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INVESTIGACIÓN

Classification of real farm conditions Iberian pigs according to the feeding regime with multivariate models developed by using fatty acids composition or NIR spectral data

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RESUMEN

Clasificación según el régimen alimenticio de cerdos Ibéricos producidos bajo condiciones no experimentales mediante modelos multivariantes generados a partir de la composición en ácidos grasos o datos espectrales NIR.

Se han desarrollado modelos multivariantes, generados a partir de la composición en ácidos grasos o datos espectrales NIR, para clasificar según el régimen alimenticio cerdos Ibéricos producidos bajo condiciones no experimentales. Se han empleado 121 muestras de grasa líquida procedentes de grasa subcutánea de canales de cerdos Ibéricos pertenecientes a 5 partidas con regímenes alimenticios diferentes. A dichas muestras líquidas se les determinó el contenido en 11 ácidos grasos (C14:0, C16:0, C16:1, C17:0, C17:1, C18:0, C18:1, C18:2, C18:3, C20:0 and C20:1) y se obtuvo su espectro NIR. Los modelos de clasificación multivariantes se desarrollaron mediante Análisis Discriminante Lineal. Dichos modelos presentaron un error de clasificación del 0,0% al emplear como variables los datos espectrales NIR y un error del 1,7% al generarse a partir de la composición de ácidos grasos. Estos resultados confirman la capacidad de discriminar muestras de grasa líquida de cerdos Ibéricos producidos bajo condiciones no experimentales ya sea a partir de su composición en ácidos grasos o de su espectro NIR. El error de clasificación obtenido por el modelo al emplear los datos espectrales NIR fue menor que en el caso de emplear la composición en ácidos grasos.

PALABRAS CLAVE: Ácidos grasos – Cerdo Ibérico – Espectroscopía en el Infrarrojo Cercano – Grasa – Modelos multivariante – NIRS.

SUMMARY

Classification of real farm conditions Iberian pigs according to the feeding regime with multivariate models developed by using fatty acids composition or NIR spectral data.

Multivariate Classification models to classify real farm conditions Iberian pigs, according to the feeding regime were developed by using fatty acids composition or NIR spectral data of liquid fat samples. A total of 121 subcutaneous fat samples were taken from Iberian pigs carcasses belonging to 5 batches reared under different feeding systems. Once the liquid sample was extracted from each subcutaneous fat sample, it was determined the percentage of 11 fatty acids (C14:0, C16:0, C16:1, C17:0, C17:1, C18:0, C18:1, C18:2, C18:3, C20:0 and C20:1). At the same time, Near Infrared (NIR) spectrum of each liquid sample was obtained. Linear Discriminant Analysis (LDA) was considered as pattern recognition method to develop the multivariate models. Classification errors of the LDA models generated by using NIR spectral data were 0.0% and 1.7% for the model generated by using fatty acids composition. Results confirm the possibility to discriminate Iberian pig liquid samples from animals reared under different feeding regimes on real farm conditions by using NIR spectral data or fatty acids composition. Classification error obtained using models generated from NIR spectral data were lower than those obtained in models based on fatty acids composition.

KEY-WORDS: Iberian pig – Fat – Fatty acids – Multivariate methods – Near Infrared Spectroscopy – NIRS.

1. INTRODUCCIÓN

Nowadays, it has been sufficiently proven that the characteristics of the Iberian pig fat basically depend on the type of feeding that the pig has received at the end of its fattening period, and that the use of different diet types, (ie. acorns, acorns and feed concentrates, only concentrates) have a significant repercussion on the fatty acid composition of the adipose tissues of its carcass (Flores, Biron, Izquierdo and Nieto, 1988; De Pedro, 2001).

The Near Infrared Spectroscopy (NIRS) literature highlights the variety of chemometric algorithms used to develop models for classification, authentication and discrimination purposes (Mark, 1992; Massart, Vandeginste, Buydens, De Jong, Lewi and Smeyers-Verdeke, 1997). Some of these pattern recognition algorithms are more sophisticated than others and some of them are implemented as part of routine NIRS software. One

of this classification algorithm is the Linear Discriminant Analysis (LDA). This method develop a linear function (discriminant function) that provides an accurate discrimination between the different classes or categories of the data set (Massart, Vandeginste, Buydens, De Jong, Lewi and Smeyers-Verdeke, 1997). LDA has been a pattern recognition method used by several authors to classify fats and oils according to different criteria (Garrido-Varo, García-Olmo and Pérez-Marín, 2004).

Previous works has shown the possibility to discriminate Iberian pig carcasses with different type of feeding based on spectral NIR data (Hervás, Garrido, Lucena, Garcia and De Pedro, 1994; De Pedro, Garrido, Lobo, Dardenne and Murray, 1995). They used 118 Iberian pig fat samples obtained from 3 batches of animals with different feeding regimes. The three batches can be designated as animals fed on acorns plus low and medium levels of compound feeding stuffs and animals fed only on compound feeding stuffs. NIRS spectra were obtained using the transmission mode in a monochromator instrument, in the range 1100 to 2500 nm. Results were obtained by several chemometrics algoritms as Linear Discriminant Analisys and Artificial Neural Network (ANN), using as variables the composition in terms of 11 FAs obtained by Gas Chromatography (GC) and Principal Component Analysis (PCA) scores from NIR spectral data.

Comparison of the data allows them to conclude that the classification strategies produce errors of different magnitude in the validation set samples. The LDA models based on fatty acids composition yielding the greatest classification errors and the LDA models using PCA scores from NIR data yielded greater classification errors than the ANN models tested in the same set.

However, the samples used by Hervás *et al.* (1994) and De Pedro *et al.* (1995) in their classification studies were obtained from animals with well-defined and tightly-controlled feeding regimes, as is usual in research projects (Garrido-Varo et al, 2004) . In order to confirm the previous results and to be able to apply the models obtained for carcasses classification at the slaughterhouse, it is necessary to develop discrimination models using samples from other batches of animals more representative of real farm conditions.

The objective of this work is to compare the performance of pattern recognition models developed by using fatty acids composition or NIR spectral data to classify Iberian pig samples representative of real farm conditions, according to the feeding system of the animals.

2. MATERIAL AND METHODS

2.1. Samples

Subcutaneous fat samples were taken from 121 carcasses of Iberian pigs belonging to 5 batches of Iberian pigs reared under different feeding systems in different farms in Andalusia (Spain). Table 1 contains the number of animals and the type of feeding for each of the five batches. It is important to highlight that the quality and the price of the derived products from each batch of animals decrease from batch 1 to batch 5.

Fat samples were taken from the tail insertion area in the coxal region of the carcass. That is the same location used by the Iberian Pig Designation of Origin committees and laboratories when taking samples for the quality control of Iberian pig carcasses (Boletín Oficial del Estado 2004).

Each subcutaneous adipose tissue sample containing all the fat accumulation, with the skin and lean parts removed, was divided into 2 subsamples. One of the subsample was processed individually to extract a unique liquid fat sample from each animal of the batches. Thus, a total of 121 individual liquid fat samples were therefore available to carry out the present work.

The other subsample was mixed with the rest of the subsamples belonging to animals of the same batch. Every subsamples pool was processed to extract a mean liquid fat sample for each batch according to Boletín Oficial del Estado (2004). Thus, it was obtained a total of 5 mean liquid fat samples.

Individual and mean liquid fat samples were obtained after melting in a microwave the oven the subcutaneous adipose tissue subsamples according to the methodology described by De Pedro *et al.* (1996) and included into Boletín Oficial del Estado (2004). Subsamples were placed in a glass container and heated in a microwave oven for a long enough period of time to melt the fat (namely, 3 minutes for each 100 g of sample at 700 W power). From the melted fat, and once the remains of the subcutaneous tissue supernatant had been removed, the appropriate liquid sample was taken.

Table 1
Characteristics of the batches included on the data set

Batch	N	Type of feeding
1	41	Only acorns during 6 months
2	36	Only acorns during 3 months
3	20	Only acorns during 1 months
4	11	Acorns plus medium levels of compound feedingstuffs during 6 months
5	13	Only compound feedingstuffs

[a] Use. [b] Las notas.

2.2. Fatty acids composition

Mean liquid fat samples were analysed by gas chromatography (GC) with a capillary column to determine the fatty acids composition in accordance with the UNE 5508 standard method and Boletín Oficial del Estado (2004). The methyl esters were prepared following, in general terms, the cold methylation procedure indicated in section 5 of the IUPAC No. 2301 procedure. A small amount of fat was dissolved in hexane to which a solution of KOH in Methanol was added. After vigorous shaking for several seconds, it was left to decant, taking an aliquot from the organic phase for its analysis. All the characteristics of the chromatographic method (equipment, columns, reagents, operation and maintenance conditions) used by each laboratory for the separation and quantification of the methyl esters were included in the recommendations given by the UNE 5508 standard method.

Individual liquid fat samples were analysed by NIRS to predict the fatty acids composition. There were used calibration equations to determine the percentage of 11 fatty acids (C14:0, C16:0, C16:1, C17:0, C17:1, C18:0, C18:1, C18:2, C18:3, C20:0 and C20:1) (García-Olmo, Garrido and De Pedro, 2001; García-Olmo, 2002). NIR Spectra were predicted by using the WinISI software ver. 1.50 (WinISI, 2000).

2.3. NIRS Analysis

Individual liquid fat samples were analysed by folded transmission in a Foss-NIRSystems 6500 monochromator equipped with a spinning module. It was used a ring cup with a pathlenght of 0.1 mm (ref. IH-03459). A diffuse reflecting surface placed at the bottom of the cup reflects the radiation back trough the sample to the reflectance detectors (Shenk and Westerhaus, 1995a). Spectra were collected by repacking of two subsamples using the ISI NIRS 3 software ver. 3.11 (Shenk and Westerhaus, 1995a; Shenk and Westerhaus, 1995b).

2.4. Data treatment

Classification models were developed by using NIR spectral data of 121 individual liquid fat samples of Iberian pigs.

A multivariate pattern recognition method, namely linear discriminant analysis (LDA), were considered in this study to classify Iberian pig carcasses according to the type of feeding. LDA models were obtained by using SAS software ver. 8.2 (SAS, 2000). LDA models were generated by using a number of PCA scores that explain more than the 99% of the original variance for the data set.

Cross validation method was selected to evaluate the different multivariate models developed (Mark, 1992; Massart, Vandeginste, Buydens, De Jong, Lewi and Smeyers-Verdeke, 1997). The statistic calculated to compare the performance of the different classification methods was the classification error or percentage of misclassified samples by the pattern recognition model (Mark, 1992).

3. RESULTS AND DISCUSSION

Table 2 shows the fatty acids composition of the mean liquid fat sample for each batch analysed by GC. As can be seen on this table, the 5 batches have a very different fatty acids composition mainly the main fatty acids (C16:0 range from 18.9% to 24.0%, C18:0 range from 8.4% to 12.8%, C18:1 range from 57.3% to 47.6% and C18:2 range from 8.4 to 11.1). These results are in accordance with the type of feeding described on Table 1 and results obtained by De Pedro (2001).

Table 2 also includes mean NIRS predicted fatty acids composition of the individual liquid fat samples for each batch. This table shows that mean fatty acids composition obtained by NIRS or GC for each batch is very similar. For the main fatty acids, the maximum difference between the mean NIRS predicted values and the GC value was 0.7 for%C16:0 (batch 4), 1.0 for%C18:0 (batch 1), 0.4 for%C18:1 (batch 2) and 0.6 for%C18:2 (batch 1). It is important to highlight that these difference values were obtained on different samples analysed with different methodologies and in 2 different laboratories.

These difference values are lower than the reproducibility values (maximum absolute difference between 2 results, and only 2, obtained on the same sample with the same methodology in 2 different laboratories) for these fatty acids according with the results obtained by Garcia-Olmo *et al.* (2002). The reproducibility values described by these authors were 2.0 for%C16:0, 1.1 for %C18:0, 2.5 for %C18:1 and 0.8 for %C18:2. Thus, the difference between the mean NIRS predicted values and the GC value showed on Table 2 are similar or even lower than the difference values obtained analysing the same sample by GC in 2 different laboratories.

Table 3 and 4 include a summary of the classification results of LDA models by using as variable the predicted fatty acids composition (Table 3) or the NIR spectral data (table 4) of the liquid fat samples. These tables show the classification matrix obtained after the allocation of each sample into one of the considered batches by using the LDA models generated. As can be seen, models generated by using NIR spectral data (Table 4), classify correctly all the samples (121 of 121) into its original batch (41 samples into batch 1, 36 into batch 2, 20 into batch 3, 11 into batch 4 and 13 samples into batch 5).

However, in the case of models generated by using fatty acids composition (Table 3), two samples were misclassified: one sample of the

Table 2
Mean fatty acids composition of the batches obteined by NIRS[a] and GC[b]

Batch	1		2		3		4		5	
Analysis[a]	NIRS[b]	GC[c]	NIRS[b]	GC[c]	NIRS[b]	GC[c]	NIRS[b]	GC[c]	NIRS[b]	GC[c]
C14:0	1.3	1.2	1.3	1.3	1.3	1.2	1.3	1.3	1.4	1.4
C16:0	19.0	18.9	20.4	20.5	21.3	21.2	20.2	19.5	24.1	24.0
C16:1	2.2	2.0	2.0	1.9	1.9	1.6	2.2	1.9	2.2	2.3
C17:0	0.3	0.2	0.3	0.2	0.3	0.2	0.3	0.3	0.3	0.4
C17:1	0.4	0.3	0.4	0.2	0.4	0.2	0.4	0.3	0.4	0.4
C18:0	7.4	8.4	8.4	8.8	10.5	11.1	8.8	9.1	12.2	12.8
C18:1	57.3	57.3	54.6	55	53.1	53.4	53.8	53.7	47.8	47.6
C18:2	9.8	9.2	9.7	9.6	8.5	8.4	10.7	11.1	9.2	8.8
C18:3	1.1	1.0	1.2	1.0	1.0	0.8	1.1	1.2	0.9	0.8
C20:0	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.1	0.2	0.2
C20:1	1.5	1.5	1.3	1.3	1.6	1.7	1.5	1.4	1.4	1.3

[a] Results are expressed as % of total fatty acids analyzed.
[b] Mean NIRS predicted fatty acids composition of the individual liquid fat samples for each batch.
[c] Fatty acids composition of the mean liquid fat sample for each batch analysed by Gas Chromatography.

batch 1 (2.4%) were classified as belonging to batch 4 and one sample of batch 4 (9.1%) were asigned to batch 1. According to these results, the classification errors obtained on the LDA models generated by using NIR spectral data were 0,0% while this statistic had a value of 1.7 % for the model generated by using fatty acids composition. These classification error were lower than those obtained by Hervás *et al.* (1994) and De Pedro *et al.* (1995). They obtained classification errors of 15.7% and 11.3% for LDA models generated by using fatty acids compositions and NIR spectral data respectively. In the case of ANN models developed with NIR spectral data as variables, the classification error was 3.7%.

Thus, the low classification errors obtained at the present work confirm the possibility to discriminate Iberian pig carcasses from animals reared under different feeding regimes by using NIR spectral data and fatty acids composition. At the same time, these results show that classification errors, obtained by means of cross validation of the calibration set, are lower in the models generated using NIR spectral data instead of predicted fatty acid data.

Moreover, the results obtained by Hervás *et al.* (1994) and De Pedro *et al.* (1995) were confirmed, since the errors obtained using models generated from spectral data (regardless of the chemometric method of analysis used) were lower than those obtained in models based on fatty acids data. It should be because NIR spectral data is a spectral fingerprint data of each individual sample. This spectral fingerprint not only has chemical

Table 3
Classification results obtained by LDA model generated by using fatty acids composition (11 fatty acids) as variable

		CLASSIFIED AS				
		Batch 1	Batch 2	Batch 3	Batch 4	Batch 5
Origin	Batch 1	40	0	0	1 (2,4%)	0
	Batch 2	0	36	0	0	0
	Batch 3	0	0	20	0	0
	Batch 4	1 (9,1%)	0	0	10	0
	Batch 5	0	0	0	0	13

Table 4
Classification results obtained by LDA model generated by using NIR spectral data of liquid fat as variable

		CLASSIFIED AS				
		Batch 1	Batch 2	Batch 3	Batch 4	Batch 5
Origin	Batch 1	41	0	0	0	0
	Batch 2	0	36	0	0	0
	Batch 3	0	0	20	0	0
	Batch 4	0	0	0	11	0
	Batch 5	0	0	0	0	13

information of the fatty acid composition but also chemical information of all the analytical component included on the sample.

4. CONCLUSIONS

Pattern recognition models obtained confirm the possibility to discriminate Iberian pig liquid samples from animals reared under different feeding regimes on real farm conditions by using NIR spectral data or fatty acids composition.

Moreover, the classification errors obtained using models generated from NIR spectral data were lower than those obtained in models based on fatty acids composition.

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