Authentication of Iberian pork official quality categories using a portable near infrared spectroscopy (NIRS) instrument

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Abstract

A portable near infrared spectroscopy (NIRS) instrument was evaluated for the discrimination of individual Iberian pig carcasses into the four official quality categories (defined by a combination of genotype and feeding regime). Spectra were obtained scanning four anatomical locations (live animal skin, carcass surface, fresh meat and subcutaneous fat samples) at a commercial abattoir, using a handheld micro electro mechanical system instrument. The best assignments into official quality categories with the NIRS measurements in the carcass surface and subcutaneous fat were able to correctly classify 75.9% and 73.8% of the carcasses, respectively. Moreover, 93.2% and 93.4% of carcasses were correctly classified according to feeding regimes by using the spectra from fresh meat and subcutaneous fat samples. The results suggest that, using subcutaneous fat samples, a portable NIRS could be used in commercial abattoirs as a tool to support the control of official quality category assignment in Iberian pig carcasses.

Keywords: carcass; classification; Duroc; fat; meat; pig

1. Introduction

Iberian pork products enjoy great prestige worldwide due to their exceptional organoleptic properties (Ventanas, Ruiz, García, & Ventanas, 2007). Traditionally, Iberian pigs have been raised in extensive production systems known as *'Dehesa'*, a natural system where the animals graze natural resources and use the acorn from *Quercus* genus in the final fattening phase. Due to geographical limitations of the Dehesa and to annual variations of acorns productions in this area, Buxadé (2002) estimated that only about 15% of Iberian pigs were produced using purebred animals and fed with acorns and grass. In recent years, demand of Iberian pig products has increased (Pugliese & Sirtori, 2012), leading to a rise in the Iberian census and the development of new strategies for meat production, including crossbreeding with Duroc pigs (Ramírez & Cava, 2007) and the use of concentrate during finishing.

In order to ensure the traceability in policy procedures for labelling and authentication of Iberian pig products, a new official classification system was developed considering both genotype and feeding regime (BOE, 2014). The regulation contemplates four quality categories: ***Black*** (100% Iberian pigs finished on acorns); ***Red*** (at least 50% Iberian pigs finished on acorns); ***Green*** (at least 50% Iberian pigs fed on grass and supplemented with concentrate; outdoor systems) and ***White*** (at least 50% Iberian pigs fed on concentrate; indoor systems). Rigorous inspections on farms and abattoirs are carried out to control authentication of breed purity and feeding systems. However, these controls are costly, require manpower and cannot provide individual animal information.

Several studies have confirmed the ability of near infrared spectroscopy (NIRS) to predict meat quality attributes (Prieto, Roehe, Lavín, Batten, & Andrés, 2009). Handheld NIRS instruments have been previously applied on live Iberian pigs and

carcass backfat for the classification of individual carcasses based on feeding regimes (Pérez-Marín, De Pedro, Guerrrero-Ginel, & Garrido, 2009; Zamora-Rojas, Pérez-Marín, De Pedro-Sanz, Guerrero-Ginel, & Garrido-Varo, 2012). Recently, Prieto, Juárez, Dugan, Zijlstra, & Aalhus (2016) proposed the use of NIRS on pig ears as a rapid method to discriminate live animals according to total fat content and fatty acid composition. Hence, NIRS has the potential to become a powerful tool for carcass classification and sorting for different markets (Pérez-Marín et al., 2009).

The aim of the present study was to evaluate the effectiveness of using a handheld NIRS instrument to classify individual carcasses within the four official quality categories defined for Iberian pork, including for the first time a combination of genetic purity and feeding regime, through the scanning of four anatomical locations (live animal skin, carcass surface, subcutaneous fat and fresh meat). Furthermore, those discrimination results for official commercial categories were compared to the ability of the instrument to discriminate according to feeding regime using the same spectral data set.

2. Materials and methods

2.1. Animal population

Seven hundred and sixty-three Iberian pigs (purebred Iberian and Iberian-Duroc crossbreds) from six commercial farms (south-western Spain) were used for this study. Animals were slaughtered in two commercial abattoirs (Council Regulation (EC) Nº 1099/2009 of 24 September, 2009), during two consecutive winter seasons (2014/2015 and 2015/2016). Individual carcasses were classified within the four official categories for Iberian pig carcasses (BOE, 2014), as following: ***Black*** (n=176), ***Red*** (n=182), ***Green*** (n=196) and ***White*** (n=185). Certification of feeding regimes was performed in

86 farm by staff accredited in on-field inspection and Iberian breed purity of the ***Black***
87 group was confirmed by DNA analysis (Carrodeguas, Burgos, Moreno, Sánchez,
88 Ventanas, Tarrafeta, et al., 2005).

89 *2.2. Spectra collection*

90 NIRS spectra were collected using a handheld micro electro mechanical system
91 spectrophotometer (MicroPhazir 1624, Polychromix, Inc., Wilmington, MA, USA).
92 Reflectance spectra were obtained through non-constant intervals of approximately
93 8 nm across the NIR wavelength range of 1600 to 2400 nm, with integration time of 600
94 ms and with a window area of about 4 mm^2. Performance of measures was checked
95 every 10 min through white reference measurements, using Spectralon as standard.
96 Anatomical locations tested in this study (Figure 1) included live animal skin (duplicate
97 spectra were collected at about 4ºC over the skin without shearing or clipping of hair in
98 the caudal loin region), carcass surface (one hour after slaughter, three spectra were
99 collected on the surface of the left carcass side, over the ***semimembranosus*** muscle),
100 fresh meat (four hours after slaughter, three spectra were collected on the left ***psoas***
101 ***major*** muscle) and subcutaneous fat (two hours after slaughter, three spectra were
102 collected in range 8-10 ºC directly on the backfat of the left carcass side).

103 *2.3. Management of outlier samples and selection of training and validation sets*

104 Spectra processing and transformation, as well as development and validation of
105 discriminant models, were performed using WINISI v.1.5 (Infrasoft International, State
106 College, PA). During the development of discrimination models, outlier data were
107 identified and removed. Principal component analysis was carried out for each of the
108 four anatomical locations in order to calculate the centre of the spectral populations. The
109 Mahalanobis distance (H) between each sample and the center of the spectral population
110 was calculated, identifying as outliers those samples with a H value greater than 3

(Shenk & Westerhaus, 1995). The remaining sub-spectra were averaged and the final sample set comprised 666, 737, 738 and 739 spectra corresponding to live animals, carcass surface, fresh meat and subcutaneous fat, respectively (Table 1).

During the spectra collection phase, a similar number of animals was included in each quality group and anatomical location, in order to achieve a balanced calibration model (Sánchez, Flores-Rojas, Guerrero, Garrido-Varo, & Pérez-Marín, 2010), while trying to include largest possible number of samples in order to maximize individual variability in the models (Pérez-Marín et al., 2009). The smallest number of selected spectra corresponded to live animals (87.3% of total studied animals), due to the more complicated conditions for spectra collection. The percentage of spectra selected from carcass surface, fresh meat and subcutaneous fat were 96.6%, 96.7% and 96.8%, respectively (Table 1).

Adequate selection of representative samples into model and test is crucial, as both of them should include all the variables affecting spectral features, they have to be placed at boundary of the category and filling the group space uniformly (Pieszczek, Czarnik-Matusewicz, & Daszykowski, 2018). In this work samples for training and validation sets were selected according to Zamora-Rojas et al. (2012) by calculating the Global Mahalanobis (GH) distance to the centre of the spectral population after spectral pre-treatment (SNV and Detred and derivative 1,5,5,1), sorting all samples from lower to higher GH values and selecting one of every four samples in each quality category (*Black*, *Red*, *Green* and *White*) for the validation set. Selection of samples for calibration and validation sets were performed for subcutaneous fat, the most homogeneous spectral data set. Afterwards, the same samples were selected for the other three anatomical locations.

2.4. Discriminant analysis algorithm

Discriminant analysis was selected to perform qualitative modes because, in this particular study, the number of quality categories is finite (Pieszczek et al., 2018). There are only four quality categories for Iberian pigs in accordance with Spanish regulations. Therefore, each of the animals that arrive at the slaughterhouse must be assigned to one of the four quality groups. In order to classify samples into each of the four categories for each anatomical location, a partial least squares discriminant analysis (PLS2-DA) was applied. PLS2-DA algorithm correlates spectral variations and category classes, trying to maximize the covariance between both of them. In our case, the dependent variables (quality categories) were not continuous, but categorical "dummy" variables defined by assigning different values to the different groups to be discriminated (Naes, Isaksson, Fearn, & Davies, 2002). The optimum number of PLS factors for the models was selected by 4-fold cross-validation.

Development of discriminant models was carried out using raw spectra and different spectral pre-treatments for the correction of scatter phenomena, like Standard Normal Variate (SNV) and De-trending (DT) (Barnes, Dhanoa, & Lister, 1989). Furthermore, four derivate mathematical treatments were tested: 1,5,5,1; 2,5,5,1; 1,10,5,1 and 2,10,5,1; where the first digit is the number of the derivative, the second is the gap over which the derivative was calculated, the third is the number of data point in a running average or smoothing, and the fourth is the second smoothing segment (Shenk, Westerhaus, & Abrams, 1989). Obtained discrimination models were evaluated according to the percentage of samples correctly classified during calibration development and, subsequently, with external validation. Calibrations with lower classification errors in validation were selected. As additional figures of merit, sensitivity (SE) and specificity (SP) of discrimination models were calculated according

to the following expressions combining the number of true positives (TP), true negatives (TN), false positives (FP) and false negatives (FN) obtained in validation:

$$SE=TP/(TP+FN)$$

$$SP=TN/(TN+FP)$$

SE gives the proportion of animals belonging to one specific group that are correctly identified, while SP informs about the correct identification of animals not belonging to that group.

3. Results and discussion

3.1. Spectral features

Obtaining high-quality spectra is critical for the characterization of official quality categories and thus for the construction of effective discriminant models. Spectral data between 1600 and 2392 nm obtained from live animal, carcass surface, fresh meat and subcutaneous fat are show in Figure 2. A first look at live animal and carcass surface spectra reveals a similar spectral pattern explained by the coincidence of optical features of both scanned regions. As observed in Figures 2a and 2b, the dominating bands were mainly related to O-H stretching vibrations absorbing mainly at frequencies around 1900 nm (fundamental constituent of water molecules; Prieto et al., 2016). The same water absorption band was observed for fresh meat samples (Figure 2c). Moreover, subtle peaks around 1725 nm, corresponding to C-H stretching vibrations associated to fat, can be observed for fresh meat samples (Shenk, Westerhaus, & Workman, 1992). Whereas spectral features for live animal, carcass surface and fresh meat displayed similar shapes, subcutaneous fat exhibited a different spectral pattern (Figure 2d), similar to those showed by Pérez-Juan, Afseth, González, Díaz, Gispert, Font i Furnols et al. (2010) and Zamora-Rojas et al. (2012) for transverse and longitudinal cuts of

185 backfat from the Pietrain × Large White × Landrace or Iberian pigs, respectively. As
186 observed in Figure 2d, the dominating bands were mainly associated to sharp peaks at
187 1725 nm (C-H stretch first overtone), around 1850-1900 nm (O-H stretching vibrations)
188 and 2200-2300 nm (C-H bend second overtone). According to Shenk et al. (1992) and
189 Prieto et al. (2016), those bands correspond to C-H stretching vibrations (fat molecules)
190 and O-H (water molecules). Above 2300 nm, absorbance values for subcutaneous fat
191 ranged between 2.5 and 3.0. According to Kapper, Klont, Verdonk, & Urlings (2012),
192 an absorption value (log1/R) of 2.8 implies that only 0.16% of total emitted light
193 reaches the detector, so a limited amount of useful data is available at this spectral
194 region. In this sense, adipocytes could act as optical fibres conducting light by internal
195 reflections (Prieto et al., 2009). Transmission of light along the fat tissue might have
196 contributed to the relatively high absorption values observed above 2300 nm.

197 Spectra obtained from the surface of live animals and carcasses using the portable
198 NIRS instrument showed an irregular pattern associated to spectral noise above 2200
199 nm, while at lower wavelengths noise was not noticeable. Even though scans were
200 collected along the clean surface section chosen on live animals, spectral noise may
201 have been observed due to the presence of dirt, discrepancies in surface shape and
202 fissures of the skin (Pérez- Marín et al., 2009). Spectra of fresh meat samples (Fig. 2c)
203 displayed areas with low signal/noise ratio. Noisy trends around 2000–2400 nm, higher
204 than those observed for live animal or carcass surface, were observed, reflecting sample
205 heterogeneity rather than failure in instrument performance. Moreover, higher noisy
206 tendency in this spectral region was also affected by the presence of blood and
207 intramuscular fat, fissures of the tissue and sample surface shape (Zamora-Rojas et al.,
208 2012). The conduction of light along the muscle fibres might have also contributed to
209 the relatively high noise levels in the spectra between 2000–2400 nm. Myofibrils or

intact muscle fibres may act as optical fibres and conduct light along their fibrils and by internal reflections (Prieto et al., 2009).

In relation to subcutaneous fat samples, the spectral data displayed the lowest noise levels. However, some noisy areas were found above 2250 nm, mainly between 2290 and 2390 nm (Fig 2 d). This range is associated to the absorption of the C–H bonds of fatty acids in fat tissue (Shenk et al., 1992). Backfat is composed of two adipose tissue sub-layers with slight differences in fatty acid profiles (Daza, Ruiz-Carrascal, Olivares, Menoyo, & López-Bote, 2007) that could cause sample heterogeneity and, thus, spectral discrepancies.

3.2. Discriminant models

In a first stage, the ability of discriminant models to classify carcasses was evaluated in the four commercial quality groups. The descriptive statistics for the best calibration models developed using the handheld NIRS instrument at four anatomical locations in Iberian pig are shown in Table 2. The best validation results for live animal and carcass surface spectra were obtained with the use of raw spectra (without scatter correction/derivative pre-treatments), while SNV followed by DT, together with a second derivative (2,10,5,1) provided the best validation results for fresh meat and subcutaneous fat spectra. Thus, it can be highlighted that using carcass surface and subcutaneous fat spectra, about 75% of samples in the validation set were correctly classified according to commercial quality categories, while around 65% of samples were correctly classified using live animal and fresh meat spectra. The best results predicting quality categories were found for carcass surface, where 183 of 241 samples in validation (75.9%) were correctly classified. For fresh meat, sensitivity for the ***Black*** category was higher than for other quality categories (84.5%). This result, together with a specificity value of 82.8%, is remarkable, as ***Black*** quality products reach the highest

prices in the market. For spectra obtained from subcutaneous fat, 180 samples (~74%) were correctly classified in their quality category. The discriminant model again achieved higher sensitivity values for the *Black* quality class (77.6%) and 89.8% specificity. The higher discrimination ability observed for models developed for carcass surface or subcutaneous fat spectra could be related to the reduced noisy areas at both anatomical locations. However, the worse classification results observed for live animal or fresh meat could be explained by optical heterogeneity. As previously described, the presence of dirt on the surface of the skin of live animals could interfere with the resolution of spectra and, thus, increase classification errors. Furthermore, the content of myofibril proteins in fresh meat samples (determined by genetics factors) could interfere with the response to transmission of light signal along the muscle fibres.

The homogeneity and authenticity of the *Black* group was tested by DNA analysis, which confirmed the inclusion of 100% Iberian pig animals. A greater variability in the percentage of breed purity was observed for the *Red*, *Green* or *White* groups, ranging from 50 to 75% of breed purity. This genetic variability could be responsible for some misclassifications observed for these three categories. As was reported by Josell, Martinsson, Borggaard, Andersen, & Tornberg (2000) and McDevitt, Gavin, Andres, & Murray (2005) our results showed that genetics, feeding system and the individual variability of the animals can influence the correct assignment of commercial categories in Iberian pigs.

In a second phase, discrimination models were performed to predict feeding regimes (Table 3). In general, for all anatomical locations, the discrimination models resulted in higher percentages of correctly classified samples than for commercial categories. These results are in agreement with those reported by Zamora-Rojas et al. (2012) using feeding system categories corresponding to the previous Spanish

legislation for Iberian pig. Taking into account these findings, the use of portable NIRS instruments could be very useful for the classification of animals according to their feeding regimes. The highest predictive capacity was again obtained for the models developed with the spectra of subcutaneous fat (93.4%), followed by carcass surface (90.9%), live animals (88.7%) and fresh meat (85.2%). In fact, using subcutaneous fat, sensitivity and specificity values for the Acorn samples (corresponding to *Black* and *Red* categories) in the validation set were 93.6 and 93.3%; respectively, compared to 90.2 and 95.8% for the Concentrate samples (corresponding to *Green* and *White* categories). This is reasonable, since feeding regimes directly affect the fatty acid profile of animals. Thus, the subcutaneous fat spectra displayed clear and sharp bands associated to fat (Figure 2d) with little or no interference from water, and greater sample homogeneity. Worse results were observed for fresh meat for the classification of feeding regimes (Table 3). For this anatomical location, 83.9% of Acorn samples and 86.5% samples of Concentrate were correctly classified. Perez-Marín et al. (2009) and Prieto et al. (2016) reported favourable results for NIRS classification by feeding regime of Iberian and white pigs, respectively. When comparing predictive models to estimate fatty acid profile from the spectra collected on live animal, carcass surface and subcutaneous fat from Iberian pigs, Perez-Marín et al. (2009) reported that best predictive models were also obtained for the visible and NIR region from subcutaneous fat.

4. Conclusions

The handheld NIRS device used in this study could be successfully applied in commercial abattoirs to classify Iberian pig carcasses into the four official categories (*Black*, *Red*, *Green* and *While*) or by feeding regime. However, the discrimination

ability of the NIRS device is higher for feeding system than for the official quality categories, including genotype and productions system.

Out of the four scanning areas evaluated, carcass surface and, especially, subcutaneous fat are recommended in order to make the best assignation of quality category. The on-site portable NIRS system evaluated in this study could enable acceptable discrimination of carcasses in commercial abattoirs according to the official system, without interfering or disturbing the processing chain. Further work is in progress to develop robust NIRS models with larger populations in order to implement this system as routine analysis.

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388

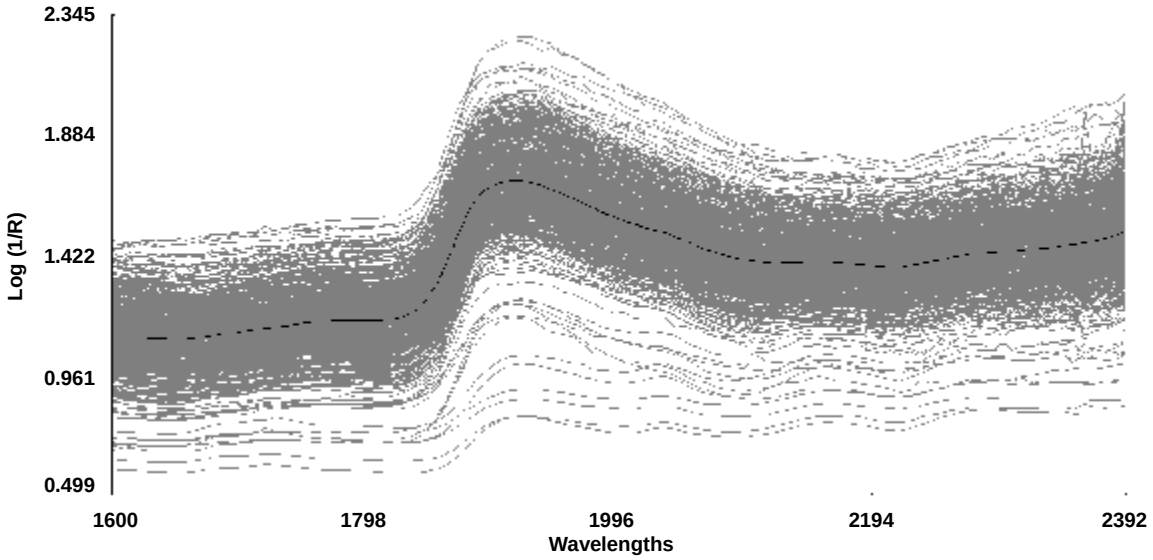
389

a) b)

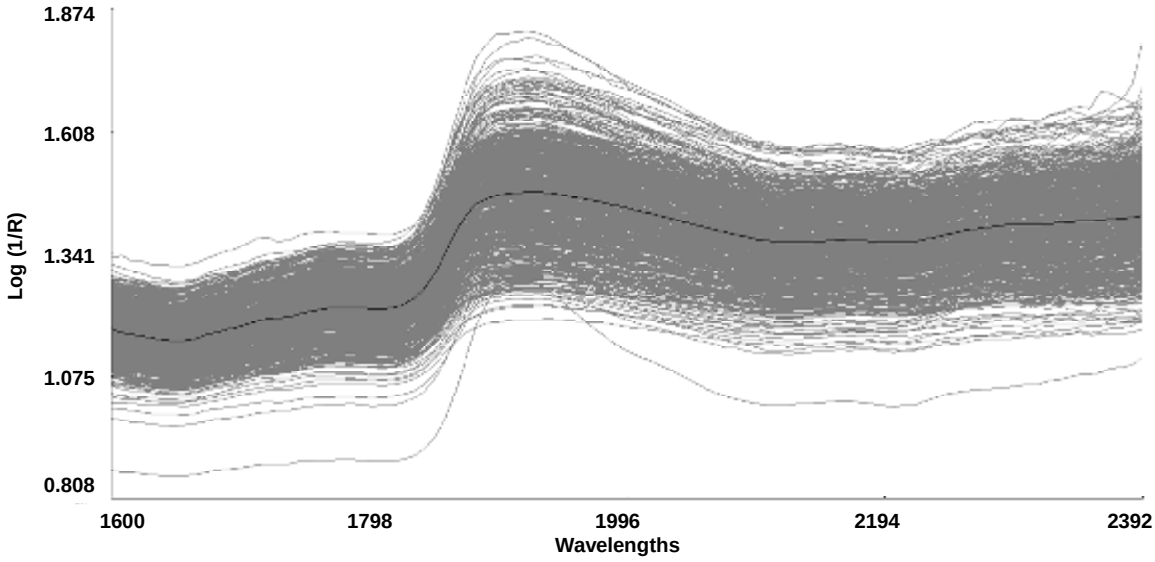
c) d)

390 **Fig. 1.** In-situ NIRS analysis performed with a handheld MEMS instrument on live
391 animal (a), carcass surface (b), fresh meat (c), and subcutaneous fat (d).

392


2.345
1.884
1.422
0.961
0.499
Log (1/R)
1600
1798
1996
2194
2392
Wavelengths

a)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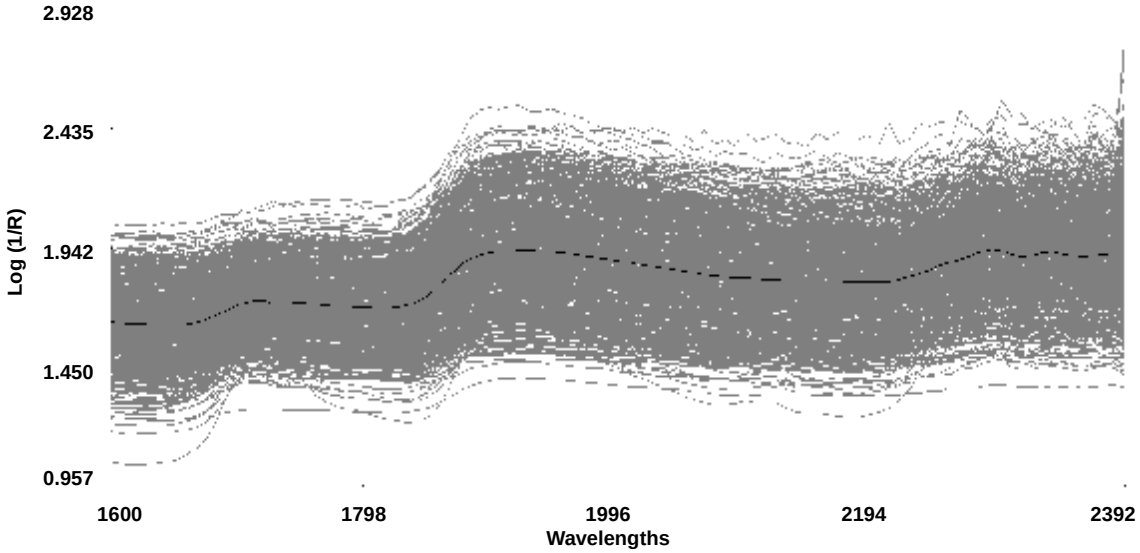
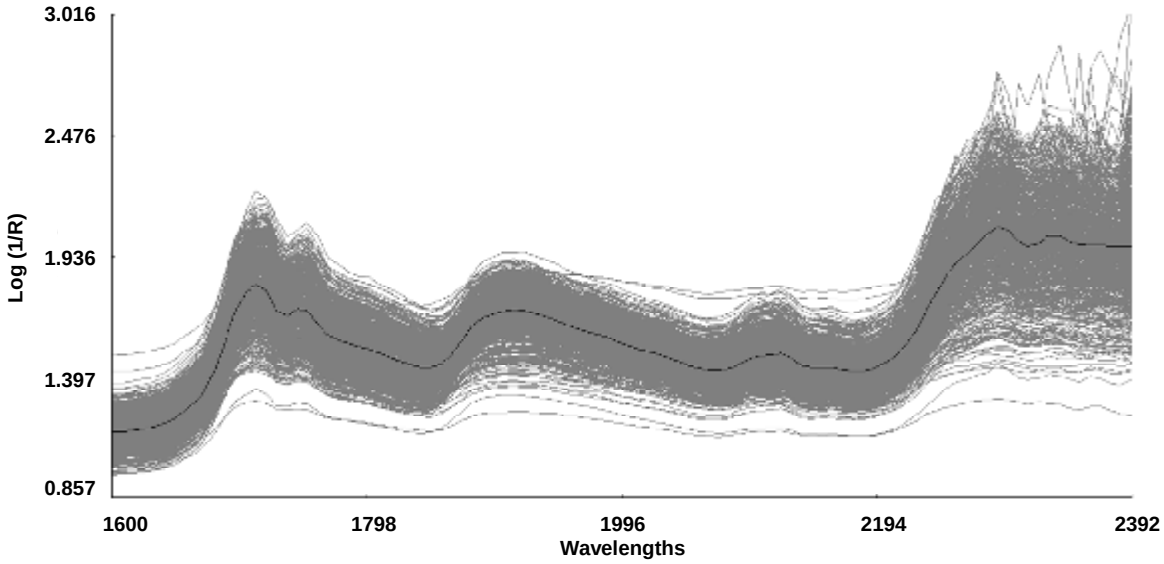
1798
1996
2194
2392
Wavelengths

b)



c)



d)

393 **Fig. 2.** NIR spectral data set and average spectra (black line) collected in four
394 anatomical location of Iberian pigs: live animal (a), carcass surface (b), fresh meat (c),
395 and subcutaneous fat (d).

Table 1
Number of samples for each anatomical location and quality category

	Quality category											
	Black			*Red*			*Green*			*White*		
	Total	Calibration	Validation	Total	Calibration	Validation	Total	Calibration	Validation	Total	Calibration	Validation
Live animal	134	91	43	176	118	58	191	122	63	165	111	54
Carcass surface	175	118	57	182	122	60	195	132	63	185	124	61
Fresh meat	176	118	58	182	122	60	196	131	65	184	123	61
Subcutaneous fat	176	118	58	182	122	60	196	131	65	185	124	61

Table 2
Classification results within the official Iberian pork quality categories, according to discriminant models determined in four anatomical locations

			Global results		Validation results by category							
			Correctly classified (%)		Black		Red		Green		White	
	Spectral pre-treatmet	PLS	Calibration	Validation	SE	SP	SE	SP	SE	SP	SE	SP
Live animal	None/0,0,1,1	9	59.3	65.1	51.2	91.1	89.6	90.9	57.9	58.2	57.4	86.1
Carcass surface	None/0,0,1,1	15	80.2	75.9	75.4	91.3	75.0	93.9	79.4	93.8	73.8	88.9
Fresh meat	SNV+DT/2,10,5,1	12	61.9	63.1	84.5	82.8	38.3	93.5	50.8	92.2	80.3	82.5
Subcutaneous fat	SNV+DT/2,10,5,1	9	66.9	73.8	77.6	89.8	71.7	91.8	73.8	92.7	72.1	90.7

PLS: number of partial least squares terms; SE: sensitivity; SP: specificity.

Table 3
Classification results within feeding regimes (acorn and feed), according to discriminant models determined in four anatomical locations

			Global results		Validation results by category			
			Correctly classified (%)		Acorn		Concentrate	
	Spectral pre-treatmet	PLS	Calibration	Validation	SE	SP	SE	SP
Live animal	SNV+DT/1,5,5,1	13	87.3	88.7	85.7	90.6	83.6	91.4
Carcass surface	None/0,0,1,1	15	92.5	90.9	88.0	92.9	90.5	91.2
Fresh meat	SNV+DT/2,10,5,1	12	82.6	85.2	79.1	88.9	82.8	86.9
Subcutaneous fat	SNV+DT/2,10,5,1	9	93.1	93.4	93.6	93.3	90.2	95.8

PLS: Number of partial least squares terms; SE: sensitivity; SP: specificity.