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The influence of myoglobin on the colour of minced pork loin

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ABSTRACT

The influence of the total and chromatic absorbance at wavelengths of 525 nm (A_{525} and A_{525p}) and 700 nm (A_{700}), and the relative content of oxymyoglobin (MbO_2), metmyoglobin (MetMb) and deoxymyoglobin (Mb), on the value of the colour parameters (L^*, a^*, b^*, C^* and $h°$) of minced pork loin, were evaluated. Lightness (L^*) depended almost entirely on variation in total absorbance at a wavelength of 525 nm. Redness (a^*) depended on the forms of myoglobin and A_{525p}, while yellowness (b^*) depended mainly on the proportions of the reduced form (Mb), the oxygenated form (MbO_2) and the oxidised form (MetMb). Yellowness (b^*) significantly increased with a decrease in the relative amount of Mb and an increase in relative amounts of MbO_2 and MetMb, although a greater impact was exerted by fluctuations in MbO_2 than MetMb. Variability of chroma (C^*) depended mainly on proportions of the forms of myoglobin. Hue angle ($h°$) depended primarily on chromatic absorbance at 525 nm (A_{525p}).

1. Introduction

The colour of pork largely depends on the amount of myoglobin and the relative content of its chemical forms – the oxygenated form (oxymyoglobin — MbO_2), the oxidised form (metmyoglobin — MetMb) and the reduced form (deoxymyoglobin — Mb) – as well as on the pork's tissue structure. This is formed mainly by the content of its basic chemical components and by the dynamics and extent of the *post mortem* pH reduction that affects both the denaturation of muscle proteins (Bendall & Swatland, 1988; Hamm, 1996; Honikel & Kim, 1985) and myofilament lattice spacing (Irving, Swatland, & Milman, 1989; Offer & Trinick, 1983). Meat colour is also significantly related to the amount of haemoglobin which depends on the extent of exsanguination. The haemoglobin present in meat has absorbing properties similar to myoglobin (Giddings, 1977). The difference between myoglobin and haemoglobin absorbance coefficient is slight and does not exceed the error of their determination (Krzywicki, 1982).

Feldhusen (1994) concludes that low correlation coefficients between the quantity of pigment in pork and the parameters of colour, particularly redness (a), indicate the important role of factors other than pigments. One of these factors is the variation in the structure of pork, and this is especially important when the naturally low quantities of pigments in pork are taken into account. The structure of pork has a significant impact on the depth of penetration of both light and oxygen, and on the values of achromatic and chromatic absorbance, although the latter also depends on the quantity of pigment penetrated by the light and on the proportions of the three chemical forms of myoglobin in the surface layer of the meat.

The methods used for the determination of the quantities of pigments in the meat, consisting of extraction and colourimetric assays, do not take into account the impact of tissue structure (Lindahl, 2005), therefore they do not accurately determine the effect of the amount of pigment and the relative content of the forms of myoglobin on pork colour parameters. Spectrophotometric methods would appear to be more effective for this. The impact on the effect of the amount of pigment on the colour parameters of raw meat can be inferred from the chromatic absorbance at a wavelength of 525 nm, i.e. at the isobestic point of the three chemical forms of myoglobin. Chromatic absorbance at 525 nm is directly proportional to the amount of pigment reached by the light, i.e. the amount of pigment involved in the formation of colour.

Krzywicki (1979) states that when the path length of light in the tissue in any two samples of meat is the same, then the value of the chromatic absorbance at a wavelength of 525 nm is directly proportional to the pigment content in these samples. In any other case, the pigment content is not directly proportional to the amount of pigment that affects colour. Therefore, in studies on the effects of the amount of pigment and the relative amount of the chemical forms of myoglobin on meat colour values, measurements of its reflectance/absorbance are more useful than the determination of the pigment content of the meat. This applies primarily to those muscles with low pigment levels and colour parameters which are largely influenced by the meat structure. One such muscle is the most widely studied muscle in pig carcasses – *m. longissimus* – whose quality indicates the quality of muscles in other parts of the carcass, especially in the ham (Warner, Kauffman, & Russel, 1993).

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The aim of this paper was to acquire more knowledge about the effects of pigment, achromatic absorbance and the relative amounts of the chemical forms of myoglobin on the colour parameters measured in the *longissimus lumborum* muscle by applying the CIELAB and CIELCh ratings, using an illuminant D65 and standard observer 10°.

2. Materials and methods

2.1. Animal and sampling

The animal material consisted of samples of the *longissimus lumborum* muscle taken from 102 carcasses weighing 70–110 kg (of which there was an equal proportion of each sex) obtained from pigs which were crosses of Polish Large White x Polish Landrace sows with Duroc x Pietrain boars, which had been slaughtered in an industrial production line. The pigs were slaughtered at about 6 months of age. Meat samples weighing about 1 kg (meat on the bone) were collected after about 24 h of chilling at 0–4 °C, from the segment between the 1st and 4th lumbars in the right half of the carcass. The samples were wrapped in foil, transported to the laboratory in a vacuum flask and stored until the following day at 4 °C.

2.2. Physicochemical characteristics

About 48 h after slaughter the meat was separated from the bone, the external fat and perimysium were removed and then the meat was ground twice on a meat grinder with a 4 mm mesh. After that, the meat colour and pH were measured. The percentages of dry matter, protein and fat were also determined.

2.2.1. Colour measurements

Colour measurements were taken after keeping the ground meat samples in measurement cells in a household refrigerator at 4 °C for 20 min to enable myoglobin oxygenation on the surface layer of the meat. The 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 ground meat, applying the CIELAB and CIELCh scales (CIE, 1976, 1978), D65 illuminant and 10° standard observer recommended for meat colour measurements (Honikel, 1998). Standardisation of the apparatus was carried out with reference to black and white colour standard references with the following coordinates: X = 78.5, Y = 83.3 and Z = 87.8 (for D65 illuminant and 10° observer).

The relative content of Mb, MbO_2 and MetMb was calculated from the reflectance curve according to Krzywicki (1979) using 700 nm (the highest wavelength of the instrument) instead of 730 nm. The instrument measures the reflectance between 400 nm and 700 nm at 10 nm intervals. Reflectance values at wavelengths not given by the instrument (473, 525 and 572 nm) were calculated using linear interpolation. Reflectance values were converted into absorbance values according to the formula: $A = 2 - \log 10R$, where A is absorbance and R is reflectance.

Colour measurements using the CIELAB and CIELCh scales and reflectance measurements were performed using duplicate standards, which permitted the values of all the colour parameters of a given sample and its reflectance values to be obtained from a single measurement.

2.2.2. pH measurement

pH measurement was performed 48 h after slaughter using a combined glass electrode (ESAgP-306W type) with a CyberScan 10 pH-metre (Eutech Cybernetics Pte Ltd., Singapore) in water extract (distilled water) with a 1:1 meat to water ratio, after 1 h of extraction.

2.3. Proximate analysis

The following chemical constituents were measured on thawed samples of ground meat according to the official methods of analysis of the AOAC (2003): moisture content by oven drying *c.* 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).

2.4. Statistical analysis

The results of the aforementioned measurements were analysed using the procedures of STATISTICA 8 (Statsoft, Tulsa, OK). Mean and standard deviations were calculated for the examined characteristics. Simple and partial correlation coefficients and coefficients of determination were also determined, as was their significance at $P \leq 0.01$ and $P \leq 0.001$.

3. Results and discussion

3.1. Determination of the relative content of the forms of myoglobin in the surface layer of pork meat according to the Krzywicki method

According to Krzywicki (1979), the absorbance value at 730 nm in bovine meat represents achromatic light absorption. This value is subtracted as a correction in the calculation of the absorbance of pigments at a wavelength of 525 nm and the relative content of the three chemical forms of myoglobin in the surface layer of meat. A common problem when applying the Krzywicki (1979) is the range of available spectrophotometers, which usually only cover the range 400–700 nm; therefore they do not permit the measurement of the reflectance/absorbance of light at a wavelength of 730 nm.

For this reason, some authors use a correction of achromatic absorption values, measured at other wavelengths, in a section of the spectrum where the impact of pigments is very small. It is known that the impact of pigments is already substantially reduced at wavelengths of 650–660 nm, and absorbance values in the section of the spectrum beyond these wavelengths show only small differences, generally concerning muscle with low pigment content and thus with poorly developed reflectance spectra and their extremes.

In this experiment, the correction of achromatic light absorption was absorbance at 700 nm, the longest wavelength on the instrument, which probably had no significant effect on the results. In addition, Lindahl, Lundström, and Tornberg (2001), using the Krzywicki (1979), replaced the value of absorbance at 730 nm with the absorbance at a different wavelength (710 nm). In certain cases those authors observed reflectance maxima at wavelengths of 690–700 nm. In this study too in several cases the reflectance values were slightly higher at a wavelength of 690 nm than at a wavelength of 700 nm.

3.2. Relationship between the content of dry matter, crude protein and intramuscular fat, pH and the colour parameters, total and chromatic absorbance at a wavelength of 525 nm, absorbance at a wavelength of 700 nm and the relative content of the forms of myoglobin

Table 1 shows the average and standard deviation of colour parameters; total and chromatic absorbance at a wavelength of 525 nm; absorbance at 700 nm; the relative content of MbO_2, MetMb and Mb, and the percentage in the meat of dry matter, crude protein, intramuscular fat, and pH. Table 2 presents the simple correlation coefficients between the percentage of dry matter, protein and fat content in the meat, pH value and the parameters of colour and total and chromatic absorbance at 525 nm (A_{525} and A_{525p}), absorbance at 700 nm (A_{700}) and the relative content of MbO_2, MetMb and Mb.