Mineral analysis of fresh Iberian pork loin samples by NIRS with a remote fibre-optic reflectance probe

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Introduction

The results obtained for the prediction of mineral elements are not as good as for organic compounds owing to the lack of a direct relation between the element involved and the spectra obtained. If NIRS can be used for determining mineral concentrations this is probably due to the association between minerals and organic functional groups^{1,2}. The prediction of trace elements by NIRS in agricultural products has been reported even less frequently and has always been used in the context of plants^{3,4}. In the case of meat, the mineral components are associated with the water and the protein fraction⁵, such that the differences seen in the NIR spectra of meat are mainly due to those found in proteins and humidity. The only works found in the literature addressing the prediction of trace elements in meat by NIR refer to the determination of heme and non-heme iron in raw meat⁶, and of sodium chloride in meat⁷. Here we set up analytical procedures using NIR techniques for the determination of the trace elements Fe, Zn, Ca, Na and K in fresh Iberian breed pork loin using ground samples and the determination of Fe, Na and K by direct application to the loin samples of a remote fibre-optic reflectance probe, using ICP as the reference method.

Experimental

Samples

Forty-two samples of Iberian breed pork loin (*Longissimus dorsi muscle*) from Ganaderos Salmantinos de Porcino, S.Coop., cut along the loin longitudinally in the zone between the third and fourth vertebrae of the animal were used. Pieces of 8 cm width , 12 cm long and 2 cm high were obtained. NIR spectra were recorded by applying the fibre-optic probe to intact loin samples and to the same samples after grinding and homogenization. For the latter, we used a Knife 1094 Mill Sample homogenizer from Foss Tecator.

Reference chemical analyses

Reference chemical measurements were carried out on samples of ground and homogenised fresh loin dried in an oven at 105°C for 24 hours. Once dried, the samples were ground and subjected to mineralisation in a microwave, measuring the mineral composition in the resulting solution by ICP. For digestion, a Milestone MLD-1200 oven was used. A 1 g portion of dried ground sample was weighed and placed in Milestone high-pressure capsules (model HPV 80) and two steps were implemented: in the first, 5 mL of HNO₃ (c) was added and a power of 300 watts was applied over 5 min. In the second, the sample was allowed to cool and a further 5 mL of HNO₃ (c) was added plus 1 mL of H_2O_2 (30%), applying a power of 30 watts over 7 min. Once the

samples had returned to room temperature, volume was brought up to100 mL with distilled water and the solutions were conserved at 4°C until analysis.

Measurements were taken using optic ICP using a Plasma II model device from Perkin Elmer. Quantification was based on standard calibration graphs. The results obtained, the means of two determinations, are expressed as ppm in the solution (1 g of dried sample /100 mL).

NIR Spectroscopy

A NIRSystems 5000 from Foss S.A, equipped with a 1.5 m fibre-optic probe (Ref. N° R6539) of the regular 210/210 Bundle type was used. The window is made of quartz, with a surface area of 5 x 5 cm, measuring reflectance in the near infrared zone of 1100-2000 nm. When working with ground samples, Transport quarte cup capsules, known as rectangular cups, were used, with a window surface area of 4.7 x 5.7 cm and an optic pathway of 1.7 cm. Measurements were made in reflectance mode between 1100 and 2498 nm, figure 1. The assignation "intact" when the spectra were obtained with the fibre-optic probe by direct application on the raw sample and the spectrum assigned "ground" when the same sample was ground and homogenised. In both cases, the recording of spectra was carried out at intervals of 2 nm, performing 32 scans for both the reference and the sample. To minimise sampling error, triplicate samples were analysed for all 42 samples. The average spectra was used for NIR analysis. The software used was WinISI 1.05.





Ground Samples

Intact samples Figure 1. Measurement of samples

Results And Discussion

Chemical analyses and spectral information

The chemical compositions of the trace elements in the loin samples used for calibration are shown in Table 1. The chemical results are expressed in ppm in the solution (1 g of dried sample/100 mL). The results obtained for the mineral composition of the loin samples studied here are consistent with the values usually found for this type of sample⁵. The high values of the standard deviation for K is in agreement with the literature^{8,9} and shows that the concentration of this element varies considerably in the muscles of the same animal. Figure 2 shows the spectrum of a loin sample, together with the mathematical treatment that proved to be optimum, for the calibration of iron, SNV-DT and 1st derivative and MSC Standard and 1st derivative, in both forms of presentation.

	Minimum	Maximum	Mean	SD
Fe	0.000	1.208	0.417	0.147
Zn	0.167	1.218	0.692	0.175
Ca	0.598	1.835	1.217	0.206
Na	9.204	16.291	12.747	1.181
Κ	87.534	144.187	115.86	9.440

Table 1. Statistical overview of chemical analyses.

The best results were obtained by applying mathematical treatments such as the Multiplicaitve Scatter Correction (MSC) method, a technique initially used by Geladi¹⁰ that prevents the effects of dispersion in ground samples from becoming superimposed over the chemical signals and the SNV-DT and 1st derivative spectral treatment. Those transformations have been introduced to correct problems appearing in the log (1/R) form associated with sample particle size¹¹. The SNV-DT were introduced not only for reduction of multicollinearity but also to calculate spectral differences by reducing the confounding effects of baseline shift and curvature.



Figure 2. A: NIR spectrum, ground sample. B: Corrected spectrum using 1st derivative and SNV-DT. C: NIR spectrum, intact sample. D: Corrected spectrum using 1st derivative and Standard MSC.

The spectral information obtained and compared with the data from the literature defined absorption bands at 1114, 1230, and 1474 nm related to the iron content, absorption at 1470 nm for total iron to the first overtone of NH stretching vibration in peptide⁶. The absorption bands at 1510, 2060, 2172-2186 nm, related to the protein content, would be associated with elements such as Ca and Zn. In the case of K,^{2,12} consider that this component would be associated with organic acids (in the case of plants) and indirectly related to the amount of water retained by meat¹³. This accounts for the marked variability sometimes seen in meat samples, depending on the previous treatment of the samples. The NIR spectrum of this type of sample and the determination of K is due to the rotation or vibrational energies associated with hydrogen bonds².

Determination of the mineral composition in loin samples.

Calibration equations.

To obtain the calibration equations for trace elements in Iberian pork loin, 42 samples were used. The procedure was carried out separately in intact samples with the help of the fibre-optic probe and in the same samples after grinding. Once the number of principal components had been determined, anomalous spectra were detected using the Mahalanobis distance (H statistic), establishing H=3 as the limit value. Thus, those spectra whose H distance was greater than 3.0 were considered different from the spectral population and were discarded. Calculation of the statistical parameters of the calibration equations for each component is shown in Tables 2 and 3, together with the best mathematical treatment. On analysing samples of ground loin, 5 principal components were used, accounting for 99.2% of the variability of the samples with the PCA method, after obtaining the calibration equations by MPLS for the trace elements Fe, Zn, Ca. Na and K. When measurements were carried out with the fibre-optic probe, using intact samples, it was only possible to obtain calibration equations for Fe, Na and K, using 8 principal components, accounting for 95.5% of the variability of the samples.

The results obtained in the determination of the mineral composition are very acceptable for Fe, Zn, Ca, and Na but not so acceptable for K, as shown in the statistical descriptors, SEC and SECV.

Component	Mathematical treatment	RSQ	SEC	SECV	Nºof Principal component	Variability explained
Fe	1/4/4/1 Standard+MSC	0.807	0.064	0.143	5	99.20 %
Zn	2/10/10/1 Standard+MSC	0.643	0.105	0.151	5	99.35 %
Ca	1/4/4/1 SNV + DT	0.719	0.109	0.164	5	99.20 %
Na	1/4/4/1 DT	0.596	0.751	0.996	5	99.20 %
K	2/10/10/1 DT	0.748	4.738	6.872	5	99.35 %

 Table 2.Ground samples. Calibration statistical descriptors for the NIR determination of Fe, Zn, Ca, Na and K.

Validation

Internal validation (prediction).

Assessment of the calibration model was accomplished by cross-validation (prediction).

Table 3.Measurement with the fibre-optic probe. Intact samples. Calibration statistical descripted	ors
for the NIR determination of Fe, Na and K.	

Component	Mathematical treatment	RSQ	SEC	SECV	Nºof principal component	Variability explained
Fe	1/4/4/1 SNV + DT	0.877	0.09	0.245	8	95.55 %
Na	1/4/4/1 SNV	0.721	0.74 7	1.200	8	95.52 %
K	1/4/4/1 DT	0.750	7.13	9.440	8	95.48 %

In this method, the calibration sample set was divided into 7 groups, using one of them to check the results (prediction) and the other 6 to construct the calibration model. The model was repeated as many times as there groups are, such that all groups passed through the calibration group and through the prediction group. The predicted values gave validation errors were combined into SEP (C). In this stage, samples with high residuals values were eliminated, using the T> 2.5 criterion.

The results of the statistical descriptors of the internal validation in NIR for the trace elements in 42 samples of ground and intact Iberian pork loin with fibre-optic probe measurements are shown in tables 4 and 5 shows the correlation of the values obtained at the laboratory with respect to what was predicted with NIR for the elements Fe, Na and K in ground and intact samples.

Table 4. Ground sa and K in samples of	mples.Validatio	on statistical in	descriptors for	NIR determinat	ion of Fe, Zn,	Ca, Na
Commente	C1	DCO	CED	DIAC	OED(O)	

Components	Slope	RSQ	SEP	BIAS	SEP(C)
Fe	1.000	0.842	0.058	0.000	0.058
Zn	1.000	0.695	0.096	0.000	0.097
Ca	1.000	0.761	0.100	0.000	0.101
Na	1.000	0.639	0.701	0.000	0.710
K	1.000	0.781	4.358	0.000	4.415

In the light of these results it may be deduced that the NIR technique is an excellent alternative for the determination of the composition of trace elements such as Fe, Zn, Ca, Na and K in samples of Iberian pork loin

External validation

Once the calibration equation for the determination of the composition in the trace elements Fe, Zn, Ca, Na and K in loin samples had been determined, they were subjected to external validation, applying them to a set of 8 samples that had not been included in the calibration set.

The procedure followed was as follows: the spectra were recorded in triplicate and the spectral mean was taken. The calibration equations obtained in the development of the work were applied and the predicted values were compared with the laboratory results obtained later.

Table 5	. Measurements	with	fibre-optic	probe.	Intact	samples.	Validation	statistical	descriptors	for
NIR det	ermination of Fe	, Na a	nd K in san	nples o	f Iberia	n pork loi	in			

Components	Slope	RSQ	SEP	BIAS	SEP(C)
Fe	1.000	0.898	0.083	0.000	0.084
Na	1.000	0.757	0.689	0.000	0.698
K	1.000	0.775	6.675	0.000	6.760

The differences found in the external validation between the predicted and reference values vary on average between 25% for Fe, 18% for Ca, 15% for Zn, 7% for Na and 2% for K, showing that the NIR technique may be an alternative for the determination of the mineral composition of loin samples for elements such as K, Na and Zn.

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