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Evaluation of a handheld near infrared (NIR) spectrometer for the discrimination of Iberian pigs according to their feeding regime

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Abstract. The aim of this study was to evaluate a handheld near infrared spectroscopy (NIRS) instrument to classify Iberian pig carcasses in different commercial categories for instantaneous quality control and authentication in the industry. Sixty intact subcutaneous adipose tissue samples taken from the tail insertion area in the coxal region of the body were analyzed, twenty per category ("Bellota", "Recebo" and "Cebo"). A discriminant analysis based on the algorithm PLS2 was performed and different spectra pretreatment were evaluated. A Principal Component Analysis (PCA) showed more clear differences between "Cebo" samples and the other classes, while "Bellota" and "Recebo" were spectrally more similar within them. The external validation of the classification models based on a second derivative showed only one "Recebo" sample misclassified as "Bellota". These results show the high potential of the handheld NIRS device evaluated for the individual authentication of Iberian pig carcasses to discriminate the different feeding regime followed by the animal during the growing-finishing period.

Keywords. Iberian pigs – NIR Spectroscopy – Classification – MEMS spectrometer – Adipose tissue.

Évaluation d'un spectromètre portatif dans le proche infrarouge (NIR) pour la discrimination des porcs Ibériques selon leur régime

***Résumé**. L'objectif de cette étude était d'évaluer un instrument portatif de spectroscopie dans le proche infrarouge (NIRS) pour classer les carcasses de porcs Ibériques selon les différentes catégories commerciales pour le contrôle de la qualité instantanée et de l'authentification dans l'industrie. Soixante échantillons de tissu adipeux sous-cutané intact prélevés dans la zone d'insertion de la queue dans la région coxale du corps ont été analysés, vingt par catégorie ("Bellota", "Recebo" et "Cebo"). Une analyse discriminante basée sur l'algorithme de PLS2 a été réalisée et les différents spectres de prétraitement ont été évalués. Une analyse en composantes principales (PCA) a montré plus de différences claires entre les aliments des échantillons "Cebo" et les autres classes, tandis que "Bellota" (gland) et "Recebo", spectralement, étaient plus semblables en leur sein. La validation externe des modèles de classification basés sur une dérivée seconde montre un seul échantillon "Recebo" classé à tort comme "Bellota". Les résultats montrent le potentiel élevé de l'appareil de poche NIRS évalué pour l'authentification individuelle des carcasses de porcs Ibériques, afin de discriminer les différents régimes d'alimentation suivis par les animaux pendant la période d'engraissement.*

***Mots-clés.** Porcs Ibériques – Spectroscopie NIR – Classement – Spectromètre MEMS – Tissu adipeux.*

I – Introduction

The feeding regime of the animals during the final period of growing plays an important role in Iberian pig products quality (Garrido and De Pedro, 2007), within other factors that also influence such as the genotype (Ramírez and Cava, 2007), age or rearing conditions (Bonneau and Lebret, 2010). Those factors have a significant impact on the fatty acid composition and

mainly in the high unsaturated/saturated fatty acid ratio which at the end is responsible of the exceptional organoleptic and healthy properties of the Iberian meat products (Cava *et al.*, 2000; Ventana *et al.*, 2007). The Spanish legislation classifies the animals into four commercial categories depending on the feeding regime and production system (BOE, 2007): "Bellota" (i.e. animals in free range fed exclusively with grass and acorns), "Recebo" (i.e. animals fed with acorns and grass supplemented with compound feeds in a outdoor system), "Cebo" (i.e. animals fed with compound feed in an intensive system) and "Cebo de campo" (i.e. animals fed exclusively with compound feed in free range).

Official classification methods (on-farm inspections and gas chromatography of melted fat) are high cost and time-consuming, not providing individual items but batches information of animals groups that can have individual variability. Fast, accurate, objective, low cost and individual analysis methods are demanded by consumers and industry for quality control and authentication of these high market prices products. Since 1992, the potential of Near Infrared Spectroscopy (NIRS) has been highlighted for the analysis of melted fat (De Pedro *et al.*, 1992; García-Olmo *et al.*, 2009), intact adipose tissue (De Pedro *et al.*, 2007) and live Iberian pigs (Pérez-Marín *et al.*, 2009) as a tool for classifying Iberian pig animals into different commercial categories on the basis of the feeding regime. Few applications have been evaluated for on-line analysis (Perez-Marín *et al.*, 2009) and nowadays there are appearing in the market new handheld devices of low cost and easy analysis presentation, providing instantaneous results readily-available. In this study is evaluated a handheld micro electron mechanical system (MEMS) NIRS spectrometer for classifying Iberian pigs carcasses according to their feeding regime.

II – Materials and methods

1. Sample Set

Sixty Iberian pig adipose tissue samples were measured, taken from the tail insertion area in the coxal region of the body, where traditional gas chromatography biopsy is taken (De Pedro, 2001). The samples were stored at -20ºC until 24 hours before the NIRS analysis. The sample set was composed of 20 samples of each commercial category studied ("Bellota", "Recebo" and "Cebo"). Animal feeding regime was controlled by trained personnel.

2. NIRS measurements

A handheld MEMS-NIRS instrument (Phazir 2400, Polychromix Inc., Wilmingon, MA, USA) was used to collect reflectance spectra in the range 1600-2400 nm with a resolution of 8 nm (resolution-pixel 8nm, resolution-optical 12nm). A quartz protection was used for preventing dirt accumulation in the instrument. Three spectra per sample were collected and the mean spectrum per sample was used for further analysis.

3. Data modelling

As spectral pre-treatments, Standard Normal Variate (SNV) plus Detrending (DT) (Barnes *et al.*, 1989) was used to remove the multiplicative interferences of scatter and two derivative mathematical treatments were performed: window-wise filtering (1,10,5,1) and (2,5,5,1) (ISI, 2000). A Principal Component Analysis (PCA) was performed in order to detect spectral outlier samples and observe possible groups tendency. After outlier detection, the data set was divided in two: a training and validation (5 samples of each category) sets using the SELECT algorithm of the WinISI software. Discriminant analysis based on Partial Least-Squares (PLS2) was applied to classify the subcutaneous adipose tissue in the different commercial categories studied. The optimum number of model factors was selected by cross-validation using 4 groups. Those chemometric analyses were performed using the software WinISI II ver 1.50 (Infrasoft International, Port Matilda, PA, USA). The classification models were statistically evaluated, by calculating the number of animals correctly classified.

III – Results

Figure 1 shows the mean spectra of each commercial category analyzed. It was observed a similar pattern for all the groups, although it seems that there is a difference in absorbance range. Fat peaks were recorded at around 1720-1760, 2150 and 2310-2340 nm (Williams and Norris, 1987; Osborne, Fearn and Hindle, 1993); characteristic absorption bands at around 1940 nm were water-related (Williams and Norris, 1987; Osborne, Fearn and Hindle, 1993).

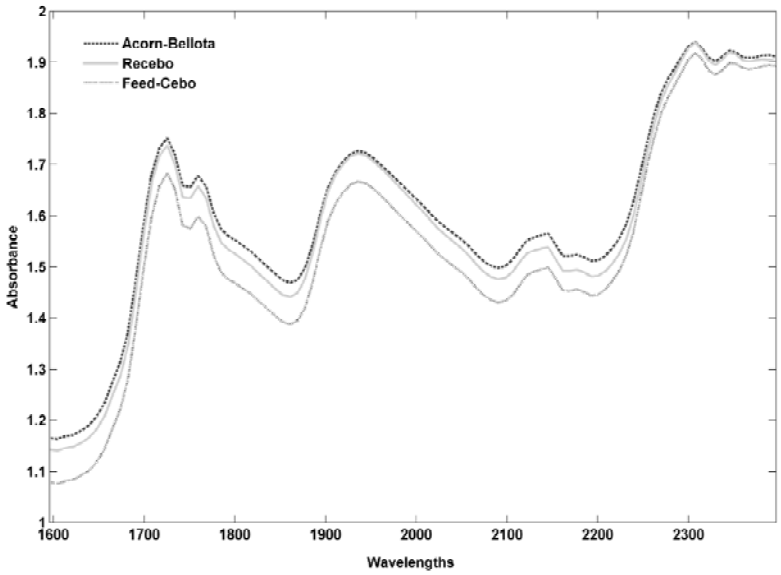

Fig. 1. Raw spectra (mean spectra) for each Iberian pig category studies.

PCA was performed to visualize the main structure of the data set and detect spectral outlier samples. It was observed 7 samples far from the centre of the population or with possible classification error due to the individual variability of the animals. After being removed, a new PCA analysis was performed using 9 Principal Components (PCs) explaining the 98.86%. The score plot (Fig. 2) shows clear differences between “Cebo” cluster and the other categories. “Recebo” and “Bellota” showed an overlap cluster, probably due to they are samples with a fatty acid profile more similar since those animals have eaten both acorns and grass.

Table 1 shows the statistics and number of samples correctly-classified of the training set, after outlier detection, for PLS discriminant. Two spectra pre-treatments were evaluated and a second derivative provided better classification results. A 91.66% of the “Bellota” samples were correctly classified, 63.6% of the “Recebo” samples and 100% of the “Cebo” samples. Table 2 shows the external validation of the model performed with a second derivative. Only one sample of the “Recebo” group was misclassified as an “Bellota” sample.

It should be remarked that any sample of the group “Cebo” (products with lower prices) was misclassified in the other groups (“Bellota” or “Recebo”, products with higher prices) or vice versa. These results confirm the possibility to discriminate Iberian pig adipose tissue from animals reared under different feeding regime using a handheld MEMS-NIRS instrument that can be implemented in on-line applications in the industry.

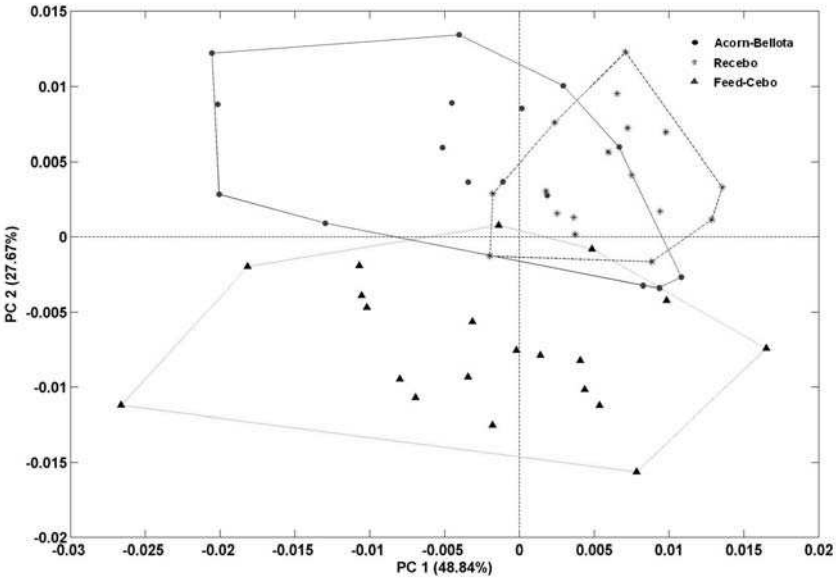

Fig. 2. PCA score plots for the two principal components.

Table 1. Classification results obtained by the PLS discriminant with a second derivative

Origin	No. of samples	Classified as		
		Bellota	Recebo	Cebo
Bellota	12	11	1	0
Recebo	11	4	7	0
Cebo	15	0	0	15

Table 2. External validation of the second derivative PLS discriminant model

Origin	No. of samples	Classified as		
		Bellota	Recebo	Cebo
Bellota	5	5	0	0
Recebo	5	1	4	0
Cebo	5	0	0	5

IV – Conclusions

The classification of Iberian pig intact adipose tissues using the handheld NIRS device evaluated was successful. PLS discriminant performed better classification predictions with a second derivative with only one sample of the “Recebo” group misclassified for the external

validation. These results indicate the feasibility of performing an individual authentication of Iberian pig carcasses according to the feeding regime of the animals in a fast and with possibility of on-line applications. Further work is required in order to develop robust classification models with larger data sets and transfer the technology from laboratory to an on-line monitoring system.

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