Determination of proteins and infiltrated fat in Iberian pork loin by near infrared spectroscopy with a fibre-optic probe

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Introduction

Iberian breed pork loin is widely accepted by consumers owing to the special characteristics of the swine. The pork loin comes from Iberian breed swine, or the same crossed with Duroc breed animals, fed for two months on a range regime, mainly with acorns and grass (*montanera* swine), or over one month on a *montanera* regime and another month with commercial swine feed (*recebo* swine) or on an intensive regime with commercial feed (*feed* swine).[1,2]

The amount and composition of the fat of Iberian breed swine are determinant factors in the quality of the meat and derived meat products such as cured pork loin and ham[3]. These products are highly appreciated for their sensory qualities in comparison with those from other breeds of swine[4,5] and this is why the price of Iberian pork products is far higher than that of products from white breeds.[6,7]

Near infrared (NIR) spectroscopy with a fibre-optic probe has mainly been applied in processed meat or beef homogenates for the determination of the chemical composition of fat and protein[8] of sensory qualities such as textures, flavour and tenderness,[9] or the differentiation of pork, mutton, beef and poultry meat.[10]The work carried out on pork has addressed the water-retaining capacity of the meat and intramuscular fat[11] and the on-line determination of fat, water and protein in ground pork.[12] However, no references have been found in the literature relating to commercialised meat cuts from Iberian breed swine such as pork loin. Thus, the need exists for an analytical technique able to differentiate Iberian breed meat (in this case pork loin) safely and rapidly.

Here we set up procedures employing NIR analytical techniques aided by a fibre-optic probe that allow instantaneous analysis at production level (on-line) and the classification of commercially marketed cuts, such as pork loin, from Iberian swine.

Experimental

Samples

We examined 56 samples of pork loin (*longissimus dorsi* muscle) from Iberian swine purchased from "*Ganaderos Salmantinos de Porcino Ibérico S. Coop.*", cut longitudinally from the zone between the third and fourth vertebrae of the animals to provide a slice measuring 8 × 12 × 2 cm. NIR spectra were measured by applying the fibre-optic probe to intact pork loin samples and to the cut slices after the latter had been previously ground and homogenised. To this end, we used a Knife 1095 Mill Sample homogenizer from Foss Tecator.

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Chemical analyses

Reference chemical measurements were performed with ground and homogenised samples[13]. The content of infiltrated fat in the pork loin was determined, following the ISO-1443, by extraction of the fat from previously hydrolysed and desiccated samples using petroleum ether, removal of the solvent by evaporation, desiccation of the residue and later weighing after cooling. Total protein was determined by the Kjeldahl method: ISO-R-937.

In the analysis of both fat and protein, three determinations were made, taking the means as values and eliminating the values in which there was an error of ± 5%. The chemical compositions are presented in percentages of weight.

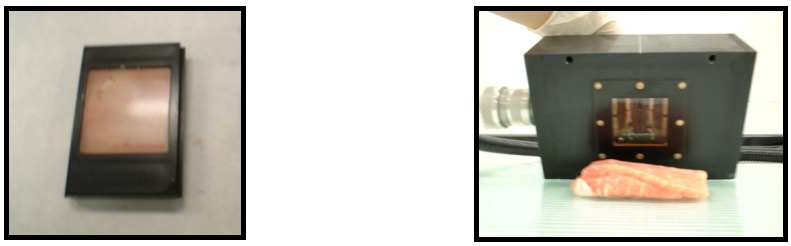
NIR spectroscopy

A Foss NIRSystems 5000 with a standard 1.5 m 210/210 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 (Figure 1), measuring reflectance in the IR zone close to 1100–2000 nm.

When working with ground samples, we used "transport quarter cups" called "rectangular cups", with a window surface of 4.7 × 5.7 cm and an optic pathway of 1.7 cm, usually used for pasty solid samples (Figure 1), measuring reflectance between 1100 and 2498 nm.

In both cases, the spectra were recorded at intervals of 2 nm, performing 32 scans both for the reference and for the sample. To minimise sampling error, all 56 samples were analysed in triplicate. The average spectrum was used for NIR analysis.

The software used was WinISI 1.05, installed in a Hewlett Packard Pentium III computer.



A **B**

Figure 1. Measurements of samples. A: ground samples, B: intact samples.

Statistical analysis

The Mahalanobis distance (*H* statistic) was calculated from principal component analysis scores. The results indicate how different a sample spectrum is from the average sample of the set.[14] A sample with an *H* statistic of ≥ 3.0 standardised units from the mean spectrum was defined as a global H outlier and was then eliminated from the calibration set.

Calibrations were performed by modified partial least squares regression (MPLS). To optimise the calibration accuracy, several scattering corrections and mathematical treatments were tested [standard normal variate, (SNV); de-trending, (DT); multiplicative scatter corrections, (MSC); first derivative and second derivative]. The best one was selected for each constituent on the basis of the highest *RSQ* (multiple correlation coefficient) and the lowest standard error of calibration and cross-validation (*SEC* and *SECV*, respectively).

Assessment of the calibration model was performed by cross-validation. In this method, the set of calibration samples is divided in groups, using one of them to check the results (prediction) and the remaining to construct the calibration model. The model is repeated as many times as there are groups in such a way that all pass through the calibration set and the prediction set. The same process was followed for the ground and intact samples.

Samples from the validation set were then analysed with these equations, which gave a standard error of prediction corrected (*SEPC*) and bias (mean of residuals, defined as the difference between the laboratory value and the value predicted by the equation) for each constituent. In this step, samples with high residual values were eliminated, using the $T > 2.5$ criterion.

After the calibration equations for the fibre-optic probe had been obtained, they were subjected to external validation by application to a set not involved in the calibration process, checking the functioning of the fibre-optic probe for instantaneous analysis at production level at the slaughterhouse of "*Ganaderos Salmantinos de Porcino Ibérica* S. Coop." in Salamanca (Spain). The spectra were recorded by direct application of the probe on intact pieces of Iberian pork loin in which a longitudinal cut had been made so that the samples would be free of the fat surrounding them. Spectra were recorded in triplicate and the spectral average was taken. The calibration equations obtained in the development of the procedure were applied and the predicted values were compared with the laboratory results obtained later.

Results and discussion

Chemical analyses and spectral information

The chemical compositions of the pork loin samples used for calibration are shown in Table 1.

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

	Minimum	Maximum	Mean	*SD*
Fat	2.88	18.40	8.76	3.21
Protein	21.62	31.13	26.37	1.59

The results obtained for protein contents ranged between 21.0 and 31%, which is usual for this type of sample. Regarding the fat contents, the chemical values ranged between 2.9 and 18.4%. These are not the usual values found in Iberian swine (normally between 8 and 11%) and the reason for such high variability must be sought in the food regime and genetics of the animals (the Iberian swine used in this sector are of the subgenus "*Sus mediterraneus"* crossed with *"Duroc-Jersey"*, Iberian genetics varying between 50 and 75%). An increase in the "*Duroc-Jersey*" component gives rise to a lower percentage of infiltrated fat. The great variability in the values of infiltrated fat is an important advantage when obtaining the calibration equations**.**

Figure 2 show the spectra of a pork loin sample together with the mathematical treatments that proved to be optimal for the calibration, MSC Standard and 1st derivative and SNV-DT and 1st derivative. In both forms of presentation, the term "intact" was used when the spectra were obtained with the fibre-optic probe by direct application on the gross sample and the term "ground" was used when the sample of pork loin was ground and homogenised.

The spectral information defines the absorption bands at 1510, 2060, 2172–2186 nm associated with the content in protein, whereas those appearing at 1722, 1760, 2308–2348 nm are related to fat.[14,15]

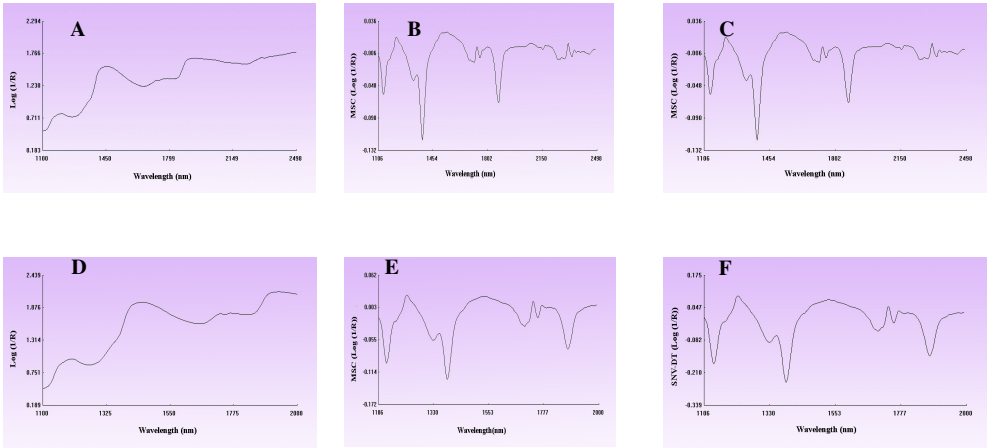


Figure 2. Ground samples: (A) NIR Spectrum, (B) corrected spectrum using 1st derivative and standard MSC, fat. (C) corrected spectrum using 1st derivative and standard MSC, protein. Intact samples, (D) NIR Spectrum, (E) corrected spectrum using 1st derivative and standard MSC, fat and (F) corrected spectrum using 1st derivative and SNV-DT, protein.

NIR determination of fat and protein

Calibration equations

For the calibration of infiltrated fat and protein, 56 samples of Iberian pork loin were used. Calibration was done in ground and intact samples with the aid of the fibre-optic probe.

When ground pork loin samples were used, one sample from the population was eliminated. When the form of presentation was "intact" and the sample was measured with the fibre-optic probe, no samples were eliminated, in both cases taking into account the $H = 3$ criterion. Calculation of the statistical parameters of the calibration equations for each component is shown in Table 2.

Table 2. Calibration statistical descriptors for the NIR determination of infiltrated fat and protein.

Sample		*N*	Mathematical treatment	*RSQ*	*SEC*	*SECV*	Range
Fat	Ground	51	Standard MSC /1st derivative	0.988	0.360	0.440	0.00–18.72
	Intact	56	Standard MSC /1st derivative	0.939	0.795	1.258	0.00–18.40
Protein	Ground	53	Standard MSC /1st derivative	0.941	0.389	0.467	21.55–31.16
	Intact	55	SNV-DT /1st derivative	0.877	0.557	0.827	21.62–31.13

As may be seen in Table 2, the best mathematical treatments for fat were found on applying Standard MSC, 1st derivative. In the calibration equations of protein, Standard MSC, 1st derivative, was used in ground samples and SNV-DT when the measurements were made with the probe.

The results obtained for fat were better than those found for protein in both forms of sample presentation. This is because it is difficult to relate the results obtained by reference analytical methods with the spectroscopic data in the determination of crude protein considered as % N × 6.25,

since nitrogen does not elicit a vibrational response in NIR. However, in the near IR it is possible to measure the vibrations of N–H bonds, which are part of the protein molecule. In conclusion, it can be said that the NIR and Kjeldahl reference method do not measure the same type of protein and the correlation between both may vary as a function of the type of samples studied.[16]

Internal validation

Six cross-validation groups were used, both for intact samples and for ground ones. Using the $T > 2.5$ criterion, no samples were eliminated.

The results concerning the statistical descriptors of the validation in NIR for the parameters of infiltrated fat and protein in 56 samples of ground Iberian pork loin are shown in Table 3.

Table 3. Statistical validation descriptors for the determination by NIR of infiltrated fat and protein.

Sample		Slope	*RSQ*	*SEP*	*SEP*(*C*)	Bias
Fat	Ground	1.014	0.974	0.524	0.518	–0.109
	Intact	1.000	0.945	0.744	0.750	0.000
Protein	Ground	0.990	0.990	0.444	0.448	0.002
	Intact	0.974	0.762	0.806	0.807	–0.083

In the light of the results obtained, it may be deduced that the NIR technique, aided by the fibre-optic probe, is a very good alternative for the determination of protein and fat in Iberian pork loin.

External validation

The predicted results for infiltrated fat in the 20 pork loin samples measured with the probe at the slaughterhouse had values ranging between 4 and 15%. On later measuring the infiltrated fat at the laboratory using reference methods, differences of 6.5% were seen in the measurements. From this, it may be deduced that use of the fibre-optic probe and NIR technique is an optimum method for the determination of infiltrated fat and protein in Iberian pork loin without destruction of the sample.

Conclusions

An NIR spectrometer equipped with a remote reflectance fibre optic probe allow the immediate determination, without expense or destruction to the original samples, of protein and infiltrated fat in Iberian pork loin by direct application of the probe to the piece of loin.

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