Determination of fatty acids in the subcutaneous fat of Iberian breed swine by NIR with a fibre-optic probe

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Introduction

For current classification of Iberian swine on the basis of their feeding regime, analyses are made of the fatty acids C16:0, C18:0, C18:1 and C18:2 from the subcutaneous fat by gas chromatography,[1] with mean maximum and minimum values established each year and published by the Spanish Ministry of Agriculture, classifying the animals and their products under the terms "*bellota*-acorn", "*recebo"* and "*pienso-feed"*.

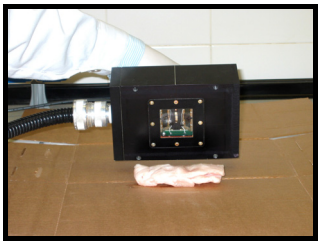
In 1992, De Pedro *et al*.[2] demonstrated that near infrared (NIR) technology permits the prediction of the composition in oleic, linoleic, palmitic and stearic acids from subcutaneous fat obtained from fresh Iberian breed hams and compared their results with those obtained with gas chromatography.[3] The work carried out on pork with NIR has addressed the water-retaining capacity of the meat and intramuscular fat[4] and the on-line determination of fat, water and protein in ground pork.[5] Few works have used NIR and fibre-optic probes directly on Iberian swine carcasses.[6,7]

Here, we established analytical procedures employing NIR technology and a fibre-optic probe that would allow instantaneous analysis at on-line level of fatty acids (C14:0, C16:0, C18:0, C18:1, C18:2 C18:3, and C20:1) for the classification of the meat products of Iberian swine on the basis of their feeding regime.

Materials and methods

Samples

We used 157 samples of subcutaneous fat from Iberian breed swine. The samples obtained contained the hide/skin, the fat between the hide and the lean meat and some lean meat. NIR spectra were measured by direct application of the fibre-optic probe onto intact samples of subcutaneous fat and with cam-lock cups, measuring the total lipid extract from the same sample. A Foss NIRsystems 5000 with a standard 1.5 m 210/7210 bundle fibre-optic probe, Ref. no. R6539-A, was used. The probe employs a remote reflectance system and uses a ceramic plate as reference. The window is of quartz with a 5 × 5 cm surface area, measuring reflectance in the IR zone close to 1100–2000 nm. When working with samples of extracted subcutaneous fat, "*Cam-lock cups*" were used. These are circular capsules with an optical pathlength of 0.1 nm and are normally used for liquid samples. Measurements were carried out in reflectance mode between 1100 and 2498 nm, (Figure 1). The software used was Win ISI 1.05, installed on a Hewlett-Packard Pentium III computer.



Remote reflectance fibre-optic probe. intact samples

Cam-lock extracted samples

Figure 1. Measurement of samples.

Chemical analyses

The reference method used was gas chromatography. Chemical determinations were carried out using diethyl ether extracts of total lipids from samples of subcutaneous fat. Total lipids were extracted with diethyl ether at room temperature. The methyl esters of the fatty acids were obtained by reaction with a solution of potassium hydroxide, then carrying out analysis by gas chromatography (ISO Norm 5508:1990).

Results and discussion

Chemical analyses and spectral information

The chemical composition of the samples of subcutaneous fat used for the calibrations are shown in Table 1. The causes of such great variability must be sought in the feeding regime and genetics of the animals (the Iberian swine used in this work were Iberian swine of the subgenus "*Sus mediterraneus*" crossed with Duroc–Jersey, with Iberian genetics varying between 50 and 75%).

Table 1. Statistical overview of chemical analysis (all units in %).

	Minimum	Maximum	Mean	*SD*
C14:0	0.78	1.77	1.28	0.17
C16:0	15.87	29.74	22.80	2.31
C18:0	4.61	15.90	10.25	1.88
C18:1	43.50	61.27	52.38	2.96
C18:2	2.03	13.94	7.98	1.99
C18:3	0.13	1.14	0.64	0.17
C20:1	0.45	2.32	1.38	0.31

The spectral information defines a series of characteristic absorption bands. Thus, the C–H bond, which is a fundamental constituent of fatty acid molecules, absorbs clearly at wavelengths close to 1200, 1400, 1750, 2310 and 2340 nm.[8] Moreover, the 2310–2340 region corresponds to the combination bands of the C–H bond and the absorption produced in the 1720–1760 region correspond to the first overtone of that bond.[9] Osborne *et al.*,[10] 1993, has attributed the absorption produced at a wavelength of 1210 nm to be the consequence of the second overtone of the CH_2 bond. The absorption in the 2150–2190 region at 1680 nm indicates the presence of *cis* double bonds in the molecules; that is, the existence of unsaturated fatty acids[11] in the samples analysed.

Determination of the fatty acid composition

Calibration equations

To calibrate the fatty acids, we employed 157 samples of subcutaneous fat from Iberian breed swine. Calibration was performed in the extracted subcutaneous fat and intact samples of subcutaneous fat using the fibre-optic probe. Calculation of the statistical parameters of the calibration equations for each component is shown in Table 2. Having calculated the number of principal components, the detection of anomalous spectra was accomplished using the Mahalanobis distance (H statistic), establishing a limit values of $H = 3.0$ such that the spectra whose H distance was greater than this figure were considered different from the spectral population and were discarded. On using extracted subcutaneous fat samples, two samples were removed form the population, except for C18:0, for which ten were removed. In the case of using the fibre-optic probe, four samples were eliminated from the population except for the fatty acids C14:0, C16:0 and C20:1, for which six were removed.

Table 2. Calibration Statistical Descriptors for the NIR determination of fatty acid

Fatty acid	Intact sample				Extracted samples			
	RSQ	*SEC*	*SECV*	Range	*RSQ*	*SEC*	*SECV*	Range
C14:0	0.673	0.095	0.103	0.78–1.77	0.703	0.091	0.109	0.78–1.78
C16:0	0.938	0.576	0.735	15.87–29.74	0.968	0.405	0.551	15.91–29.51
C18:0	0.865	0.692	0.796	4.61–15.90	0.935	0.447	0.610	5.01–15.51
C18:1	0.892	0.975	1.187	43.50–61.27	0.937	0.741	1.060	43.60–61.37
C18:2	0.953	0.431	0.522	2.03–13.94	0.955	0.437	0.593	1.90–14.27
C18:3	0.612	0.105	0.128	0.13–1.14	0.773	0.086	0.123	0.10–1.18
C20:1	0.543	0.210	0.240	0.45–2.32	0.684	0.181	0.267	0.43–2.36

The results obtained were very good for the fatty acids C16:0, C18:0, C18:1 and C18:2, and good for C14:0, C18:3 and C20:1, both in extracted subcutaneous fat samples and for intact samples, In general, similar calibration results were obtained in both cases, although with some exceptions such as C18:3 or C14:0.

Internal validation (prediction)

Assessment of the model is performed by cross-validation; that is in this method the set of calibrations samples is divided into a series of groups, in our case four, and from these groups three are chosen for the calibration set and one for prediction. The process is repeated as many times as there are groups so that all pass through the calibration set and through the prediction set. Using this process, we validated the model used and checked its prediction capacity. This process was performed for both intact samples and for extracted subcutaneous fat samples.

The predicted values gave validation errors that were combined into *SEP*(*C*). In this step, samples with high residual values were removed using the T > 2–5 criterion. Thus, for example, 1 sample was removed in the case of C16:0 and 7 in the case of C18:0.

Figure 2 shows the correlation of the values obtained at the laboratory with respect to those predicted by NIR for measurement with fibre optic.

In the light of the results obtained, it may be deduced that the NIR technique, using a fibre-optic probe, is a good alternative for the determination of the content in fatty acids in samples of subcutaneous fat from Iberian breed swine.

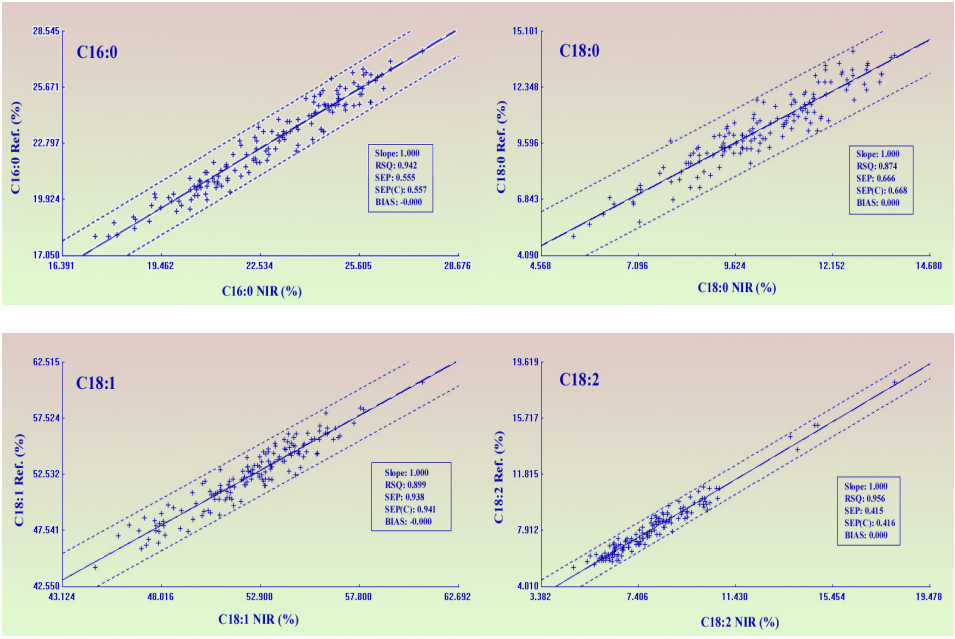


Figure 2. Comparison of the reference values with the values predicted by the calibration equations. Measurements with fibre optic probe.

External validation

Having obtained the calibration sequences, we checked the robustness of the method by applying the fibre-optic probe to 23 samples in a slaughterhouse. These samples did not correspond to the calibrations set. The procedure was as follows: spectra were recorded in triplicate and the spectral mean was taken; the calibrations equations obtained during the work were applied and the predicted values were compared with those obtained using gas chromatography performed later, confirming the goodness of the proposed procedure.

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