The use of visible and near infrared reflectance spectroscopy to predict colour on pork muscle

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Introduction

Colour is an important component of quality throughout the agricultural and food industries. In both the agricultural and the food industry the most popular numerical colour space systems is the L^*, b^* and a^*, which is also referred as the CIELAB system, originally defined by the CIE in 1976. [1] In the CIE system L^* is the measurement of the lightness of the colour of the sample, a^* measures the red and green characteristics, and b^* measures the yellow and blue characteristics. One of the most important quality traits of fresh pork in the decision to purchase by the consumer is the appearance or colour of meat. [2, 3] Several factors affect the meat colour: the post mortem glycolysis rate, the intramuscular fat, the pigment level (myoglobin) and the oxidative status of the pigment. [2] The perception of colour is very dependent on the observer, and for these reason it is important to know the value of relating objective colour measurements to the subjective judgement of acceptable pork colour. Recently, reflectance spectrophotometers had span the near infrared range to the visible range and they have been available for research and industrial purposes. Combining both the visible and near infrared region in one instrument makes a vast improvement in efficiency related to instrumental, sampling and analytical cost It was reported by several authors elsewhere that the spectra obtained would be related to chemical properties and taste panel assessments of meat. [4, 5] Routine assessment of meat composition using such optical devices is now widely used in the oilseed, milk and cereal industry for quality purposes. [6] Because many quality characteristics are associated with visible characteristics such as colour, the visible region may soon prove to be as useful as the near infrared region. [1] The use of NIRS as a rapid method by the industry could gave the capability to increase control checks during meat processing and retailing. The aim of this work was to explore the use of visible and near infrared spectroscopy to predict CIElab values in pork muscle samples.

Materials and Methods

Forty-four (n=44) pork muscle (*longissimus thoracis*) samples were obtained after a feeding trial which compared the use of both commercial feeds and pastures to finishing pigs (T1 = 100% commercial feed; T2, T3 and T4 different proportions of commercial feed and pastures) (data not presented). Pigs were slaughtered (aprox. 100 kg live weight) under commercial conditions (stunned

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electrically, exsanguinated, scalded, de-haired, eviscerated and split into sides), where no treatments at slaughter were carried out. Slaughter procedures conformed to the National Meat Institute of Uruguay (INAC - Uruguay). Carcass weight and length were measured after 24 hours of slaughter. The samples were taken after slaughter (carcass weight after 24 hours aprox. 84 kg). Pork muscles were taken from the 10^{th} rib, wrapped in aluminium foil and kept on freezer (two weeks) before analysis. About 100 to 200 g of muscle was thawing at room temperature (20 – 22 ^{0}C) and homogenised during one to two minutes with a food multiprocessor blender (Philips RI - 3142, Brazil). The blender cup was washed first with hot water, followed by cold water and towel dried between samples. Before colour measurement, samples were thawed at room temperature (20 - 22 ^{0}C) for 12 hours. The CIE L^*, CIE a^* and CIE b^* values were determined on the intact muscle using a digital camera chroma meter (CR 10 Minolta Co. Ltd, Osaka, Japan). The camera averages the colour reading from an 8-mm measured area, with a standard illuminant D and has an 8/d geometry. Each data was the mean of three applications. Intact samples were prepared by cutting slices parallel to the longitudinal orientation of the muscle fibres (60 to 80 mm x 20 to 50 mm x approximately 20 mm thick) from the thawed muscle sample. Samples were scanned both intact and minced in the reflectance mode (400 – 2500 nm) in a scanning monochromator NIRS 6500 (NIRSystems, Silver Spring, MD, USA). Spectra collection and multivariate analysis were performed using NIRS 2 software, version 3.01, from Infrasoft International (ISI, Port Matilda, PA, USA). Each spectrum was collected in the visible and near infrared range at 2 nm intervals. First, intact samples (aprox. 15 - 20 mm thickness) were scanned in a rectangular cup (100 mm x 50 mm) (Part number IH – 0395, NIRSystems, USA). Immediately after homogenisation, minced samples were scanned in a circular cup (50 mm diameter, 10 mm depth) (Part number IH – 0307, NIRSystems, USA) sealed with disposal paper back. Reflectance data were stored as log (1/R) (where R: reflectance) at two nm intervals. Spectra for both minced and intact samples were collected without rotating samples. Two pairs of lead sulphide detectors collected the reflectance spectra and were referenced to corresponding readings from a ceramic disk. The spectrum of each muscle sample is the average of 32 successive scans. Predictive equations were developed using modified partial least square (MPLS) regression with internal cross-validation and scatter correction Standard Normal Variate (SNV) and detrend transformations. [7, 8] Cross validation estimates the prediction error by splitting the calibration samples into groups (four in this study). One group (n: 11) was reserved for validation and the remaining groups were used for calibration (n: 33). The process was repeated until all groups have been used for validation once. The outlier elimination pass was set to allow the computer program to remove outliers twice before completing the final calibration. [7] Two mathematical treatment were applied to the spectra (1,4,4,1) and (2,5,5,2). The first number indicates the order of derivative (one is the first derivative of log 1/R), the second number is the gap in data points over which the derivative is calculated; the third number is the number of data points used in the first smoothing and the fourth number refers to the number of data points over which the second smoothing is applied. Calibration statistics calculated included the standard error of calibration (SEC), the coefficient of determination in calibration (R^2_{cal}), the standard error of cross validation (SECV) and the coefficient of determination in cross validation (R^2_{val}). [8] The optimum calibrations were selected based on minimising the standard error of cross validation (SECV).

Results and Discussion

Figure 1 shows the visible and near infrared mean spectrum of pork muscles on both intact and homogenised presentation. In the visible region, absorption band at 418 nm was associated with Soret absorption and absorption bands at 542 nm and 572 nm were associated with respiratory pigments, principally myoglobin. [9, 10] Absorption bands in the near infrared region at 980 nm and at

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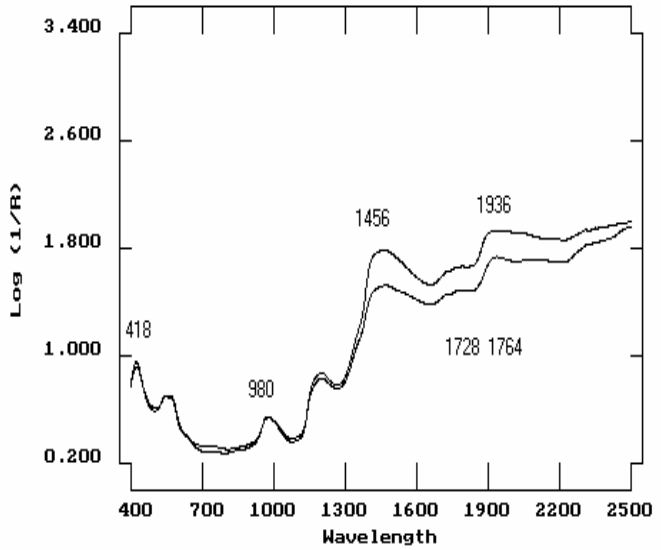

Figure 1. VIS and NIR mean spectrum of INTACT and HOMOGENISED pork samples.

Table 1. Near infrared calibration statistics CIE *L*, *a* and *b* on intact pork samples

	n	SD	Mean	SEC	R^2	SECV
VIS + NIR						
CIE *L*						
1st D + SNVD	43	5.6	48.7	4.7	0.30	5.6
2nd + SNVD	43	5.6	48.7	4.6	0.30	5.3
CIE *a*						
1st D + SNVD	42	2.2	6.9	1.0	0.80	1.5
2nd + SNVD	43	2.3	7.1	1.2	0.72	1.9
CIE *b*						
1st D + SNVD	40	1.5	8.6	0.9	0.60	1.1
2nd + SNVD	42	1.6	8.6	1.3	0.30	1.5

1456 nm were related with OH second and first overtone respectively. [11, 12] Absorption bands at 1728 nm and 1764 nm were related with CH first overtone, at 1936 nm with OH first overtone (water) and both at 2308 nm and 2348 nm with CH combination tones. [11, 12] High correlation were found between wavelength and CIElab values at 538 nm, 638 nm and 1108 nm for L^*, at 592 nm and 632 nm for both a^* and b^* values respectively, on intact presentation. Correlation's at 924 nm, 1452 nm and 1934 nm with L^* value, at 580 nm with a^* value and at 536 nm, 570 nm and 810 nm

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with b^* value on homogenised presentation. CIElab L^* value was highly correlate with water absorption bands on homogenised presentation. Although no individual pigments were measured in this work, high correlation was found between the CIElab values and the corresponding absorption of the individual pigment in the visible region. Table 1 shows the NIRS calibrations and cross validation statistics for CIE L^*, CIE a^* and CIE b^* values in both intact and homogenised presentation. On the intact presentation, the CIE a^* values gave the best coefficients of determination on both visible and visible + near infrared region ($R^2_{cal} > 0.60$). In the intact samples, the different levels of organisation of tissue affect the information obtained from the sample such as: myofibrillar birefringence, myoglobin and protein precipitation in the sarcoplasm, macroscopic surface reflectance properties of cut meat, and the sarcomere length. [10, 12] The structure of the muscle affects how the light is trapped or scattered from the sample and probably interferes for information that the instrument could read from the sample. In bulk meat samples (intact samples) the muscles fibers or myofibrils themselves may act as "optical fibers" tending to conduct light along their length by a series of internal reflections. As well as muscle structure, the pH, intramuscular fat patches collagen, protein coagulation surface, and moisture, causing light scattering. [10, 12] Packing of tissue behind a quartz window will also deform muscle fibre orientation effects and alters the penetration of the light through the muscle sample. These factors could explain the low correlation obtained for both L^* and b^* values. However, the VIS/NIR region showed high correspondence with respiratory pigments measured as a^* value. The homogenise presentation shows the best calibration statistics for both the CIE L^* and CIE a^* values, coefficients of determination (R^2_{cal}) > 0.70. Homogenisation of the sample severely alters the structure of the muscle, destroying and randomising the fiber arrangement of the muscle as well as averaging the effects of scattering by the fibers in the tissue. [10, 12] In muscle homogenates, the sample preparation, the chemical analysis and the subsampling to perform the chemical analysis remaining the variables that can affect the accuracy and reproducibility of the near infrared analysis. In particular this is true for the determination of intramuscular fat and pigments. The results obtained in these study suggest that VIS/NIR instruments (400 - 2500 nm) have an excellent potential to provide information related with the CIE system (L^* and a^*) on pork muscles samples. In the future VIS/NIR colour measurements combined with other meat characteristics (chemical and physical) could provide a more accurate information of pork meat quality.

Table 2. Near infrared calibration statistics CIE *L*, *a* and *b* on homogenised pork samples.

	n	SD	Mean	SEC	R^2	SECV
VIS + NIR						
CIE *L*						
1st D + SNVD	44	5.6	48.4	3.6	0.58	4.5
2nd + SNVD	44	5.6	48.3	3.4	0.62	4.5
CIE *a*						
1st D + SNVD	40	2	6.3	1.2	0.70	1.5
2nd + SNVD	41	2.1	6.8	1.5	0.46	1.7
CIE *b*						
1st D + SNVD	43	1.7	8.8	1.6	0.10	1.7
2nd + SNVD	42	1.6	8.7	1.4	0.24	1.5

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Conclusions

Measuring colour on visible/near infrared spectrophotometer allows to simultaneously determine multiple meat characteristics. Combining colour and chemical constituents in one instrument increase efficiency of analysis of muscles and meat under industrial conditions. It must be pointed out that the pork meat muscles studied in this work arose from a small number of animals (n= 44) and that more trial is required to accurate report the general predictive ability of NIR to predict colour. Further work will be done to incorporate more samples as well as different muscles to perform near infrared calibrations for both colour and chemical characteristics.

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