



## Prediction of pH and color in pork meat using VIS-NIR Near-infrared Spectroscopy (NIRS)

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### Abstract

The potential of near-infrared spectroscopy (NIRS) to predict the physicochemical characteristics of the porcine longissimus dorsi (LD) muscle was evaluated in comparison to the standard methods of pH and color for meat quality analysis compared to the pH results with Colorimeter and pH meter. Spectral information from each sample ( $n = 77$ ) was obtained as the average of 32 successive scans acquired over a spectral range from 400 - 2498 nm with a 2 - nm gap for calibration and validation models. Partial least squares (PLS) regression was used for each individual model. An  $R^2$  and a residual predictive deviation (RPD) of 0.67/1.7, 0.86/2, and 0.76/1.9 were estimated for color parameters  $L^*$ ,  $a^*$ , and  $b^*$ , respectively. Final pH had an  $R^2$  of 0.67 and a RPD of 1.6. NIRS showed great potential to predict color parameter  $a^*$  of porcine LD muscle. Further studies with larger samples should help improve model quality.

**Keywords:** carcasses; color analysis; *longissimus dorsi*; partial least squares regression; pH analysis.

**Practical Application:** Development of prediction curves for evaluation of pork meat quality.

## 1 Introduction

Meat is one of the most nutritional foods in the human diet (Kamruzzaman et al., 2012) thus, clear parameters of pork meat quality are necessary for the consumer to be able to evaluate the quality of the product at the point of purchase (Joo et al., 2013). Meat quality depends on several factors, including animal nutrition, environmental conditions, genetics and sex of the animal, production system, pre-slaughter management and slaughter procedure (Rosenvold & Andersen, 2003), as well as post-mortem glycolysis, which may influence the physical characteristics of the meat (van Oeckel et al., 1999). There is no predefined meat quality standard: the quality of meat is associated with characteristics that are closely related to muscle pH (Pearce et al., 2011), and acceptance of the meat by the consumer often results from the visual evaluation of its color (Barbut et al., 2008). However, standard methods for quality evaluation of meat products, such as color and pH, are often rather imprecise and time consuming. Near-infrared spectroscopy (NIRS) is fast, reliable, accurate and inexpensive (Prevolnik et al., 2004; Teye et al., 2013) and has great potential as a substitute for chemical composition analysis of meat and meat products (Andrés et al., 2007).

A number of studies on the chemical composition of pork meat used NIRS, in the reflection and absorption modes (Barlocco et al., 2006; Chan et al., 2002; Horiuchi et al., 1999;

Lanza, 1983; Savenije et al., 2006; Tøgersen et al., 1999). The differences observed across studies may be related to differences in instrument calibration, sample size, sampling location, statistical methodology, and physical conditions at the sampling locations (industry or laboratory) (Kapper et al., 2012a). Other studies used NIRS for meat color evaluation (Kang et al., 2001; Liu et al., 2004; Roza-Delgado et al., 2013; Xing et al., 2007). In addition, NIRS was used to predict the pH of pork meat (Prieto et al., 2009). The objective of this work was to develop and evaluate prediction equations, based on measurements obtained in the laboratory, for a rapid definition of the parameters of pH and color in pork quality.

## 2 Materials and methods

### 2.1 Meat samples

Seventy-seven male and female commercial hybrid swines with average live weight of 110.6 kg were slaughtered according to Brazilian regulations, and their carcasses were processed according to standard slaughter guidelines. Carcasses were then chilled (0-2 °C) for 24 h and meat samples were collected from the (LD) muscle between the 12<sup>th</sup> and 13<sup>th</sup> ribs. Samples were transported under refrigeration to the Universidade Estadual de Londrina (Londrina State University – UEL) Animal Science Laboratory, Londrina, Paraná State, Brazil. Samples were sliced

Received 17 Aug., 2017

Accepted 02 Apr., 2018

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to 25 - mm thick slices for measuring color and final pH using the conventional methodology (see below) and NIRS.

## 2.2 Conventional measurements of color (CIE L\*a\*b\*) and pH

Meat color was evaluated with a portable Minolta® CR-10 colorimeter (Konica Minolta, Inc., Osaka, Japan) at 48 h post-mortem, when the biochemical changes in meat can be perceived (Kim et al., 2014). Meat color was expressed in the three parameters (lightness [L\*], redness [a\*], and yellowness [b\*]) developed by the Comission Internationale de l'Eclairage (CIE) (Commission Internationale de l'Eclairage, 1978; Honikel, 1998; Karamucki et al., 2011). Final pH values were measured 24 hours after slaughter using a Testo 205 pH meter (Testo AG, Lenzkirch, Germany).

## 2.3 NIR spectral measurement

Spectral information from each sample was obtained as the mean of 32 successive scans in the reflectance mode ( $\log 1 / R$ ) over a 400 - 2498 nm spectral range, using an XDS™ Rapid Content Analyzer (Foss A/S, Hillerød, Denmark). The surface cell moisture was cleaned after each measurement by cleaning with ethanol (70% v / v) and following of distilled water. The dataset included 77 meat samples divided in two groups, the first consisting of 50 samples for the calibration model (training dataset) and another group of 27 independent samples for the validation model (testing dataset).

## 2.4 Statistical analysis and partial least squares (PLS) calibration model

Spectral data were analyzed with Unscrambler software version 9.7 (Camo, Trondheim, Norway). Partial least squares (PLS) regression was used for statistical evaluation of spectral measurements and the data are expressed as mean and standard deviation and variation coefficient.

PLS regression is a statistical method used to predict a set of independent variables, framing a predictor matrix (n samples + w wavelengths), by reducing the number of original predictors and the dimensionality of the regression problem, compacting the number of predictors to a new variable latent thus denominated (Geladi & Kowalski, 1986).

The calibration model developed was based on equation 1 ( $y = Xb + E$ ), where  $y$  is the unit matrix for instrumental (n samples  $\times$  1),  $X$  is the predictor unit matrix (n samples  $\times$  w wavelengths),  $b$  demonstrate the coefficient unit matrix obtained from the PLS analysis, and  $E$  signify the unit matrix with the residual information that is not contained in the previous predict (Osborne et al., 1993). The ideal value of latent variables was found by the minimum prediction value of the summation of squares prediction error (PRESS) (Elmasry et al., 2011).

The data quality of the statistical set was analyzed for the analysis of the validation set for each model, based on the proportion coefficient of determination ( $R^2$ ), the mean square error of calibration (RMSEC), mean square error of cross-validation (RMSECV) (Geesink et al., 2003). The equations of evaluation of the prediction model were also determinants for assessing the quality of the model in cross-validation (Balage et al., 2015).

The PLS regression models were constructed so as to maximize the data variation capacity with all preformulation data using the leave-one-out cross-validation (LOOCV), which is a model validation technique to evaluate the validity External. The corresponding quadratic cross-validation error (RMSECV), cross-validation prediction errors and PLS regression are respectively obtained (Chen et al., 2005). The residuals of each model were used to construct the prediction model and calculate the (RMSECV) to determine the best predictive efficiency model for each characteristic, being the lowest value for each formula (Hubert & Branden, 2003).

In the cross-validation process, the model was evaluated for its prediction capability using the determination coefficient of calibration ( $R^2c$ ), standard error of calibration (SEC), determination coefficient of cross-validation ( $R^2cv$ ), and standard error of cross-validation (SECV) (Qu et al., 2005).

The predictive capacity of the equations were evaluated using residual prediction deviation value (RPD) (Williams, 1987). The residual predictive deviation (RPD), calculated as the ratio of the standard deviation of the reference parameters chemistry (SD) and the standard error of cross-validation (SD/SECV) its considered excellent when  $\geq 3$ . In addition, the RPD should ideally be at least three, taking into account that the variation in the reference data is low, the values for  $R^2$  and RPD cannot be high (Pérez-Marín et al., 2004; Williams & Sobering, 1996).

The range error ratio (RER) is the range of reference techniques values without a predictive set for RMSEP (Pérez-Marín et al., 2004). The (RER) value is obtained by calculating the division of the concentration amplitude of an analyte by the mean square error of calibration (RMSECV), where a model with ratio of error range (RER) values  $< 3$  has small predictive capability, models with RER between 3 and 10 have low to moderate practical utility, and RER values  $> 10$  indicate good practical utility (Williams, 1987). According to Millmier et al. (2000), RER values  $> 12$  indicate high predictability.

An  $R^2$  value of 0.80 is a referential measure for concrete multiple regression models (Shiranita et al., 2000). Reliability analysis indicates that a RPD  $> 3$  and RER  $> 10$  are required to improve the quality analysis indicate a good classification (Dagnew et al., 2004).

## 3 Results and discussion

Descriptive statistics of meat quality attributes are presented in Table 1. The color parameter  $a^*$  presented a better variation of data than the others, and the standard deviation (SD) was 27.8% of the difference between the maximum and minimum

**Table 1.** Descriptive statistics for color parameters ( $L^*$ ,  $a^*$ ,  $b^*$ ) and final pH ( $pH_u$ ) of porcine *longissimus dorsi* muscle determined by conventional methods (colorimeter).

Parameter	Mean $\pm$ SD (%)	CV <sup>a</sup> (%)	Range (min-max)
$L^*$	54.9 $\pm$ 3.3	5.9	46.2-63.9
$a^*$	6 $\pm$ 1.7	27.8	2.8-9.7
$b^*$	11.3 $\pm$ 1.3	11.2	8.6-14.9
$pH_u$	5.5 $\pm$ 0.1	2.3	5.3-5.8

<sup>a</sup>CV (%) = coefficient of variation.

**Table 2.** NIRS calibration and prediction statistics for color parameters ( $L^*$ ,  $a^*$ ,  $b^*$ ) and final pH of porcine *longissimus dorsi* muscle.

Parameter	LVc <sup>b</sup>	LVcv <sup>c</sup>	R <sup>2</sup> c <sup>d</sup>	R <sup>2</sup> cv <sup>e</sup>	SD <sup>f</sup>	SEC <sup>g</sup>	SECV <sup>h</sup>	SEC–SECV <sup>i</sup>	RPD <sup>j</sup>	RER <sup>k</sup>
$L^*$	5	3	0.67	0.63	3.3	1.7	1.9	0.9	1.7	9.4
$a^*$	16	11	0.86	0.72	1.7	0.6	0.9	0.7	2	8
$b^*$	6	6	0.76	0.65	1.3	0.6	0.7	0.8	1.9	9.2
pH <sub>u</sub> <sup>a</sup>	5	5	0.67	0.62	0.1	0.1	0.1	0.9	1.6	6.2

<sup>a</sup>pH<sub>u</sub> = final pH; <sup>b</sup>LVc = latent variable of calibration; <sup>c</sup>LVcv = latent variable of cross-validation; <sup>d</sup>R<sup>2</sup>c = calibration coefficient; <sup>e</sup>R<sup>2</sup>cv = cross-validation coefficient; <sup>f</sup>SD = standard deviation; <sup>g</sup>SEC = standard error of calibration; <sup>h</sup>SECV = standard error of cross-validation; <sup>i</sup>SEC–SECV = difference between SEC and SECV; <sup>j</sup>RPD = residual standard deviation; <sup>k</sup>RER = range error ratio.

values of this parameter, indicating that the data can guarantee a significant calibration.

The mean lightness of meat samples measured with a colorimeter was 54.9 (Table 1), indicating that this sample of pork meat was pale-colored (Warner et al., 1997). The mean values for color parameters  $a^*$  and  $b^*$  (Table 1) are similar to those reported by van der Wal et al. (1988), who considered  $a^*$  and  $b^*$  values of 6.3 and 13.7, respectively, as normal for pork meat. Likewise, final pH (Table 1) had a mean value within the normal range of 5.4–5.8 suggested by Fischer (2007) as normal for pork LD.

The NIRS regression model (Table 2) showed good predictive value for color parameter  $a^*$ , with an  $R^2$  of 0.86, but a low RPD, as an RPD around 2 indicates that the equation is promising but should be improved. Those values were similar to the  $R^2$  of 0.82 and RPD of 2.6 reported by Balage et al. (2015) for  $a^*$ , also considered low by those authors. The prediction equation for  $L^*$  also yielded  $R^2$  and RPD values (Table 2) that were unsatisfactory, and similar to those reported by Kapper et al. (2012b), measuring 685 pork LD samples from four slaughterhouses ( $R^2$  = 0.70 and RPD = 1.82). Likewise, for color parameter  $b^*$  the correlation between the model standard deviation and the observed standard deviation was weak, as shown by the values of  $R^2$  and RPD in Table 2. Similar weak correlation equation values were reported by Čandek-Potokar et al. (2006), using NIRS to predict pork quality traits ( $R^2$  = 0.76 and RPD = 1.6). Finally, the poor performance of the calibration parameter for final pH (Table 2) may have been due to the small variability of final pH in the samples, as Hoving-Bolink et al. (2005) argued that a large number of samples are needed to minimize the effects of pork meat variation and to improve the quality of prediction equations. Andersen et al. (1999) found similar values in the prediction model for pH ( $R^2$  = 0.62 and RPD = 1.66) of processed pork meat.

As shown in Table 2, the protein prediction model required 16 latent variables for prediction and 11 for cross validation for the parameter of model  $a^*$ , which could be an indication of overlap and presence of noise in the model. As for the other parameters of color and pH, the orthogonal data presented lower values of latent variables for validation and cross-validation in the different models of latent variables of color  $L^*$  and  $b^*$  and pH, being  $L^*$  5 and 3,  $b^*$  6 and 6 and 5 and 5, respectively. The value obtained for latent variables can be changed by the measured attribute difference (Burger & Geladi, 2006).

The models developed for color  $L^*$ ,  $a^*$ ,  $b^*$  and pH presented mean values for RER with 9.4, 8, 9.2 and 6.2, respectively. Second,

a classification description by Millmier et al. (2000), the model with values between 8 to 12 can describe models for analyzing data from parameter indicators.

#### 4 Conclusion

The conventional methods used for meat analysis at laboratory scale are laborious and time-consuming, and difficult to use under production plant conditions. NIRS showed reasonable predictive potential for meat quality traits, especially color parameter  $a^*$ , yielding moderate to good predictive value. Further research with larger samples and different experimental conditions should help improve model quality.

#### Acknowledgements

We would like to thank the funding agencies that supported this study, CAPES and FAEPE/UEL (PUBLIC 2016).

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