Non-destructive *in-vivo* classification of Iberian pigs measured with a hand-held NIR digital transform spectrometer: a viability study

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Introduction

Iberian pig products are well known over the world for their exceptional organoleptic characteristics and healthy properties, and because they are produced in a sustainable and natural ecosystem, called *The Dehesa*. The genotype[1] and feeding regime[2] of the animals influence meat quality because they are directly related with the fatty acid profile and mainly with the high unsaturated/saturated fatty acid ratio. Furthermore, the marbling, oiliness, brightness, aroma and flavour are related with the fatty acid composition of the intramuscular fat.[3] The Spanish legislation classifies the animals into four commercial categories, depending on the feeding regime during the final growing-finishing period and the system of production.[4] In fact, a product of the highest quality category "*Acorns"* (i.e. animals in free range fed exclusively with natural resources in the Dehesa) costs approximately double that of a product of animals fed with compound feeds in an intensive system, "*Concentrates*".

The official quality control systems for determining the feeding regime of the animals are based on on-farm inspection and fatty acid composition analysis of the subcutaneous fat by gas chromatography.[4] However, these methods are expensive and time-consuming, and provide information only of batches of animals.[5] Consumers are demanding objective authentication systems for these high market priced products. The industry requires new rapid screening techniques for individual certification of animals to enable payment to the livestock farmer for individual

animals of each commercial category, instead of paying by batches, that can have variability of the type of animals within each group.

Near Infrared (NIR) Spectroscopy has shown its potential for the authentication of Iberian pig products according to the feeding regime over the past 17 years. First attempts focused on the analysis of melted fat, and later on intact fat or adipose tissue.[2] More recently a viability study has been carried out using a spectrometer provided with a fiber optic probe on carcasses and live animals in the slaughterhouse.[5] However, handheld devices are appearing in the market during the past few years.

The aim of this work was to investigate the feasibility of using a handheld micro electron mechanical system (MEMS) NIR spectrometer for classifying live Iberian pigs according to their feeding regime.

Materials and methods

Animal set

Ninety-five Iberian pigs were measured before being slaughtered at commercial Spanish slaughterhouses. These animals belonged to two commercial categories concerning the feeding regime during the growing-finishing period: one group of 47 animals was designated "*Acorns*" and a group of 48 was designated "*Concentrates*".

NIR spectroscopy measurements

The reflectance spectra of live animals were collected using a hand-held MEMS-NIRS instrument (Phazir 2400, Polychromix Inc., Wilmingon, MA, USA). The instrument operates between 1600–2400 nm with 8 nm intervals (resolution-pixel 8 nm, resolution-optical 12 nm). A quartz protection was used for preventing dirt accumulation in the instrument.

Spectra measurements were taken from the tail insertion area in the coxal region of the body; the site used for the traditional gas chromatography based control procedure.[6] Three spectra per animal were collected, and the mean spectra were used for model development.

Data treatment

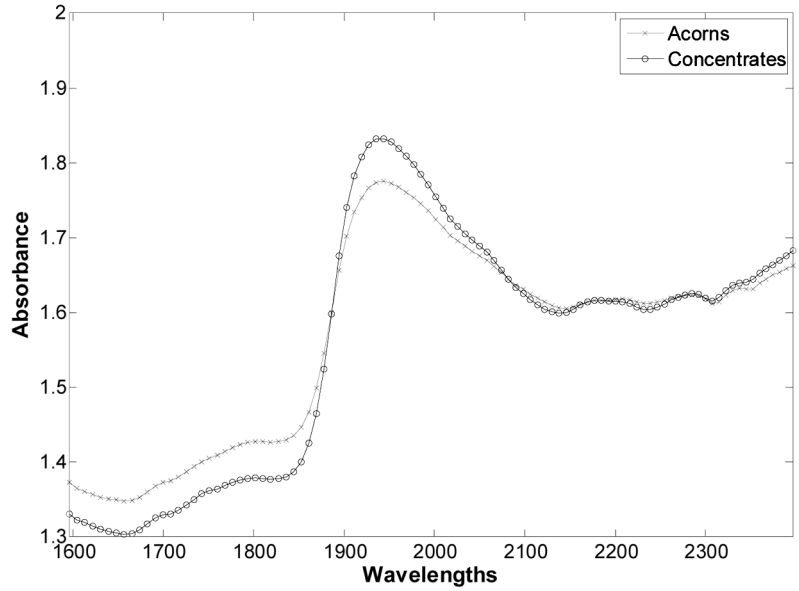
All the chemometric calculations were performed using MATLAB ver. 7.6 (The MathWorks, Natick, USA) and the PLS_toolbox (Eigenvector Research, Manson, USA).

Before averaging the spectra of each animal, the spectral repeatability was evaluated using the Root Mean Squared (RMS) statistic for removing spectra that clearly deviated from the median spectrum.

Multivariate analyses based on Partial Least-Squares Discriminant Analysis (PLS-DA)[7] were applied to classify the animals analyzed in the different commercial categories studied. The optimum number of model factors was selected by leave-one-out cross-validation. Outlier detection was performed based on the Residuals (Q) and Hotelling (T^2) values for the detection of animals with atypical spectra.[8]

The classification models were statistically evaluated, by calculating the number of animals correctly classified.

(a)



(b)

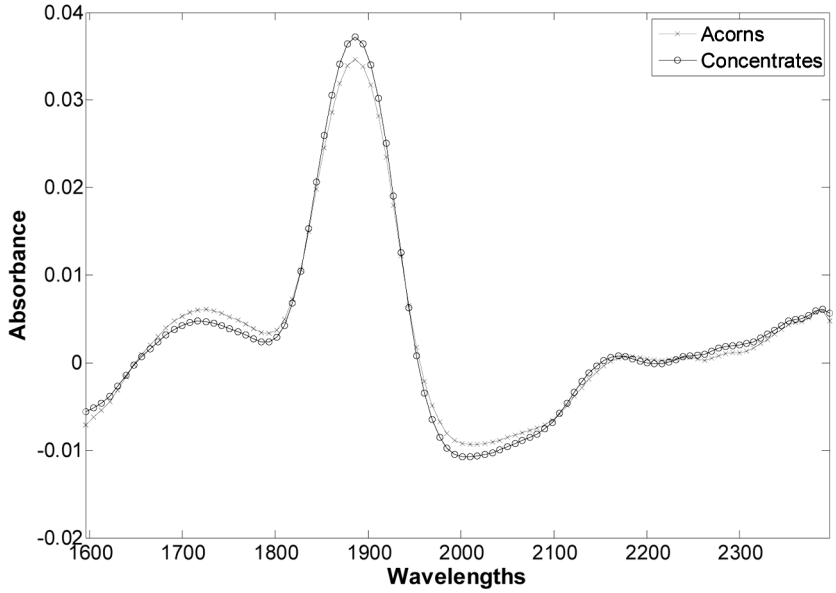


Figure 1. Mean spectra for each Iberian pig category. a) raw spectra; b) first derivative; c) second derivative.

(c)

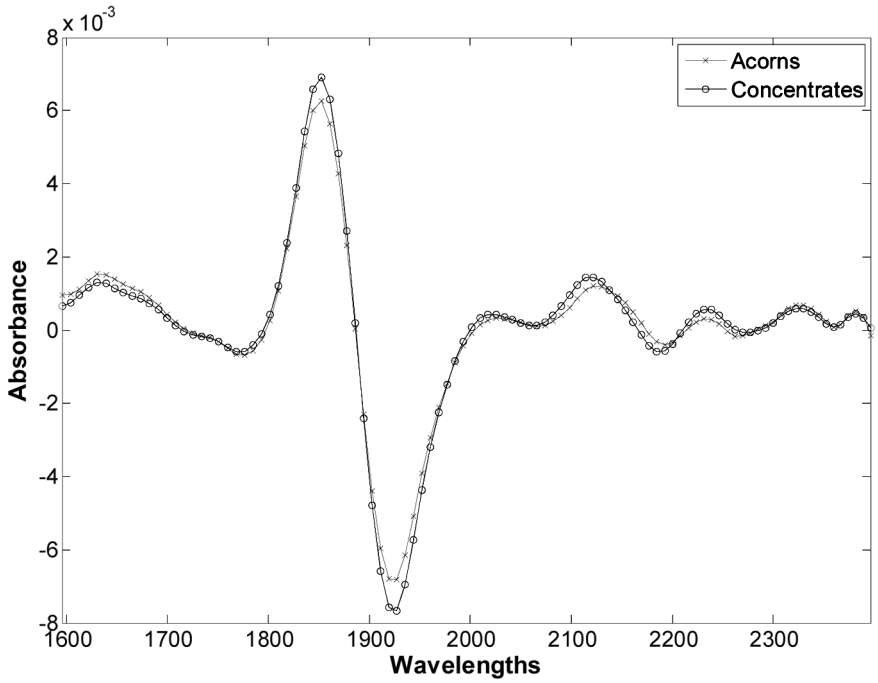


Figure 1. (*continued*)

Results and discussion

In order to reduce noise and baseline shifts that may be due to movement of the live animals during the measurements, or poor contact between the sample window and the animal, NIR spectra repeatability was calculated with the different replicate spectra of the same animal, for checking the quality of the spectra. Before averaging the replicate spectra taken from each animal body, RMS values exceeding 700,00 µlog 1/*R* were considered unacceptable. Once these scans were

Table 1. Classification results obtained by PLS-DA model and 1st derivative.

MSC/1st der/Mean center		Classified as	
		Acorns	Concentrates
Origin	Acorns	42 (41)	1 (2)
	Concentrates	0 (2)	45 (43)

In brackets () corresponds with the results for cross-validation.

Table 2. Classification results obtained by PLSDA model and 2nd derivative.

MSC/2nd der/Mean center		Classified as	
		Acorns	Concentrates
Origin	Acorns	42 (42)	1 (1)
	Concentrates	1 (2)	44 (43)

In brackets () corresponds with the results for cross-validation.

removed, the mean spectra of each animal were calculated, and different mathematical pretreatments were evaluated. Seven animals were detected as outliers, 4 of the category *"Acorns"* and 3 of the class *"Concentrates"*.

Figure 1 shows the average spectra of the each animal category after different mathematical pre-treatments.

An important absorbance region around 1900 nm was observed, identified as area of water absorption that can be related with the presence of dampness on the animals' skin. Small differences can be seen between the average spectra of each animal category around 2150–2300 nm, which may be related with protein and/or fat [Figure 1(c)].

Tables 1 and 2 show the statistics and number of animals correctly-classified of the training set, after outlier detection, for a first and second derivative respectively.

Both derivative pre-treatments provided similar classification results. A first derivative correctly classified 100% of the animals of the "*Concentrates*" category, while one animal of the category "*Acorns*" was incorrectly classified. The cross-validation results showed also a high percentage of animals correctly classified for both classes (95.55% for *"Concentrates"* and 95.34% for *"Acorns"*). Furthermore, the misclassified animals from the category "*Acorns*" as belonging to the class "*Concentrates*" could be explained by the fact that the animals hadn't eaten enough natural resources from the Dehesa to obtain that category.

Conclusions

This viability study demonstrated the high potential of the handheld NIRS device evaluated for the individual monitoring of *in-vivo* Iberian pigs, to discriminate the different feeding regime followed by the animal during the growing-finishing period. Further work is required in order to study the effect of the presence of hair and skin in the NIRS measurements, to improve the spectra repeatability and to develop more robust discrimination models with larger number of animals.

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