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Assessing the Value of a Portable Near Infrared Spectroscopy Sensor for Predicting Pork Meat Quality Traits of “*Asturcelta* Autochthonous Swine Breed”

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Abstract Sixty-one intact meat samples from *Asturcelta* autochthonous swine breed were scanned in the slaughterhouse in reflectance mode. A handheld microelectromechanical system digital transform (Phazir1624, Polychromix Inc.), with a window sampling area of 0.8×1 cm and wavelengths ranging from 1,600 to 2,400 nm, was used. With the spectra database recorded were developed different chemometrical models assaying first and second derivatives as math treatment and standard normal variate (SNV) and multiplicative scatter correction for minimizing scattering effect. The greatest predictive capacity was achieved after applying SNV and first derivative for moisture, intramuscular fat (IMF) content, and pH parameters and second derivative for CIE L^*, a^*, b^* colorimetric values, and the Warner–Bratzler force (instrumental texture). The coefficients of determination for calibration ranged from 0.63 to 0.89. The ratio between the standard error of the laboratory and the standard error of calibration ranged from 0.8 to 2.5 for all parameters (1.7 on average) with the exception of *b* and pH with ratios of 3.5 and 4.1, respectively. The statistical values obtained for the models developed to estimate IMF, CIE L^*, a^*, b^*, moisture, and pH, displayed acceptable predictive capacity. For instrumental texture, the model could be able to discriminate among tender, medium, and hard meat in carcasses for characterization slaughter purposes.

Keywords NIR spectroscopy · Portable NIR sensor · Quality parameters · Pork meat · *Asturcelta* swine breed

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Introduction

There are several and different autochthonous swine breeds all over countries, such as Cinta Senese breed (Crovetti et al. 2007), Nero siciliano (D’Alessandro et al. 2007), Mangalica (Hungary) (Egerszegi et al. 2003), or Celtic-Iberian Bísara (Royo et al. 2007), between others. In the northwest of Spain (Asturias), we can find a particular breed known as *Gochu Asturcelta*, a local swine breed that was almost extinguished in the late nineties. In 2007, with the purpose of gathering the last few specimens spread by several villages in this area and recover this unique breed, this local breed was recognized by the National Register (BOE 2007) through the Pig Breeders Association (ACGA) at the Ministry for Agriculture and Fishery’s request. With the combined effort of the Regional Institute for Agro-food Research and Development (SERIDA) and the ACGA, in 2011, a total of 1,711 animals were supposed to exist (SERIDA 2012).

The *Gochu Asturcelta*—indigenous designation for this specimen—breed has long been known for its efficiency in gaining weight and its peculiar physiognomy. Characterized by their small and shiny eyes, long and pendent ears, elongated hair with black and white spots, these animals can achieve a body length of 1.5 m from the head to the tail insertion. At 12 months age, the average weight was between 180 and 190 kg (de la Roza-Delgado et al. 2012). Raised in a semiextensive system, these animals have possessed their excellent carcass quality from primeval times. The quality of the *Asturcelta* meat is distinctive because it has a greater proportion of lean meat intermixed with streaks of fat. This intramuscular fat content (IMF) gives more marbling to the meat as compared to other more popular breeds in meat consumption taking into consideration that IMF content of pork muscle is considered to be a key factor that influences the sensory characteristics of both meat and meat products (Fernández et al. 1999; Morlein et al. 2005).

The Regional Government from Asturias (Spain) has established a systematic and structured program to avoid extinction and enhance the population of *Asturcelta* autochthonous swine breed, which is necessary in carrying out a methodology to establish a fast and recognized standard quality of this autochthone meat, using innovative technologies for large-scale screening of meat quality traits. The use of rapid spectroscopic techniques for quality inspection of meat has been successfully demonstrated for a number of quality aspects of pork. Amongst these are the fatty acid composition of pork fat (García Olmo et al. 2001), IMF (BrØrdum et al. 2000), drip loss, tenderness (Geesink et al. 2003), and pH (Savenije et al. 2006). Most of these applications were from moderately successful to successful, depending on instrumentation, wavelength range, or scanning meat samples intact or minced.

Recently, an innovative near infrared reflectance spectroscopy (NIRS) technology based on a combination of microelectromechanical system (MEMS) and digital transform spectroscopy has been employed to develop a portable spectrometer. This new device allows the compilation of spectra data “in situ,” working on reflectance mode. Several recent studies have evaluated this technology in the agro-food area (fruits and feeds) (Pérez-Marín et al. 2009, 2010; Fernández-Pierna et al. 2010) and pork meat (Zamora-Rojas et al. 2012a). Nevertheless, this cutting-edge technology needs to be assessed in its application to the analysis of different samples and/or presentations for non-destructive analysis, because, in intact meat samples, the muscle fibers or myofibrils themselves may act as optical fibers tending to conduct NIR light along their length by a series of internal reflections, absorbing more energy and giving less reflectance when comparing with homogenized meat.

The purpose of the present study was to investigate the usefulness of a novelty handheld MEMS-NIRS device for predicting pork meat quality traits of *Asturcelta* autochthonous swine breed on intact samples in the slaughterhouse to assess the suitability of NIRS technology to establish a physicochemical characterization of the *Asturcelta* meat.

Materials and Methods

Animals and Samples

This study has involved 61 female and male *Asturcelta* swine (n=61) breed animals with an average live weight of 185 kg, collected between 2010 and 2012. These animals belonged to the SERIDA located in Villaviciosa, Spain (43°28′50″ latitude and 5°26′27″ longitude). The animals were reared in a semi-extensive system including a (25:75) barley–rye concentrate. The animals were transported to a local abattoir, stunned in the head with a captive bolt, slaughtered, and dressed according to current European Union regulations. The carcasses were cooled at 4 °C (relative humidity, 98 %) for a day, according to the routine slaughterhouse procedure. After that, the samples were obtained from the 7th to the 11th rib on the longissimus dorsi (LD) muscle. These samples were placed in a clean bag and transported under refrigeration conditions to the laboratory.

Instrumental and Chemical Analysis

The pH of the half left carcass was measured directly after a 24-h post-mortem period on the seventh rib using a Mettler Toledo SG2 (0.01 pH resolution (1 mV) with an accuracy of ±0.01 pH (±1 mV) with a temperature probe. Regarding the color parameters by the CIE L^*, a^*, b^* values, three measures were collected on intact meat on the LD muscle using a Minolta CM-200 colorimeter. To measure the instrumental texture (Warner–Bratzler shear force, WBSF), meat samples were placed into a bag in a heated water bath (70 °C at the core of the muscle). After 1 h in a cold bath and 30 min at room temperature, the muscles were cut (the cut line was parallel to the direction of the muscle fibers) in pieces of about 3 cm long and 1 cm^2 wide and the maximum shear force measured (six repetitions per sample) in kilogram force with an *InstronTA.XT.Plus* device equipped with a Warner–Bratzler cell.

Chemical analyses were performed using traditional standard methods. Moisture content was measured by oven-drying at 102 °C (ISO 1442:1997), and IMF was estimated by Soxhlet extraction (ISO 1443:1973).

The standard error of the laboratory (SEL) procedure has been estimated as standard deviation of reproducibility/repeatability of the measures of each sample (the standard deviation of sampling distribution). All the analyses have been repeated three times with the exception of texture, repeated six times.

Near Infrared Spectroscopy Analysis

Intact meat samples were scanned in reflectance mode with a nonconstant interval of around 8 nm (pixel resolution, 8 nm; optical resolution, 12 nm) over the NIR spectral range (1,600–2,400 nm) using a handheld MEMS digital transform (Phazir 1624, Polychromix Inc., Wilmington, MA, USA), with a window sampling area of 0.8×1 cm. This instrument has been equipped with a special quartz protection to avoid dirt accumulation. Ten spectra were taken for each meat sample, and each spectrum was the mean of ten scans. All spectra were recorded as log (1/*R*), *R* being the reflectance and collected over the transverse section of muscle to obtain sufficient representative information of the full sample.

Data Analysis

The collected data were converted into a data matrix. The *X* and *Y* variables were defined as: *X* wavelength and *Y* log 1/*R*. Calibration development was performed in two parts; pretreatments and mathematical treatments were applied to the spectra using the Unscrambler v. 9.8 software (Camo, Inc. 2008). As spectral pre-treatments, the standard normal variate (SNV) and multiplicative scatter correction (MSC) (Barnes et al. 1989) procedures were assayed, together with first and second derivatives as mathematical treatment. The regression model was performed using partial least squares. The validation of models was made using a random cross-validation method with 20 segments and three samples per segment. To select the best equations, the statistics evaluated were: the lowest standard error of calibration (SEC) and standard error of cross validation (SECV), the highest coefficient of determination for calibration (R^2) and coefficient of determination for cross validation (r^2), and the ratio SECV/SEC≤1.33 to facilitate the comparison of the results obtained (Williams 2001).

Results and Discussion

The ranges, means, standard deviations, and standard error of the laboratory for chemical composition and technological parameters of *Asturcelta* pork meat samples are given in Table 1. Descriptive statistics provide an overview of the structure of the samples used to develop the model. This sample set should ideally ensure uniform distribution of composition across the range of the studied parameter. For chemical composition, the ranges were similar to those recorded in previous work for predicting meat quality on ground Iberian pork muscles (Zamora-Rojas et al. 2011). In all measured parameters, the standard deviation (SD) was 18–26 % of the difference between the maximum and minimum values of that parameter. This indicates that the data set contains a sufficiently large variation to allow a meaningful calibration.

Lightness (*L**), redness (*a**), and yellowness (*b**) mean values indicate that the meat of *Asturcelta* pig breed provides darker, redder, and yellower meat than commercial pig breeds (Vieira et al. 2012). The moisture content is lower and the fat content is higher than commercial pig breeds. Regarding WBSF, mean values obtained correspond with tender meat, in relation with pH values, they are within the range considered normal and characteristic of animals that have not undergone stress long before slaughter.

Table 1 Statistics for meat quality attributes of *Asturcelta* pork samples (*N*=61)

	Range	Mean	SD	CV	SEL
*L**	36.69–81.49	48.13	10.33	0.215	8.27
*a**	0.93–9.75	4.44	1.912	0.431	3.95
*b**	3.97–9.66	7.12	1.453	0.204	4.03
Moisture (%)	63.70–72.87	68.80	1.888	0.027	2.17
IMF (%)[a]	3.86–12.93	7.30	2.073	0.284	1.46
Texture (kgf)	1.93–9.23	4.46	1.282	0.287	2.68
pH 24 h	5.34–6.17	5.75	0.169	0.029	0.58

IMF intramuscular fat content, *kgf* kilogram force (1 kgf=0.10197 N), *SD* standard deviation, *CV* coefficient of variation, *SEL* standard error of laboratory

[a] In wet weight

Figure 1 shows the NIR mean spectra for the sample set as log 1/*R*, and after application of the SNV algorithm and first derivative, a normal shape and similar pattern for all samples at all the wavelengths is observed. As can be seen in Fig. 1, water is the dominant tissue chromophore with specific absorbance of O–H bands around 1,940 nm (Osborne and Fearn 1986). This spectral feature along with absorption band at 1,720 and 2,200 nm (related to fat content) is the most relevant (Garrido-Varo et al. 2004; Burns and Ciurczak 1992). This constituent in pork meat represents near 75 % of total fresh matter.

The developed models, after applying first derivative and SNV, displayed the greatest predictive capacity for moisture, fat, and pH parameters. For colorimetric parameters on meat, *L**, *a**, and *b**, and for instrumental measure of texture, the calibration models constructed yielded better precision by applying the second derivative. There were found differences between maximum and minimum cross-validation errors (SECV) for all parameters, as a result of the effect of the combination of the mathematical pre-treatments applied. Statistics of the predictive models are given in Table 2. The averaged ratio between the SECV and the SEC was 1.24 for chemical and technological parameters, with the exception of *L** (SECV/SEC=1.50), indicating robust calibrations (Burns and Ciurczak 1992). The SECV values are close to the calculated SEL for the reference methods in most of the parameters. As shown in Table 1, the SECV is equal to or lower than SEL, except for IMF. The ratio between the SEL and the SECV ranged from 0.8 to

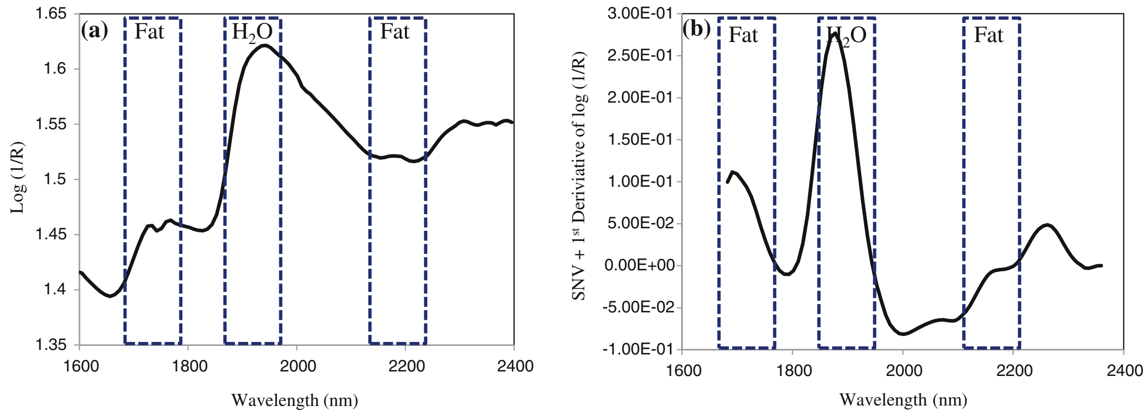

Fig. 1 NIR mean spectra of raw intact *Asturcelta* swine meat included in the calibration set: **a** log (1/*R*), **b** standard normal variate and first derivative

2.5 for all parameters (1.7 on average) with the exception of *b* and pH with ratios of 3.5 and 4.1, respectively. These ratios are very acceptable with regard to the accuracy of the calibration (Savenije et al. 2006).

Regarding the literature, results of studies evaluating NIR spectroscopy for predictions of *L**, *a**, and *b** are contradictory (Prieto et al. 2009). In this research work, the results after applying SNV and second derivative as mathematical treatments, for all color-related parameters, *L**, *a**, and *b**, displayed R^2 and r^2 values that ranged between 0.89–0.75 and 0.74–0.59, respectively, using a MEMS device with a small scan window which does not work in the optimum region for predicting color in the visible region of the spectrum (VIS), and applied on intact muscle. Despite these device limitations, our results are in accordance with Savenije et al. (2006) that working at-line with a Foss NIRSystem 6500, including VIS range (wavelength range from 400 to 2,500 nm), and scanning minced meat samples reached acceptable results, with correlation coefficients between 0.89 and 0.81. In the same way, Prieto et al. (2008) on cattle meat samples achieved coefficients of correlation up to 0.8 between absorbance data and *L** and *b** values at 1,230–1,400 and 1,600–1,710 nm, which correspond to C–H second overtone, C–H combination bands, and C–H first overtone, respectively (Burns and Ciurczak 1992). These wavelengths are related to absorbance of the C–H bonds present in IMF. Thus, *L** and *b** values could be predicted by means of NIR spectra insofar as they were correlated to IMF. However, other researches (Hoving-Bolink et al. 2005) found worse results (R^2=0.31) on intact pork muscle samples using a diode array VIS/NIR instrument equipped with a surface fiber optic probe working in the range between 380 and 1,700 nm. Probably, the most interesting results that appeared with the calibrations developed for colorimetric

Table 2 Statistics of chemometric models to predict quality parameters in the *Asturcelta* pork samples and cross-validation parameters

	Derivative+SNV	SEC	R^2	SECV	r^2
*L**	2nd	4.45	0.89	6.71	0.74
*a**	2nd	1.22	0.75	1.48	0.60
*b**	2nd	0.95	0.75	1.16	0.59
Moisture (%)	1st	1.32	0.67	1.46	0.58
IMF (%)[a]	1st	1.62	0.63	1.77	0.53
Texture (kgf)	2nd	0.80	0.73	1.06	0.48
pH 24 h	1st	0.11	0.75	0.14	0.55
	Derivative+MSC	SEC	R^2	SECV	r^2
*L**	2nd	4.26	0.92	7.51	0.74
*a**	2nd	1.42	0.71	1.76	0.50
*b**	1st	1.09	0.60	1.29	0.38
Moisture (%)	1st	1.49	0.61	1.62	0.52
IMF (%)[a]	1st	1.66	0.54	1.79	0.43
Texture (kgf)	2nd	1.30	0.26	1.47	−0.24
pH 24 h	2nd	0.10	0.73	0.13	0.56

IMF intramuscular fat content, *kgf* kilogram force (1 kgf= 0.10197 N), *SNV* standard normal variate, *MSC* multiplicative scatter correction, *SEC* standard error for calibration, *SECV* standard error for cross validation, R^2 determination coefficient for calibration, r^2 determination coefficient for external validation

[a] In wet weight

parameters are, firstly, the possibility of analyzing an intact meat sample and, secondly, the use of portable devices.

For moisture, the best calibration model displayed values for R^2 and r^2 of 0.67 and 0.58, respectively. The water specific absorbance band in the NIR spectra of meat explains NIR predictability for this component. For IMF content, the statistical values obtained indicate that the model could be able to discriminate between high, medium, and low IMF meat content in carcasses for classifications slaughter proposes. In the same way, moderate correlation was found for moisture and IMF parameters by Barlocco et al. (2006) on intact samples of pork muscles using an at-line scanning monochromator (400–2,500 nm).

The results obtained in this research work are in accordance with those coming from other researches and with other NIR instruments. Prieto et al. (2009) established that the ability of NIR spectroscopy to predict the content of main chemical components such as moisture, crude protein, and fat is more accurate in minced meat samples than in intact tissues. In intact samples, the muscle fibers may conduct light along their length and they could cause internal reflections. To avoid this effect, Geladi et al. (1985) proposed a chemometrical strategy to separate the chemical light absorption from the physical light scatter of meat; it was the use of MSC. This algorithm was also applied to spectral information, collected in this research work, before carrying out NIR calibrations. Models constructed after applying MSC leave worse statistics than SNV, except for L^* and pH parameters (see Table 2).

In relation with the prediction of pH value, day 1 postmortem, the model provided statistics for this parameter of R^2=0.75 and r^2=0.55. pH is very interesting to evaluate the stress long before slaughter, but the results obtained with calibration models constructed confirmed a limited viability of NIRS technology for estimating this parameter.

It was observed that the statistical values obtained with the model constructed to predict instrumental measure of texture indicate that they cannot be used routinely for quantitative prediction, but they could be applied to discriminate among tender, medium, and hard meat, establishing ranges of quality for this parameter in carcasses for classification slaughter purposes.

In order to evaluate all the statistical results of the developed models, Williams (2001) recommend that if the sample set is small (lower than 60), the evaluation of the calibration must be done by cross-validation procedure, using parameters such as ratio error range (RER) values (range/SECV). Williams (2001) and Pérez-Marín et al. (2005) highlight the importance of both sample set size and sample distribution within the calibration set, noting that sample sets for calibration should ideally ensure uniform distribution of composition across the range of the study parameter in question. The predictive capacity of the developed models achieved good or moderate classification for all the parameters evaluated, with averaged RER values of 5.97. These results highlight the difficulty in correlating destructive measurements made to an intact pork meat and non-destructive in situ NIR measurements. In addition, it must be pointed out that the *Asturcelta* pork meat studied in this work arose from a small number of animals and that further experimental work is required to accurately report the general ability of NIR to characterize carcasses of this autochthonous swine breed according to chemical and technological parameters. Thus, Zamora-Rojas et al. (2012b) showed the possibility to transfer large databases from a NIRS scanning monochromator to a handheld MEMS-NIRS to quantify fat, moisture, and protein composition instrument employing ground pork samples. It is worth noting in this respect that NIRS analysis in this study was carried out on intact meat samples in the slaughterhouse.

In summary, the ability of NIR spectroscopy to predict chemical characteristics using intact samples had been widely demonstrated (Prieto et al. 2009), but nowadays, the implementation of quality controls (in situ or on-line) such as in the slaughterhouse line and at industrial level by using a handheld MEMS instrument is of great importance. In this context, it is significant that improvements and progress into NIR instruments more adapted for on-site analysis allow to develop new applications with real time response.

Conclusions

The specific absorbance band in the NIR spectra of intact meat of the *Asturcelta* pork breed explains the results, showing statistics (SECV and R^2) that allow us to predict simultaneous meat parameters quantitatively. The statistical values obtained for the models developed to estimate IMF, CIE L^*, a^*, b^*, moisture, and pH displayed acceptable predictive capacity indicating that the models could be applied routinely to quantify the quality parameters of carcasses of this autochthonous swine breed. For Warner–Bratzler shear force, the model could be able to discriminate among tender, medium, and hard meat in carcasses for characterization slaughter proposes.

Over the coming years, however, after increasing the number of animals, further researches are necessary to develop more robust and new chemometrical models, especially for texture, using larger sample sets with wide ranges according to chemical and technological reference parameters, allowing us to characterize carcasses of this autochthonous swine in the abattoir. The consumers demand well-known specific characteristics of meat that differ from the most commonly consumed meat. Raised in a semiextensive or extensive system, it is presumable that the carcass and meat obtained from the *Asturcelta* swine breed could be different from the most common pork commercialized.

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Conflict of Interest Begoña de la Roza-Delgado, Ana Soldado, Antonio F. Gomes de Faria Oliveira, Adela Martínez-Fernández, and Alejandro Argamentería declare that they have no conflict of interest and that all institutional and national guidelines for the care and use of laboratory animals were followed.

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