 1979). At a high pH of muscle tissue, light penetrates deeper, but oxygen penetration is shallower. With a longer path of penetration, light absorption increases and more pigments (mainly their reduced forms) are reached by the light. In pork with a low pH, and a more open and less translucent structure, light penetration is shallower, and the amount of exposed pigment is generally lower, while the oxygen penetration is higher, and the intensity of pigment oxidation and oxygenation is higher.

The results of this study show the significance of the choice of standard observer (2° or 10°) in the measurement of raw pork colour using illuminants C and D65. Standard observers influence the chromatic parameters and show clear differences in their suitability to measure colour stability, especially when determining changes in redness (Δa^*) and hue ($\Delta h°$), which, according to Karamucki et al. (2011), are more useful in assessing the quality of pork in comparison with the total colour change (ΔE^*).

The variability of individual chromatic parameters depends on the amount of pigments reached by light in the measurement, and on the relative amounts of chemical forms of myoglobin (Hernández et al., 2016; Karamucki et al., 2013; Lindahl et al., 2001). Variability of redness (a^*) and hue ($h°$) depend mainly on the

effect of the amount of pigments, and the variability of chroma (C^*) is influenced to a greater extent by the content of chemical forms of myoglobin. However, the variability of yellowness (b^*) depends almost exclusively on the relative amounts of the chemical forms of myoglobin – Table 3.

Before illumination, with a relative share of 49.1% MbO_2 and 15.9% MetMb (Table 1), the total effect of the relative amount of myoglobin chemical forms (MbO_2 + MetMb + Mb) on the variation (R^2) of redness (a^*) was greater when using the 10° standard observer (Table 3), while after illumination, with a relative proportion of 32.4% for MbO_2 and 26.9% for MetMb (Table 1), it turned out to be higher and significant only for the 2° standard observer (Table 3). On the other hand, the total effect of the relative amount of myoglobin chemical forms on hue variation ($h°$) was greater both before and after illumination of the samples when a 2° standard observer was used, while the effect increased significantly after illumination, which was not observed in the case of the 10° standard observer (Table 3).

This was reflected in higher simple correlation coefficients (r) between hue ($h°$) and pH_{48} (Table 2), with pH_{48} having a significant effect on the oxidation and reduction of muscle pigments (Boler et al., 2010; Krzywicki, 1979; Lindahl, 2005). These coefficients turned out to be higher in the case of the 2° observer. This was similar to the correlation coefficients between pH_{48} and redness (a^*) after illumination of the samples, where the relative amount of MbO_2 decreased and the relative amount of MetMb increased.

The results presented in Table 3 also show that for a 2° standard observer, changes in redness (Δa^*) and hue ($\Delta h°$) depended more on changes in the relative quantity of myoglobin chemical forms (ΔMbO_2 + ΔMetMb + ΔMb) than on changes in the quantity of pigments reached by the light during the measurement (ΔA_{525p}). At the same time, changes that took place during the illumination consisted mainly of pH_{48}-dependent changes in the relative amount of myoglobin chemical forms. As a result, the simple correlation coefficients (r) between pH_{48} and Δa^* and $\Delta h°$ turned out to be higher when the 2° standard observer was used (Table 2).

These results show that, from the point of view of the relationship between pH_{48} and hue ($h°$) and between pH_{48} and Δa^* and $\Delta h°$, the 2° standard observer is more useful for colour measurement in the evaluation of pork meat quality.

Karamucki et al. (2006) compared the usefulness of illuminant/observer combinations C/2° and D65/10° for pork colour measurements, and found that the application of illuminant/observer system C/2° proved to be more suitable for $h°$, which is consistent with the results presented in this study. They also noted that, when illuminant/observer D65/10° was used to measure the colour of pork, changes in yellowness (b^*) had no significant effect on hue ($h°$), whereas in the case of C/2° they had a significant influence (r = 0.408).

The results presented in Table 4 show that, before illumination of the meat samples, the effect of the variation in b^* on $h°$ was very small for D65/10° and statistically insignificant for C/10°. However, after illumination, correlation coefficients between yellowness (b^*) and hue ($h°$) increased but were still significantly lower for the 10° observer (Table 4). Since the variation in colour yellowness (b^*) depends almost

Table. 4. Simple correlation coefficients (r) for the colour parameters (n = 180)

Feature	Illuminant/observer	a^*	b^*	C^*	$h°$
1	2	3	4	5	6
	Before illumination				
L^*	C/2°	–0.393***	0.722***	0.269***	0.776***
	C/10°	–0.276***	0.731***	0.484***	0.592***
	D65/2°	–0.370***	0.719***	0.227**	0.789***
	D65/10°	–0.263***	0.729***	0.219**	0.891***

Table 4 – cont.

1	2	3	4	5	6
*a**	C/2°	–	0.172*	0.722***	–0.820***
	C/10°	–	0.280***	0.625***	–0.913***
	D65/2°	–	0.203**	0.772***	–0.783***
	D65/10°	–	0.301***	0.672***	–0.891***
*b**	C/2°		–	0.805***	0.419***
	C/10°		–	0.924***	0.129
	D65/2°		–	0.778***	0.446***
	D65/10°		–	0.908***	0.160*
*C**	C/2°			–	–0.199**
	C/10°			–	–0.257***
	D65/2°			–	–0.212**
	D65/10°			–	–0.266***
			After illumination		
*L**	C/2°	–0.743***	0.756***	0.235**	0.897***
	C/10°	–0.550***	0.741***	0.515***	0.764***
	D65/2°	–0.726***	0.757***	0.171*	0.897***
	D65/10°	–0.785***	0.748***	0.483***	0.785***
*a**	C/2°	–	–0.363***	0.330***	–0.913***
	C/10°	–	–0.104	0.262***	–0.930***
	D65/2°	–	–0.343***	0.421***	–0.890***
	D65/10°	–	–0.081	0.334***	–0.909***
*b**	C/2°		–	0.756***	0.708***
	C/10°		–	0.932***	0.458***
	D65/2°		–	0.703***	0.730***
	D65/10°		–	0.912***	0.484***
*C**	C/2°			–	0.077
	C/10°			–	0.106
	D65/2°			–	0.031
	D65/10°			–	0.084

*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$.

exclusively on the effect of the relative amount of myoglobin chemical forms, these results indicate that the effect of the relative amount of myoglobin chemical forms on the hue ($h°$) is significantly greater with the 2° standard observer.

This is confirmed by the determination coefficients (R^2) and correlation coefficients (r) between pH_{48} and hue ($h°$), and between pH_{48} and redness (Δa^*) and pH_{48} ($\Delta h°$) (Table 2, Table 3), which were higher for the 2° observer, regardless of the illuminant used (C or D65). These results are also in line with the results of Table 1, which show that the application of the 2° observer emphasizes the differences in redness (Δa^*) and hue ($\Delta h°$) caused by changes in the relative amount of myoglobin chemical forms, especially an increase in the relative amount of MetMb and a decrease in the relative amount of MbO_2, i.e. the two myoglobin forms with the highest differences in redness (a^*) and the smallest differences in yellowness (b^*; Karamucki et al., 2013).

CONCLUSIONS

The use of a 2° standard observer compared to a 10° observer for pork meat colour measurements increased the effect of the relative amount of myoglobin chemical forms on hue ($h°$), which increases the correlation coefficients between hue ($h°$) and pH_{48}. In addition, the effect is greater with a higher proportion of MetMb and a lower proportion of MbO_2. When the 2° standard observer is used, higher correlation coefficients between pH_{48} and redness (Δa^*) and between pH_{48} and hue ($\Delta h°$) are found in the colour change measurements. These results indicate that, in assessing the quality of raw pork meat, the 2° standard observer may be more useful for colour measurement, especially with regard to colour stability.

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