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# COMPARISON OF D65/10° AND A/10° ILLUMINANT/OBSERVER SYSTEMS FOR COLOUR MEASUREMENT OF RAW PORK

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## ABSTRACT

**Background.** In pork colour measurements, the value of each parameter depends on the type of illuminant used. The spectra of D65 and A illuminants show great differences, with illuminant A having greater emission in the red part of the visible spectrum. Therefore, its application in the colour measurements of the pork *longissimus* muscle should result in larger changes in redness ($\Delta a^*$) and hue angle ($\Delta h°$) and higher correlation between $\Delta a^*$ and $\Delta h°$ and $pH_{48}$ and WHC. The purpose of this study was to compare the suitability of the illuminant/observer systems D65/10° and A/10° for colour measurements of pork *longissimus* muscle, using the CIELAB and CIELCh systems.

**Material and methods.** The study involved 168 samples of *longissimus lumborum* muscle taken from 168 carcasses (mean weight 90.2 ±6.0 kg) of pigs slaughtered on an industrial processing line. The moisture content, crude protein, intramuscular fat content, WHC, and $pH_{48}$ were determined. Colour measurements using CIELAB and CIELCh scales were carried out with both D65/10° and A/10° illuminant/observer systems and reflectance measurements. The chromatic absorbance value at 525 nm ($A_{525p}$) and the relative amounts of $MbO_2$, MetMb, and Mb were calculated according to methods proposed by Krzywicki (1979) and AMSA (2012). Meat samples were illuminated and differences in the values of colour parameters ($\Delta L^*$, $\Delta a^*$, $\Delta b^*$, $\Delta C^*$, $\Delta h°$), chromatic absorbance at 525 nm ($\Delta A_{525p}$), and in the relative amounts of chemical forms of myoglobin ($\Delta MbO_2$, ΔMetMb, ΔMb) were determined. In addition, hue difference ($\Delta H$) and total difference ($\Delta E$) were calculated.

**Results.** The values of correlation coefficients between moisture content (especially crude protein and intramuscular fat, and colour parameters) were low and often statistically insignificant. Higher and mostly significant values of correlation coefficients were found between colour parameters and WHC and $pH_{48}$. The A/10° system resulted in higher values of correlation coefficients than D65/10° between (I) WHC, $pH_{48}$ and (II) $h°$, $\Delta a^*$ and $\Delta h°$. At the same time, in the A/10° system the combined effect of the relative amounts of myoglobin chemical forms on the variation of $h°$ values and the combined effect of changes (Δ) in their amounts on the variation of $\Delta h°$ and $\Delta H$ and $\Delta E$ were greater than in D65/10°, with the greatest effect of these changes (Δ) in the amount of MetMb.

**Conclusion.** Replacement of the illuminant/observer D65/10° system with the A/10° system in colour measurements of raw pork *longissimus* muscle changed the proportion of pigments and the relative number of chemical forms of myoglobin in influencing the values of colour parameters, primarily $h°$. Therefore, using the A/10° system for colour measurements allows us to better capture the differences (Δ) in redness $\Delta a^*$ and especially in hue angle ($\Delta h°$), as well as hue difference ($\Delta H$) and total difference ($\Delta E$), with an increase in the relative amount of MetMb becoming the main determinant of these differences (Δ). At the same time, measurements using the A/10° system increased the correlation coefficients between WHC and $pH_{48}$ and changes in redness ($\Delta a^*$) and hue angle ($\Delta h°$). Therefore, the A/10° system compared to the D65/10° system may be more useful for measuring the colour stability of raw pork, especially the determination of $\Delta h°$ and $\Delta H$ and $\Delta E$.

**Keywords:** colour of meat, illuminant, pork quality, CIELAB, CIELCh

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## INTRODUCTION

Colour is one of the most important quality characteristics of meat – it is a good indicator of both its technological quality and degree of freshness. In the retail trade, it has an important influence on the consumer's decision to purchase meat (Brewer and McKeith, 1999; Mancini and Hunt, 2005). Colour also plays a key role in research on meat quality, along with pH and water holding capacity (WHC). In technical measurements, meat colour is represented by three components in each of the measuring scales, namely, in the CIELAB scale (CIE, 1976) lightness ($L^*$), redness ($a^*$), and yellowness ($b^*$), and in the CIELCh scale (CIE, 1978) lightness ($L^*$), chroma ($C^*$), and hue angle ($h°$). The different spectral characteristics of the illuminant used in the meat colour measurement produce different values of these parameters. The most commonly used illuminant for colour measurement is D65 (daylight) combined with a standard 10° observer, as recommended by Honikel (1998), and less frequently illuminant C (mean daylight), illuminant A (incandescent), and even less frequently, fluorescent illuminants (Tapp et al., 2011), several of which are used to illuminate meat in retail displays (Sáenz et al., 2004). The spectra of illuminant A has greater emission in the red part of the visible spectrum, producing increased values of $a^*$ and $C^*$ and lower hue angle ($h°$).

Measurements of meat colour also reflect its durability and consist in determining changes in parameter values during meat storage, where the colour is under constant change due to oxygenation and deoxygenation of muscle pigments. Meat colour is also affected by the texture, which often varies greatly (Chmiel et al., 2014), and determines many meat quality characteristics (Boler et al., 2010; Hughes et al., 2014; Purslow et al., 2020; Swatland, 2004), such as the thickness of the surface layer that is penetrated by light and oxygen, and thus the amount of light absorbed by the tissue, the pigments exposed to light, and the chemical forms of myoglobin. When measuring the colour stability of pork *longissimus* muscle, changes in redness ($\Delta a^*$) and hue angle ($\Delta h°$) are most significantly correlated with colour, wateriness and firmness, as assessed in the sensory evaluation, and $pH_u$ and WHC (Karamucki et al., 2011). The changes were more intense in pork with a low pH, roughened texture, and greater water holding capacity (pale, soft, exudative meat – PSE) than in normal or dark, firm and dry meat (DFD). This shows that colour stability is related to the quality of the pork meat.

There are few publications in the literature on meat colour measurements using illuminant A (Brewer et al., 2001; Garcia-Esteban et al., 2003). According to AMSA (2012), illuminant A is recommended for samples where detection of redness differences between treatments is the priority and where small differences in redness may not be as easily detected with illuminant D65. Therefore, the use of illuminant A to measure colour stability in pork *longissimus* muscle should yield greater differences in redness ($\Delta a^*$) and hue angle ($\Delta h°$) than with illuminant D65 as well as higher correlation coefficients between $\Delta a^*$ and $\Delta h°$ and $pH_{48}$ and WHC. At the same time, the use of illuminant A should make it possible to compare the effect of the number of pigments and the relative number of chemical forms of myoglobin on the variation of colour parameter values with both illuminants.

The purpose of this study was to compare the differences between illuminant/observer D65/10° and A/10° systems for colour measurements of raw pork *longissimus* muscle using both the CIELAB and CIELCh systems.

## MATERIALS AND METHODS

### Materials

The material for the study consisted of *longissimus lumborum* muscle samples taken from 168 pork carcasses of six-month old porkers (average weight of 90.2 ±6.0 kg) from an industrial processing line. Four crossbreeds were selected (42 individuals each); Deutsche Landschwein × Deutsche Edelschwein sows × Pietrain boars, Polish Large White × Polish Landrace sows × Duroc × Pietrain boars, Landrace × Yorkshire sows × Duroc boars and Danbred sows × PIC boars. For each crossbreed, 14 carcasses from each class S, E, and U were selected. The carcasses were first chilled in two stages (cooled for 60 min at –20°C and stored for 24 hours at 4°C), after which meat samples (~1 kg meat with bone) were dissected from the right half-carcass between the 1st and 4th lumbar vertebrae for examination.

## Methods

**Chemical and physicochemical assessment of the meat.** Physicochemical assessment of the meat was carried out about 48 h after slaughter. Each meat sample was separated from the bone, and the external fat and perymisium were removed. Next, the meat was ground twice in a mincer using a 4 mm mesh for further determination of moisture content, crude protein and crude fat content, colour measurements, WHC, and $pH_{48}$. All the determinations were performed on freshly minced meat.

**Proximate measurements.** The following chemical constituents were determined on thawed samples of the ground meat according to official AOAC (2003) methods of analysis, namely: moisture content by oven drying a *ca*. 2 g sample at 102°C to a constant weight (950.46 B, see p. 39.1.02); crude protein content by the classical macro-Kjeldahl method (981.10, see p. 39.1.19); and lipid (crude) content by petroleum ether extraction using a Soxlet apparatus (960.39 (a), see p. 39.1.05).

**WHC.** Water holding capacity (WHC) of the meat was determined as the percentage of bound water in relation to the total water content in the meat, according to Grau and Hamm (1953) as modified by Pohja and Niinivaara (1957). A sample of meat weighing 0.3 g was placed on Whatman 1 blotting paper between two glass plates and subjected to a load of 2 kg for 5 minutes. Then, the surface areas of infiltration and the meat sample were drawn onto the glass plates. After drying the filter paper, both surfaces were planimetered ($cm^2$) and then the difference between surface areas was divided by the sample weight, thus calculating the percentage of free water in the meat. The resulting value was divided by the percentage of total water content in the meat, and after deducing this value from 100 the percentage of water holding capacity of the meat was obtained.

**pH measurement.** A CyberScan 10 pH meter was used together with a glass composite electrode ERH--12-6 (HYDROMET S.C.). The $pH_{48}$ measurement was carried out by immersing the electrode in a water extract of 1:1 meat in distilled water, after 1 hour of extraction. Calibration of the electrode was performed using pH 7.0 and pH 4.0 buffers.

**Colour measurements.** A MiniScan XE Plus 45/0 spectrophotometer was used to measure meat colour. The instrument was equipped with a 31.8 mm diameter measuring port. Before measurement, the instrument was standardized for D65 illuminant and 10° observer using a black glass and a white tile with the following parameters: $X = 78.5$, $Y = 83.3$, $Z = 87.8$. The colour of the individual ground meat samples was measured after the sample was placed in the measuring vessels, the surface of the samples was levelled, and they were kept for 20 minutes at 4°C in order to oxidize the myoglobin on their surface (Krzywicki, 1979). The samples were then placed in the spectrophotometer and colour measurements were performed using both the CIELAB and CIELCh scales (CIE, 1976; CIE, 1978) and the two illuminant/observer systems: D65/10° recommended for meat colour measurement (Honikel, 1998), and A/10°. The reflectance was also measured in 10 nm increments from 400 to 700 nm for each meat sample.

The relative content of myoglobin forms in the surface layer of the meat was determined from the reflectance according to Krzywicki (1979), but with a reflectance value of 700 nm (the largest wavelength measurable by the MiniScan XE Plus 45/0) rather than 730 nm according to AMSA (2012). Reflectance values at 473, 525, and 572 nm were determined using linear interpolation for the relative amounts of myoglobin chemical forms. The resulting reflectance values were converted to absorbance values using the formula: $A = 2 - \log 10R$, where $A$ is the absorbance and $R$ is the reflectance. The measurements were performed using the so-called duplicate standard, which made it possible to obtain the values of all colour and reflectance parameters for a given sample from a single measurement.

To induce changes in meat colour, the meat samples were exposed to fluorescent lamp light at 1250 lux for 4 hours in a closed container at room temperature (22–24°C) humidified with water vapor to prevent drying, according to Kortz (1966). After this exposure, the colour and reflectance of each meat sample were measured again. The differences in the values of the colour parameters ($\Delta L^*$, $\Delta a^*$, $\Delta b^*$, $\Delta C^*$, $\Delta h°$), chromatic absorbance at 525 nm ($\Delta A_{525p}$), and the relative amounts of chemical forms of myoglobin ($\Delta MbO_2$, ΔMetMb, and ΔMb) were then calculated from the

obtained measurements. Hue difference ($\Delta H$) and total difference ($\Delta E$) were also calculated.

Chromatic absorbance at 525 nm (absorbance of pigments) and the myoglobin redox form were calculated (according to AMSA, 2012) as follows:

$$A_{525p} = A_{525} - A_{700}$$

$$\text{Metmyoglobin (MetMb)} = 1.395 - [(A_{572} - A_{700}) / (A_{525} - A_{700})]$$

$$\text{Deoxymyoglobin (Mb)} = 2.375 \times [1 - (A_{473} - A_{700}) / (A_{525} - A_{700})]$$

$$\text{Oxymyoglobin } (MbO_2) = 1 - [(\text{Mb}) - (\text{MetMb})]$$

where:

$A_{525p}$ – chromatic absorbance at 525 nm,
$A_{572}$ – absorbance at 573 nm,
$A_{473}$ – absorbance at 473 nm,
$A_{525}$ – absorbance at 525 nm,
$A_{700}$ – absorbance at 700 nm.

Hue difference $\Delta H$ and total difference $\Delta E$ were calculated (according to CIE, 1976) as follows:

$$\Delta H = \sqrt{\Delta E^2 - \Delta L^2 - \Delta C^2}$$

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 - \Delta b^2}$$

## Statistical analysis

Statistical analyses were performed using STATISTICA v13.3 (TIBCO Software Inc.). Mean values and standard deviations were calculated, and one-way analysis of variance was performed for the colour parameters determined using the illuminant/observer systems D65/10° and A/10°. The significance of differences between the averages was estimated by Duncan's test, with levels of significance $p \leq 0.05$ and $p \leq 0.01$. In addition, simple correlation coefficients (Pearson's $r$) and adjusted coefficients of determination ($R^2$) were calculated for the colour parameters studied and their significance was estimated for probability levels $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$.

## RESULTS AND DISCUSSION

The mean values and standard deviations of the features and colour parameters are presented in Table 1. The mean of moisture content was 74.85%, total protein – 22.41%, intramuscular fat – 1.72%, WHC – 74.70, and $pH_{48}$ – 5.57. The mean values of chromatic absorbance at 525 nm ($A_{525p}$) before and after illumination were very similar – 0.352 and 0.350, respectively. The relative amounts of $MbO_2$, MetMb, and Mb both before and after illumination were respectively: 0.460 (46.0%), 0.158 (15.8%), and 0.382 (38.2%) before, and 0.323 (32.3%), 0.255 (25.5%), and 0.422 (42.2%) after – thus during illumination, the proportion of $MbO_2$ decreased and the proportion of MetMb and Mb increased. Both before and after illumination, the illuminant/observer A/10° system showed significantly higher values for the $L^*$, $a^*$, and $C^*$ parameters and significantly lower values for the $b^*$ and $h°$, as well as significantly higher values for $\Delta a^*$, $\Delta C^*$, $\Delta H$, and $\Delta E$, with the largest differences found for $a^*$, $C^*$, and $h°$. After illumination, changes in the values of chromatic parameters were mainly noted, with the values of $a^*$, $b^*$, $C^*$ decreasing and the value of $h°$ increasing, which denotes a deterioration in meat colour.

Table 2 shows the simple correlation coefficients (Pearson's $r$) between moisture content, crude protein, intramuscular fat, WHC, and $pH_{48}$ and the colour parameters measured before and after illumination between the A/10° and D65/10° systems, and the differences formed in the values of these parameters during illumination.

As moisture content increased, the values of all colour parameters decreased significantly, although the correlation coefficients were not high. On the other hand, the correlation coefficients between crude protein, intramuscular fat, and colour parameters were mostly positive but generally low and in many cases not statistically significant. This indicates that the content of these chemical components in the meat did not have a large effect on colour. On the other hand, the values of correlation coefficients between the values of colour parameters measured before and after illumination and WHC and $pH_{48}$ were found to be higher and statistically significant in most cases, with the highest correlation coefficients for lightness ($L^*$), yellowness ($b^*$), and chroma ($C^*$) before illumination and for lightness ($L^*$), yellowness ($b^*$), and hue angle ($h°$) after illumination. The values of correlation coefficients between WHC and $pH_{48}$ and redness ($a^*$) increased and became positive after illumination.

**Table 1.** Means and standard deviations of feature and parameters of colour ($n$ = 168)

| Feature | Mean | SD | Parameters of colour | Mean | SD | Mean | SD |
|---|---|---|---|---|---|---|---|
| | | | | D65/10° | | A/10° | |
| Moisture content, % | 74.85 | 0.86 | $L^*$ – lightness | 54.09$^{A}$ | 2.80 | 55.92$^{B}$ | 2.82 |
| Total protein, % | 22.41 | 0.62 | $a^*$ – redness | 7.79$^{A}$ | 1.08 | 17.25$^{B}$ | 1.12 |
| Intramuscular fat, % | 1.72 | 0.74 | $b^*$ – yellowness | 15.63$^{A}$ | 0.94 | 15.26$^{B}$ | 1.11 |
| WHC, % | 74.70 | 6.33 | $C^*$ – chroma | 17.50$^{A}$ | 1.06 | 23.05$^{B}$ | 1.32 |
| $pH_{48}$ | 5.57 | 0.17 | $h°$ – hue angle | 63.55$^{A}$ | 3.20 | 41.48$^{B}$ | 2.12 |
| $A_{525p}$ | 0.352 | 0.029 | $L^*$ – after illumination | 54.01$^{A}$ | 3.10 | 55.70$^{B}$ | 3.08 |
| $MbO_2$ | 0.460 | 0.103 | $a^*$ – after illumination | 6.90$^{A}$ | 0.89 | 15.39$^{B}$ | 1.11 |
| MetMb | 0.158 | 0.034 | $b^*$ – after illumination | 14.64$^{A}$ | 0.84 | 14.30$^{B}$ | 0.96 |
| Mb | 0.382 | 0.107 | $C^*$ – after illumination | 16.21$^{A}$ | 0.80 | 21.04$^{B}$ | 0.94 |
| $A_{525p}$ – after illumination | 0.350 | 0.029 | $h°$ – after illumination | 64.76$^{A}$ | 3.27 | 42.91$^{B}$ | 3.04 |
| $MbO_2$ – after illumination | 0.323 | 0.057 | $\Delta L^*$ | –0.08 | 0.71 | –0.22 | 0.65 |
| MetMb – after illumination | 0.255 | 0.065 | $\Delta a^*$ | –0.89$^{A}$ | 0.73 | –1.86$^{B}$ | 1.07 |
| Mb – after illumination | 0.422 | 0.103 | $\Delta b^*$ | –0.99 | 0.54 | –0.96 | 0.67 |
| $\Delta A_{525p}$ | 0.002 | 0.011 | $\Delta C^*$ | –1.28$^{A}$ | 0.79 | –2.01$^{B}$ | 1.14 |
| $\Delta MbO_2$ | –0.137 | 0.091 | $\Delta h°$ | 1.21 | 1.49 | 1.43 | 1.40 |
| ΔmetMb | 0.097 | 0.051 | $\Delta H$ | 0.45$^{a}$ | 0.37 | 0.58$^{b}$ | 0.55 |
| ΔMb | 0.040 | 0.072 | $\Delta E$ | 1.59$^{A}$ | 0.77 | 2.29$^{A}$ | 1.11 |

Means with different superscript letters differ significantly: small letters at $P \leq 0.05$; capital letters at $P \leq 0.01$.
$\Delta A_{525p}$, $\Delta MbO_2$, ΔMetMb, ΔMb, $\Delta L^*$, $\Delta a^*$, $\Delta b^*$, $\Delta C^*$, and $\Delta h$ were calculated by subtracting the value of a given parameter before illumination from the value after illumination.

This was attributed to the increase in MetMb and the consequent decrease in redness ($a^*$), especially in samples with low $pH_{48}$ and WHC. The effect of the illuminant/observer system on the values of correlation coefficients between colour parameters and WHC and $pH_{48}$ before illumination was greatest for chroma ($C^*$) and hue angle ($h°$), and after illumination for redness ($a^*$), chroma ($C^*$) and hue angle ($h°$), with the values of correlation coefficients for chroma ($C^*$) found to be higher using the D65/10° system, and for redness ($a^*$) and hue angle ($h°$) using the A/10° system. The greatest effect of the illuminant/observer system on the values of correlation coefficients was observed for the hue angle ($h°$). In addition, significant correlation coefficients were found between WHC and $pH_{48}$ and changes (Δ) in the values of all colour parameters, with the highest values of correlation coefficients recorded for $\Delta h°$ and $\Delta a^*$ and were higher when using the A/10° layout.

The lightness ($L^*$) and yellowness ($b^*$) of the colour of pork *longissimus* muscle are the parameters that show the highest values of correlation coefficients with the quality characteristics of this muscle such as $pH_u$ and WHC. Colour lightness ($L^*$) is mainly influenced by achromatic reflectance/absorbance of light, which depends on its structure (Karamucki et al., 2013). On the other hand, the reflectance/absorbance of pigments, whose content in this glycolytic muscle is low and poorly correlated with the redness ($a^*$) (Feldhusen, 1994) is influenced to a lesser extent.

**Table 2.** Correlation coefficients ($r$) for the basic chemical components, WHC and $pH_{48}$ of the meat ($n$ = 168)

| Feature | Illuminant/ observer | $L^*$ | $a^*$ | $b^*$ | $C^*$ | $h°$ |
|---|---|---|---|---|---|---|
| | | | Before illumination | | | |
| Moisture content, % | D65/10° | –0.196* | –0.231** | –0.305*** | –0.349*** | –0.095 |
| | A/10° | –0.211** | –0.143 | –0.370*** | –0.296*** | –0.233** |
| Crude protein, % | D65/10° | 0.136 | 0.022 | 0.203** | 0.170* | 0.060 |
| | A/10° | 0.141 | –0.048 | 0.220** | 0.093 | 0.254*** |
| Intramuscular fat, % | D65/10° | 0.101 | 0.242** | 0.176* | 0.251** | –0.151 |
| | A/10° | 0.114 | 0.202** | 0.235** | 0.256*** | 0.050 |
| WHC | D65/10° | –0.579*** | –0.174* | –0.524*** | –0.496*** | –0.048 |
| | A/10° | –0.594*** | –0.169* | –0.535*** | –0.408*** | –0.365*** |
| $pH_{48}$ | D65/10° | –0.671*** | –0.009 | –0.703*** | –0.562*** | –0.302*** |
| | A/10° | –0.682*** | 0.002 | –0.655*** | –0.361*** | –0.637*** |
| | | | After illumination | | | |
| Moisture content, % | D65/10° | –0.191* | –0.087 | –0.168* | –0.197* | 0.020 |
| | A/10° | –0.199** | 0.185* | –0.291*** | –0.053 | –0.300*** |
| Crude protein, % | D65/10° | 0.156* | –0.081 | 0.166* | 0.117 | 0.129 |
| | A/10° | 0.158* | –0.237*** | 0.228** | –0.065 | 0.322*** |
| Intramuscular fat, % | D65/10° | 0.072 | 0.172* | 0.057 | 0.132 | –0.133 |
| | A/10° | 0.080 | 0.040 | 0.138 | 0.131 | 0.054 |
| WHC | D65/10° | –0.624*** | 0.180* | –0.402*** | –0.299*** | –0.302*** |
| | A/10° | –0.630*** | 0.378*** | –0.441*** | –0.008 | –0.527*** |
| $pH_{48}$ | D65/10° | –0.695*** | 0.277*** | –0.646*** | –0.477*** | –0.490*** |
| | A/10° | –0.703*** | 0.471*** | –0.648*** | –0.057 | –0.724*** |
| | | $\Delta L^*$ | $\Delta a^*$ | $\Delta b^*$ | $\Delta C^*$ | $\Delta h°$ |
| Moisture content, % | D65/10° | –0.061 | –0.237** | –0.269*** | –0.269*** | –0.160* |
| | A/10° | –0.026 | –0.341*** | –0.192* | –0.299*** | –0.298*** |
| Crude protein, % | D65/10° | –0.145 | 0.132 | 0.095 | 0.110 | 0.153* |
| | A/10° | –0.140 | 0.232* | 0.035 | 0.162* | 0.313*** |
| Intramuscular fat, % | D65/10° | 0.081 | 0.150 | 0.218** | 0.204** | 0.045 |
| | A/10° | 0.118 | 0.170* | 0.189* | 0.189* | 0.042 |
| WHC | D65/10° | 0.444*** | –0.477*** | –0.287*** | –0.363*** | –0.560*** |
| | A/10° | 0.411*** | –0.568*** | –0.249** | –0.466*** | –0.596*** |
| $pH_{48}$ | D65/10° | 0.390*** | –0.349*** | –0.222** | –0.272*** | –0.429*** |
| | A/10° | 0.373*** | –0.485*** | –0.157* | –0.372*** | –0.604*** |

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

In contrast, the yellowness ($b^*$) of this muscle is primarily influenced by the relative abundance of myoglobin chemical forms (Lindahl et al., 2001). Both the lightness ($L^*$) and the yellowness ($b^*$) of the pork *longissimus muscle* thus depend on pH, which affects both its structure and the intensity of oxidoreductive processes occurring in it, as well as the formation of WHC (Zhu and Brewer, 1998). Therefore, the values of correlation coefficients between these colour parameters and $pH_{48}$ and WHC were found to be the highest. In contrast, redness ($a^*$), as well as hue angle ($h°$), measured using the illuminant/observer system D65/10°, depend primarily on the number of pigments reached by light, but to a lesser extent on the relative number of chemical forms of myoglobin (Karamucki et al., 2013; Lindahl, 2005). Hence, the values of correlation coefficients between these colour parameters and $pH_{48}$ and WHC are generally lower.

For differences (Δ) in the colour parameters, the highest values of correlation coefficients were recorded between $\Delta a^*$ and $\Delta h°$ and $pH_{48}$ and WHC, which is consistent with the results of other work (Karamucki et al., 2011), with higher values found for the A/10° system.

Table 3 presents the coefficients of determination ($R^2$) showing the effect of pigment absorbance ($A_{525p}$) and relative content of myoglobin chemical forms on the variation in colour parameter values, and the effect of changes in pigment absorbance ($\Delta A_{525p}$) and relative content of myoglobin chemical forms on changes (Δ) in colour parameter values, using both the illuminant/observer systems.

The results indicate that the illuminate/observer system differed in all colour parameters, primarily chromatic but especially the $h°$ parameter. The variation in redness ($a^*$) depended primarily on the absorbance of the pigments ($A_{525p}$), with the effect being greater for the D65/10° system, especially after illumination. In contrast, the effect of the relative amount of myoglobin chemical forms on redness ($a^*$) was markedly less, and after illumination significant only for the A/10° system. The variation in yellowness ($b^*$) depended on the effect of the relative amounts of myoglobin chemical forms, especially $MbO_2$ and Mb, while a small significant effect of pigment absorbance ($A_{525p}$) was observed for the D65/10° system before illumination – 1.76% ($R^2$ = 0.0176) and after illumination – 6.03% ($R^2$ = 0.0603). The variation in chroma ($C^*$) for the D65/10° system depended primarily on the effect of the chemical forms of myoglobin, while the effect of pigments ($A_{525p}$) appeared to be small. In contrast, for the A/10° system, the effect of pigment absorbance ($A_{525p}$) on the variation in chroma ($C^*$) was clearly greater, with a greater effect after illumination than the effect of the relative amounts of their chemical forms. The effect of pigment absorbance ($A_{525p}$) on the variation of $h°$ parameter values pre- and post-illumination were respectively 82.17% and 84.39% for the D65/10° system and 46.17% and 30.31% for the A/10° system. On the other hand, the combined effect of the relative amount of myoglobin forms ($MbO_2$ + MetMb + Mb) on the variation of $h°$ parameter values before illumination was significant only for the A/10° system at 37.42%. After illumination, the effect increased and was significant for both illuminant/observer systems, being 70.28% for the A/10° system and only 7.80% for D65/10°.

Analyzing the effect of changes in the number of pigments ($\Delta A_{525p}$) and changes in the amount of myoglobin chemical forms ($\Delta MbO_2$, ΔMetMb, ΔMb) on $\Delta h°$, it was noted that changes in the number of pigments ($\Delta A_{525p}$) had a significant effect primarily for the D65/10° system – 35.44%. In contrast, for the A/10° system, the effect was only 2.90%. On the other hand, the combined effect of changes in the relative number of myoglobin chemical forms ($\Delta MbO_2$ + ΔMetMb + ΔMb) on $\Delta h°$ was found to be greater for the A/10° system than for D65/10° – 84.03% and 44.08%, respectively. In addition, for the A/10° system, changes in the amount of MetMb (ΔMetMb) had the greatest effect – 67.53%, whilst for the D65/10° system, changes in the amount of $MbO_2$ ($\Delta MbO_2$) had the greatest effect – 43.31%. When using the A/10° system in measurements, as much as 89.81% of $\Delta h°$ could be explained by the combined effect of changes in pigment absorbance and relative amounts of myoglobin chemical forms ($\Delta A_{525p}$ + $\Delta MbO_2$ + ΔMetMb + ΔMb) – when using the D65/10° system – 78.50%. For the variables $\Delta a^*$, $\Delta b^*$ and $\Delta C^*$, the effect was also greater using the A/10° system.

In summary, the results obtained indicate that the A/10° system is more useful in measuring colour stability and determining changes (Δ) in chromatic parameters, especially changes in hue angle ($\Delta h°$).

As already mentioned, the results of studies (Karamucki et al., 2013; Lindahl et al., 2001) indicate that the variation in redness (*a**) measured using the illuminant/observer D65/10° system depends mainly on the influence of the number of pigments, and the variation in yellowness (*b**) on the influence of the relative number of myoglobin chemical forms. In contrast, chroma (*C**) and hue angle (*h*°) are calculated based on *a** and *b** – so they depend to varying degrees on the influence of the number of pigments and the relative amounts of their chemical forms. The proportions of chemical forms of pigments depend, among other things, on the intensity of oxygenation and deoxygenation and oxidation and reduction processes, which are influenced by the pH of the meat as well as temperature and light (Bekhit and Faustman, 2005; Mikkelsen et al., 1999; Zhu and Brewer, 1998). Each chemical form of myoglobin is characterized by different redness (*a**), yellowness (*b**), chroma (*C**), and hue angle (*h*°). $MbO_2$ is characterized by the highest redness (*a**) and yellowness (*b**), and chroma (*C**). Mb has the lowest yellowness (*b**), while MetMb has the lowest redness (*a**) (Karamucki et al., 2013). Therefore, decreasing the amount of $MbO_2$ and increasing the amount of MetMb deteriorates the meat colour (Hernández et al., 2016). This occurs as the pH and WHC of the meat decreases, the propensity of myoglobin to deoxygenate and oxidize increases, and consequently the amount of MetMb increases (Lindahl, 2005), resulting in a decrease in redness (*a**) and a deterioration in hue angle (*h*°) (Luciano et al., 2011). Therefore, when measuring colour stability, the greatest differences are found in redness (Δ*a**) and hue angle (Δ*h*°).

Analysis of the results in Table 3 indicates that for using the illuminant/observer A/10° system, both before and after illumination, the number of pigments ($A_{525p}$) did not affect the variation of *b**. The use of the A/10° system instead of D65/10° also increased the effect of pigment absorbance ($A_{525p}$) and decreased the effect of myoglobin chemical forms on the variation of colour chroma (*C**), while decreasing the effect of pigment absorbance ($A_{525p}$) and increasing the effect

**Table 3.** The coefficient of determination $R^2$ (corrected) for colour parameters ($n$ = 168)

| Feature | Illuminat/ observer | *L** | *a** | *b** | *C** | *h*° |
|---|---|---|---|---|---|---|
| | | $R^2$ | | | | |
| 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Before illumination | | | | | | |
| $A_{525p}$ | D65/10° | 0.3955*** | 0.7043*** | 0.0176* | 0.0608*** | 0.8217*** |
| | A/10° | 0.3509*** | 0.6768*** | 0.0000 | 0.2975*** | 0.4617*** |
| $MbO_2$ | D65/10° | 0.3190*** | 0.0950*** | 0.5158*** | 0.5123*** | 0.0000 |
| | A/10° | 0.4241*** | 0.0730*** | 0.5714*** | 0.3553*** | 0.2283*** |
| MetMb | D65/10° | 0.0542** | 0.0305* | 0.1563*** | 0.1595*** | 0.0000 |
| | A/10° | 0.0622*** | 0.0016 | 0.1991*** | 0.0900*** | 0.1176*** |
| Mb | D65/10° | 0.3940*** | 0.1312*** | 0.6854*** | 0.6838*** | 0.0000 |
| | A/10° | 0.4324*** | 0.0844*** | 0.7759*** | 0.4614*** | 0.3351*** |
| $MbO_2$ + MetMb + Mb | D65/10° | 0.3974*** | 0.1356*** | 0.7213*** | 0.7213*** | 0.0000 |
| | A/10° | 0.4376*** | 0.0790*** | 0.8284*** | 0.4770*** | 0.3742*** |
| $A_{525p}$ + $MbO_2$ + MetMb + Mb | D65/10° | 0.8101*** | 0.9389*** | 0.7428*** | 0.8011*** | 0.9161*** |
| | A/10° | 0.8023*** | 0.8928*** | 0.8304*** | 0.8386*** | 0.9254*** |

**Table 3 – cont.**

| 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|
| | | | After illumination | | | |
| $A_{525p}$ | D65/10° | 0.4527*** | 0.8847*** | 0.0603*** | 0.0370** | 0.8439*** |
| | A/10° | 0.4237*** | 0.6042*** | 0.0000 | 0.4074*** | 0.3031*** |
| $MbO_2$ | D65/10° | 0.2908*** | 0.0089 | 0.5862*** | 0.5376*** | 0.0796*** |
| | A/10° | 0.3088*** | 0.0402** | 0.6522*** | 0.1606*** | 0.4176*** |
| MetMb | D65/10° | 0.2784*** | 0.0000 | 0.2263*** | 0.1968*** | 0.0269* |
| | A/10° | 0.2929*** | 0.2625*** | 0.4396*** | 0.0000 | 0.5755*** |
| Mb | D65/10° | 0.4000*** | 0.0060 | 0.5273*** | 0.4735*** | 0.0704*** |
| | A/10° | 0.4226*** | 0.1911*** | 0.7502*** | 0.0626*** | 0.6991*** |
| $MbO_2$ + MetMb + Mb | D65/10° | 0.3976*** | 0.0000 | 0.6125*** | 0.5572*** | 0.0780*** |
| | A/10° | 0.4205*** | 0.2581*** | 0.7766*** | 0.1730*** | 0.7028*** |
| $A_{525p}$ + $MbO_2$ + MetMb + Mb | D65/10° | 0.7798*** | 0.9487*** | 0.6222*** | 0.6909*** | 0.8816*** |
| | A/10° | 0.7717*** | 0.9342*** | 0.7848*** | 0.7805*** | 0.9477*** |
| | | $\Delta L^*$ | $\Delta a^*$ | $\Delta b^*$ | $\Delta C^*$ | $\Delta h°$ |
| $\Delta A_{525p}$ | D65/10° | 0.2694*** | 0.4028*** | 0.3647*** | 0.4070*** | 0.3544*** |
| | A/10° | 0.2092*** | 0.3063*** | 0.3806*** | 0.3903*** | 0.0290* |
| $\Delta MbO_2$ | D65/10° | 0.1865*** | 0.4994*** | 0.5000*** | 0.5265*** | 0.4331*** |
| | A/10° | 0.1221*** | 0.3840*** | 0.5267*** | 0.5037*** | 0.0261* |
| ΔMetMb | D65/10° | 0.1924*** | 0.2535*** | 0.1963*** | 0.2272*** | 0.2308*** |
| | A/10° | 0.1593*** | 0.5682*** | 0.0805*** | 0.3652*** | 0.6753*** |
| ΔMb | D65/10° | 0.0501** | 0.2791*** | 0.3266*** | 0.3262*** | 0.2336*** |
| | A/10° | 0.0204* | 0.0563** | 0.4983*** | 0.2122*** | 0.1225*** |
| $\Delta MbO_2$ + ΔMetMb + ΔMb | D65/10° | 0.2324*** | 0.5058*** | 0.4974*** | 0.5260*** | 0.4408*** |
| | A/10° | 0.1737*** | 0.6083*** | 0.5598*** | 0.5495*** | 0.8403*** |
| $\Delta A_{525p}$ + $\Delta MbO_2$ + ΔMetMb + ΔMb | D65/10° | 0.5078*** | 0.8956*** | 0.8435*** | 0.9153*** | 0.7850*** |
| | A/10° | 0.3909*** | 0.9350*** | 0.9044*** | 0.9324*** | 0.8981*** |

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

of myoglobin chemical forms on the variation of hue angle ($h°$) values. On the other hand, the variation of $\Delta h°$, when measured with the A/10° system, depended almost entirely on the influence of changes in the relative number of myoglobin chemical forms ($\Delta MbO_2$ + ΔMetMb + ΔMb), consisting mainly of a decrease in the amount of $MbO_2$ and an increase in the amount of MetMb (Table 1), with changes in redness ($\Delta a^*$) and hue angle ($\Delta h°$) being the main determinants of changes ($\Delta$) in the amount of MetMb, whereas changes ($\Delta$) in the amount of $MbO_2$ were the main determinants for the D65/10° system.

**Table 4.** The coefficient of determination $R^2$ (corrected) for $\Delta E$ and $\Delta H$ ($n$ = 168)

| Feature | Illuminat/ observer | $\Delta H$ | $\Delta E$ |
|---|---|---|---|
| | | $R^2$ | |
| $\Delta A_{525p}$ | D65/10° | 0.2733*** | 0.3383*** |
| | A/10° | 0.0251* | 0.3089*** |
| $\Delta MbO_2$ | D65/10° | 0.2458*** | 0.4249*** |
| | A/10° | 0.0342** | 0.4288*** |
| ΔMetMb | D65/10° | 0.1964*** | 0.1911*** |
| | A/10° | 0.6512*** | 0.4316*** |
| ΔMb | D65/10° | 0.0920*** | 0.2560*** |
| | A/10° | 0.0972*** | 0.1248*** |
| $\Delta MbO_2$ + ΔMetMb + ΔMb | D65/10° | 0.2746*** | 0.4246*** |
| | A/10° | 0.7862*** | 0.5336*** |
| $\Delta A_{525p}$ + $\Delta MbO_2$ + ΔMetMb + ΔMb | D65/10° | 0.5488*** | 0.7493*** |
| | A/10° | 0.8372*** | 0.8499*** |

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

Table 4 presents the coefficients of determination ($R^2$) showing the effects of changes in pigment absorbance ($\Delta A_{525p}$) and changes ($\Delta$) in the relative amounts of myoglobin chemical forms on hue difference ($\Delta H$) and total difference ($\Delta E$) values, with both illuminant/observer layouts. Using the A/10° system, the effect of $\Delta A_{525p}$ on the variation of $\Delta H$ and $\Delta E$ values was smaller and the total effect of changes in the relative amounts of myoglobin chemical forms ($\Delta MbO_2$ + ΔMetMb + ΔMb) was larger, especially for $\Delta H$. The analysis of the effect of changes ($\Delta$) in the relative amounts of individual chemical forms of myoglobin on the variation of $\Delta H$ and $\Delta E$ indicates that the effect of $\Delta MbO_2$ on $\Delta H$ and the effect of ΔMb on $\Delta E$ were clearly smaller for the A/10° system. In contrast, the effect of $\Delta MbO_2$ on $\Delta E$ and the effect of ΔMb on $\Delta H$ were very similar for both systems. On the other hand, the effect of ΔMetMb on both $\Delta H$ and $\Delta E$ was found to be significantly larger for the A/10° system, with this being especially true for the effect on $\Delta H$ (65.12%).

The use of the A/10° system made it possible to explain as much as 83.72% of the variation in $\Delta H$ and 84.99% of the variation in $\Delta E$ by the combined effect of changes in pigment absorbance and relative amounts of myoglobin chemical forms ($\Delta A_{525p}$ + $\Delta MbO_2$ + ΔMetMb + ΔMb), whereas the use of the D65/10° system made it possible to explain by the effect of these variables only 54.88% of the variation in $\Delta H$ and 74.93% of the variation in $\Delta E$. This confirms previous observations and indicates that the illuminant/observer A/10° system is more useful for determining $\Delta H$ and $\Delta E$.

## CONCLUSIONS

Replacing the illuminant/observer D65/10° system with the A/10° system in colour measurements of raw pork *longissimus* muscle changes the contribution of pigment absorbance ($A_{525p}$) and the relative number of chemical forms of myoglobin in influencing the values of colour parameters, primarily the hue angle ($h$°). The use of the A/10° system for colour stability measurements better captures the difference in redness ($\Delta a^*$) and especially in hue angle ($\Delta h$°), as well as hue difference ($\Delta H$) and total difference ($\Delta E$), with the increase in the relative amount of MetMb becoming the main determinant of the variation in these differences. At the same time, measurements using the A/10° system

increase the correlation coefficients between WHC and $pH_{48}$ and changes in redness ($\Delta a^*$) and hue angle ($\Delta h°$). Therefore, the A/10° system compared to the D65/10° system may be more useful for measuring the colour stability of raw pork, especially with regard to $\Delta h°$ and $\Delta H$ and $\Delta E$.

## REFERENCES

AMSA (2012). Meat colour measurement guidelines. Kearney: American Meat Science Association.

AOAC (2003). Official methods of analysis of AOAC International (17th ed.). Gaithersburg, MD, USA: Association of the Official Analytical Chemists (AOAC) International.

Brewer, M. S., McKeith, F. K. (1999). Consumer-rated quality characteristics as related to purchase intent of fresh pork. J. Food Sci., 64(1), 171–174. https://doi.org/10.1111/j.1365-2621.1999.tb09885.x

Brewer, M. S., Zhu, L. G., Bidner, B., Meisinger, D. J., McKeith, F. K. (2001). Measuring pork colour: effect of bloom time, muscle, pH, and relationship to instrumental parameters. Meat Sci., 57(2), 169–176. https://doi.org/10.1016/S0309-1740(00)00089-9

Bekhit, A. E. D., Faustman, C. (2005). Metmyoglobin reducing activity. Rev. Meat Sci., 71(3), 407–439. https://doi.org/10.1016/j.meatsci.2005.04.032

Boler, D. D., Dilger, A. C., Bidner, B. S., Carr, S. N., Eggert, J. M., Day, J. W., ..., Killefer, J. (2010). Ultimate pH explains variation in pork quality traits. J. Muscle Foods, 21(1), 119–130. https://doi.org/10.1111/j.1745-4573.2009.00171.x

CIE (1976). Colorimetry: Official Recommendations of the International Commission on Illumination. Publication CIE no. 15 (E-1.3.1). Paris, France: Bureau Central de la CIE.

CIE (1978). Recommendation on uniform colour spaces, colour difference equations, psychometric colour terms. Supplement no. 2 to publication CIE no. 15 (e-1.3.1). Paris: Commission Internationale De L'Eclairage.

Chmiel, M., Słowiński, M., Janakowski, S. (2014). The quality evaluation of RFN and PSE pork *longissimus lumborum* muscle considering its microstructure. Ann. Anim. Sci., 14(3), 737–747. https://doi.org/10.2478/aoas-2014-0035

Feldhusen, F. (1994). Influences on postmortem surface colour change in swine muscle. Fleischwirtschaft, 74, 989–991.

Garcia-Esteban, M., Ansorena, D., Gimeno, O., Astiasarán, I. (2003). Optimization of instrumental colour analysis in dry cured ham. Meat Sci., 63(3), 287–292. https://doi.org/10.1016/S0309-1740(02)00084-0

Grau, R., Hamm, R. (1953). A simple method for the determination of water binding in muscle. Natural Sci., 40, 29–30. https://doi.org/10.1007/BF00595734

Hernández, B., Sáenz, C., Alberdi, C., Dineiro, J. M. (2016). CIELAB colour coordinates versus relative proportions of myoglobin redox forms in the description of fresh meat appearance. J. Food Sci. Technol., 53(12), 4159–4167. https://doi.org/10.1007/s13197-016-2394-6

Hughes, J. M., Oiseth, S. K., Purslow, P. P., Warner, R. D. (2014). A structural approach to understanding the interactions between colour, water-holding capacity and tenderness. Meat Sci., 98(3), 520–532. http://dx.doi.org/10.1016/j.meatsci.2014.05.022

Honikel, K. O. (1998). Reference methods for the assessment of physical characteristics of meat. Meat Sci., 49(4), 447–457. http://doi.org/10.1016/S0309-1740(98)00034-5

Karamucki, T., Gardzielewska, J., Rybarczyk, A., Jakubowska, M., Natalczyk-Szymkowska, W. (2011). Usefulness of selected methods of colour change measurement for pork quality assessment. Czech J. Food Sci., 3, 212–218. http://dx.doi.org/10.17221/191/2010-CJFS

Karamucki, T., Jakubowska, M., Rybarczyk, A., Gardzielewska, J. (2013). The influence of myoglobin on the colour of minced pork loin. Meat Sci., 94(2), 234–238. http://dx.doi.org/10.1016/j.meatsci.2013.01.014

Kortz, J. (1966). The effect of free sulfhydryl groups on the colour stability of raw meat (pork). PhD. thesis. Technical University of Gdańsk, Gdańsk, Poland [in Polish].

Krzywicki, K. (1979). Assessment of relative content of myoglobin, oxymyoglobin and metmyoglobin at the surface of beef. Meat Sci., 3(1), 1–10. http://dx.doi.org/10.1016/0309-1740(79)90019-6

Lindahl, G. (2005). Colour characteristics of fresh pork. Doctoral thesis. Swedish University of Agricultural Sciences, Uppsala.

Lindahl, G., Lundström, K., Tornberg, E. (2001). Contribution of pigment content, mioglobin forms and internal reflectance to the colour of pork loin and ham from pure breed pigs. Meat Sci., 59(2), 141–151. http://dx.doi.org/10.1016/S0309-1740(01)00064-X

Luciano, G., Vata, V., Monahan, F. J., Lopez-Andres, P., Biondi, L., Lanza, M., Priolo A. (2011). Antioxydant status, colour stability and myoglobin resistance to oxidation of *longissimus dorsi* muscle from lams fed a tannin-containing diet. Food Chem., 124(3), 1036–1042. https://doi.org/10.1016/j.foodchem.2010.07.070

Mancini, R. A., Hunt, M. C. (2005). Review: Current research in meat colour. Meat Sci., 71(1), 100–121. http://dx.doi.org/10.1016/j.meatsci.2005.03.003

Mikkelsen, A., Juncher, D., Skibsted, L. H. (1999). Metmyoglobin reductase activity in porcine *m. longissimus dorsi* muscle. Meat Sci., 51(2), 155–161. https://doi.org/10.1016/S0309-1740(98)00114-4

Purslow, P. P., Warner, R. D., Clarke, F. M., Hughes, J. M. (2020). Variations in meat colour due to factors other than myoglobin chemistry; a synthesis of recent findings (invited review). Meat Sci., 159, 107941. https://doi.org/10.1016/j.meatsci.2019.107941

Pohja, N. S., Niinivaara, F. P. (1957). The determination of water binding of meat by the constant pressure method. Fleischwirtschaft, 9, 193–193.

Sáenz, C., Hernández, B., Beriain, M. J., Lizaso, G. (2004). Meat colour in retail display with fluorescent illumination. Colour Res. Appl., 30(4), 304–311. https://doi.org/10.1002/col.20123

Swatland, H. J. (2004). Progress in understanding the paleness of meat with a low pH. South Afr. J. Anim. Sci., 34 (Supplement 2), 1–7. http://dx.doi.org/10.4314/sajas.v34i6.3816

Tapp III, W. N., Yencey, J. W. S., Apple, J. K. (2011). How is the instrumental colour of meat measured? Meat Sci., 89(1), 1–5. http://dx.doi.org/10.1016/j.meatsci.2010.11.021

Zhu, L. G., Brewer, M. S. (1998). Metmyoglobin reducing capacity of fresh normal, PSE and DFD pork during retail display. J. Food Sci., 63(3), 390–393. https://doi.org/10.1111/j.1365-2621.1998.tb15749.x