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Proximate analysis of homogenized and minced mass of pork sausages by NIRS

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Abstract

Near infrared spectroscopy was employed to analyse samples of pork sausage meat used in the manufacturing of typical Spanish sausages (minced and homogenized product). As well, two modes of analysis for the instrument were compared. Data from proximate analysis (fat, moisture and protein) were put into a calibration model by a diode array NIR spectrometer. The spectral range used was 515–1650 nm and different mathematical pre-treatments on the signal (derivatives and scatter corrections) were also compared. Different mathematical pre-treatments caused considerable changes in the statistics of the models (coefficients of determination and standard errors). R^2 (calibration) and standard errors of prediction (SEP, external validation) in minced sausage meat for fat, moisture and protein were 0.98, 0.98 and 0.93 (R^2) and 1.38%, 1%, 0.83% (SEP), respectively. These values in homogenised sausage meat for fat, moisture and protein were 0.99, 0.98 and 0.93 (R^2), and 0.94%, 0.76% and 0.87% (SEP), respectively.

Keywords: Pork processed; Proximate analysis; NIRS; Quality control; Sample presentation

1. Introduction

The meat trade plays an important role in the food and agricultural industry overall, providing added value to its economy. Specifically, in Spain, the pork trade is of particular importance, making up 30.2% of the final livestock production and 10.4% of the final agrarian production (MAPA, 2004). Thus, Spain is the second largest producing country in the European Union, after Germany, accounting for 18.6% of the total production in 2003, and 3.8% on a global scale.

Dry-cured pork sausages are widely consumed in Spain, and the production of the typical "salchichón" sausage is particularly important. This sausage is obtained in the same way as other dry-cured sausages, after mixing, casing, fermentation and ripening. In order to manufacture this

Abbreviations: CIFA, Research and Development Agrarian Center of Andalusia Government; HD, sample presentation Homogenized 'Down-view'; HU, sample presentation Homogenized 'Up-view'; IFAPA, Andalusia Institute of Research and Development Agrarian, Fishing, Food and Ecologic Production; MD, sample presentation Minced 'Down-view'; MPLS, modified partial least squared; MSC, multiplicative scatter correction; MU, sample presentation Minced 'Up-view'; PCA, principal component analysis; PCR, principal components regression; R^2, coefficient of determination in calibration; r^2, coefficient of determination in cross validation; RMS, root mean squared; RPD, residual predictive deviation; SEC, standard error of calibration; SECV, standard error of cross validation; SEP, standard error of prediction; SNDDT, Standard normal variate and detrend; STD, standard deviation.

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type of sausage, several cuts of lean and fatty meat are used, generally excluding meat that is of higher commercial value, in other words, ham, shoulder and loins. According to the Spanish quality standard (BOE 21/03/1980), the commercial category awarded to dry-cured sausages is mainly based on their fat, moisture and protein contents. The fat and protein contents depend on the raw material used, which is not homogeneous, since the *cuts of meat* are obtained when the carcasses are carved up. The moisture content, which is inversely related to the fat content, will depend on the initial moisture, and the time and conditions in which the product is ripened Therefore, it is a parameter that should be controlled during manufacturing. Hence, it is important for industry to be able to control the levels of these constituents in fresh sausage meat in an efficient way, before it is made into actual sausages, so it can be adjusted to legally required levels and internal quality standards.

Currently, quantitative information about the composition of the product is not often included on the label. This lack of information has an important effect on consumers, since they are unable to differentiate between products with different nutritional contents when purchasing.

Consumers demand accurate and fast quality control in the different phases of the manufacturing process of these sausages. Hence, near infrared spectroscopy (NIRS) has emerged as a tool for quality assurance by determining the basic nutritional compounds that make up the product: it is quick, reliable, requires no chemicals and is easy to operate (Thyholt, Indahl, Hildrum, & Ellekaer, 1997).

NIR technology is used in the meat industry for proximate analysis. Specific instruments are available to determine the fat, moisture and protein contents of ground meat and meat products (Osborne, 2000).

How the sample is handled in relation to the instrument is critical in NIR analysis. Flexibility of measurement modes may be important to the success of developing a method (Stark & Luchter, 2003). Therefore, in this study, a NIR instrument was sought that could be quickly adapted in order to analyse the various different phases in the manufacturing of the target product (''salchichón''), that is to say, minced sausage meat, homogenised sausage meat, and the cured product. Hence, we opted for a Perten diode array reflectance spectrometer (DA-7000 model) since the measurements are very quick and non-invasive, covering the range 400–1700 nm.

Solberg (2000) used reflectance measurement with a Perten DA-7000 on unskinned and filleted salmon. She stated that it might be possible to grade the whole salmon and salmon fillets on-line with greater accuracy according to the fat content. Subsequently, Solberg (2003) studied the use of this diode-array NIR measurement as a screening method to determine the crude fat content in live-farmed Atlantic salmon, making it suitable for on-line analysis. Anderson and Walker (2003) studied the incorporation ability of Perten DA-7000 VIS/NIR analysis of ground beef for process control and they concluded that the equipment is suitable for on-line measurements. Using the same diode-array NIR analysis in the meat industry, Fumière, Sinnaeve, and Dardenne (2000) investigated how to differentiate between 'slow-growing' and 'industrial' chicken strains. These authors discuss the possibility of integrating the technique into an analytical system of surveillance for certified meat products. Chan, Walker, and Mills (2002) used this NCR instrument to assess the quality of fresh pork loin. They obtained calibration models for moisture, fat and protein contents, concluding that NIR reflectance can be used to predict some quality traits for whole fresh raw pork chops. Geesink et al. (2003) also achieved high-quality predictions for pork quality attributes using NIRS, but with a different spectrometer.

Our study measured visible/near-infrared spectra in the fresh sausage meat – minced and homogenised – used for manufacturing dry-cured pork sausages (''salchichón''). Spectra from the finished product (cured, then fermented and ripened) will be discussed in a later paper. Our aim was to evaluate the accuracy of prediction models for fat, moisture and protein, using four sample presentations with a diode array Perten DA-7000 spectrometer, and to study the most suitable sample presentation.

2. Material and methods

2.1. Raw material/preparation

2.1.1. Meat and experimental design

The pork meat used consisted of lean meat from the breeds most commonly used in Spain for manufacturing sausages. These breeds were Iberian and Standard. The Iberian pigs were obtained from a set of individuals registered in the Iberian breed genealogical catalogue. The Standard pigs were obtained from known herds of Landrace pigs. As soon as the meat was received from the slaughterhouse it was immediately frozen and stored that way until the experiments began.

In order to study a wide range of variability on the raw material most commonly used in the Spanish meat industry, in this study, two trials (manufacturing stages) were designed, and treatments using different percentages were set up as follows:

Trial 1: Five treatments were set up for different combinations of meat from Iberian (I) and/or Standard(S) pork. These treatments were A (100% I), B (75%

Table 1
Iberian and Standard pork used in each treatment

	Treatment				
	A	B	C	D	E
Iberian (kg)	18 (100%)	13.5 (75%)	9 (50%)	4.5 (25%)	0 (0%)
Standard (kg)	0 (0%)	4.5 (25%)	9 (50%)	13.5 (%)	18 (100%)

I–25% S), C (50% I–50% S), D (25% I–25% S) and E (100% S).

Trial 2: The same design was repeated after 8 days.

In each trial, 45 kg of Iberian pork and 45 kg of Standard pork were used in the proportions indicated in Table 1.

2.1.2. Meat additives

Standard additives commonly used in the Spanish meat industry were also applied in our experiments and included in the sausage meat. In each treatment (18 kg) the following were included: fine salt (400 g), dextrose (18 g), thyme (36 g), nitrifier (36 g), wine (0.35 l), garlic (90 g), ground black pepper (45 g), polyphosphate (18 g) and ascorbic acid (9 g). These additives improve the ripening process and produce very specific sensorial effects on the smell, taste and flavour.

2.2. Meat processing and sampling

In this study, we used the same ingredients and standard procedures as are commonly applied by the Spanish meat industry when manufacturing "salchichón" sausage. For this reason, all the stages were carried out in the meat processing plant of the IFAPA research centre.

2.2.1. Minced meat samples

Raw meats, from both animal origins (Iberian and Standard), were thawed at room temperature, subsequently and separately, and then ground with a meat grinder (Sammic®, Mod. G85 R-Olotinox 22). The ground meat was then mixed manually for 15 min and then mechanically with a kneader-mixer (Mainca, Equipamientos cárnicos S.L., Mod. BM 35) for 20 min. At this time the additives were added, according to the Martin–Bejarano formula (Martín, 1992). The sausage meat was then left to rest at 4 °C for 24 h. Ten samples of each treatment were then immediately set up (300 g/sample), giving 50 samples for each trial or 100 samples for the whole study. After the minced samples were analyzed by NIRS, they were kept frozen (−20 °C) prior to further analysis.

2.2.2. Homogenised meat samples

In order to observe the effect of homogenisation on the accuracy of the NIR predictive models, the minced samples were thawed and homogenised using a standard commercial blender (Moulinex®) equipped with horizontal blades, and shortly afterwards they were also analyzed by NIRS. Thus, a total of 100 homogenised meat samples were made (50 samples/period).

2.3. Spectra acquisition

2.3.1. Spectrometer

The instrument used for spectra acquisition was a Diode Array NIR/VIS (Perten 7000, Perten Instrument, Huddinge, Sweden), which uses two diode arrays, simultaneously processing records of light reflected from a sample by the diode sensors. The first array detects from 400 to 950 nm, and the second from 950 to 1700 nm. The spectrometer interpolates the date to produce one point at every 5 nm from 400 to 1700 nm, giving a 261 data point spectrum. Accordingly, the DA-7000 takes a spectral measurement every 1/30 s, and 30 spectral readings were measured and averaged.

When the computer processing time is factored in, the sampling time for the instrument is approximately 2 s. The DA-7000 emits a chopped, high intensity white light from a tungsten halogen lamp that is inside a cabinet. The light is directed at an angle through a rectangular window toward the sample, which reflects light back down through the rectangular window into the detection module.

The instrument design allows two modes of analyses: "Down-view" and "Up-view". The first uses a circular capsule with a diameter of 12.7 cm, taking spectral information at different points of the sample when the capsule spins. In order to use the second mode, the instrument was inverted and the sample was placed directly over a quartz window with a diameter of 12.7 cm. Fig. 1 shows both modes of analysis.

2.3.2. Sample presentation

All the samples used in this study were analysed by both modes of analysis explained above.

Two types of products were scanned with the two modes of analysis (minced and homogenised). Therefore, four sample presentations were used. First, the usage meat was mixed with the additives and then minced, thus obtaining "minced fresh sausage meat". This was the first product, which used for sample, presentations MD and MU (Minced 'Down-view' and Minced 'Up-view', respectively). Next, the "minced fresh sausage meat" was homogenised, giving "homogenised fresh sausage meat" for the HD (Homogenised 'Down-view') and HU (Homogenised 'Up-view') presentations.

2.3.3. Spectra

Since the experiment was repeated twice in different periods or phases, a total of 100 samples of each sample presentation were scanned. Thus, 400 samples were analysed by 197 NIRS.

All sample spectra were collected five times; thus a total of 2000 spectra were taken for this study. Subsequently, the spectral data treatment was performed on the average spectra.

When taking spectra in the Up-view mode, each sample was placed on the uppermost spectrometer window as a flat coating, 2–3 cm thick, and the minced sausage meat was spun after every scan. For the Down-view mode, samples were inserted into the round spinning capsule, and the minced sausage meat was shaken after each scan. A similar procedure was followed to obtain spectra from the homogenised sausage meat.

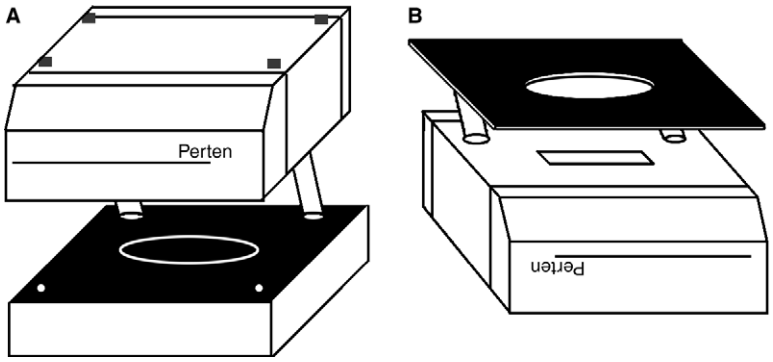

Fig. 1. Perten DA-7000 instrument modes of analysis (A: Down-view; B: Up-view) used in the spectral acquisition.

2.4. Chemical analysis

Once the spectral information was acquired, a representative amount of each sample was obtained (50 g), and then transported to the Regional Laboratory of the Department of Agriculture and Fishing (Andalusia's Regional Government), located at the "Alameda del Obispo" campus, where the nutritional parameters fat, moisture and protein were determined. Official meat and meat product analysis methods were used (MAPA, 1993), in accordance with the ISO-1443 standard in order to determine fat, and the international standards ISO-R-1442 and ISO R-937, in order to determine moisture and protein respectively.

2.5. Data analysis

2.5.1. General

Spectra were exported to WinISI III file (v1.50, Infrasoft International, Port Matilda, PA, USA) for data processing and statistical techniques were applied. The chemometric analysis procedure consists of the following steps.

2.5.2. Spectral data quality

In order to obtain a representative and quality spectrum per sample, the RMS (root mean squared) statistic was used (Shenk & Westerhaus, 1995a, 1995b), which calculates the similarity between different spectra of a sample, minimizing the various sources of error. In the present study, five spectra per sample were obtained, using the following expression in order to calculate them

$$\mathrm{RMS}_j = \sqrt{\frac{\sum_{i=1}^{n}(Y_{ij} - \overline{Y}_i^2)}{n}}$$

where n is the number of data (absorbance readings), Y_{ij} is the absorbance value $\log(1/R)$ for the sub-sample j at wavelength i (λ_i) and $\overline{Y}_i$ is the absorbance value $\log(1/R)$ for the average spectrum of a sample at wavelength $i(\lambda_i)$.

The RMS value obtained in each case was multiplied by 10^6 in order to avoid working with excessively small values.

In order to determine a RMS cut-off value in each sample presentation, the average RMS value was calculated, along with the standard deviation (STD) per sample

$$\mathrm{STD} = \sqrt{\sum_{j=1}^{N}(\mathrm{RMS}_j)^2/N - 1}$$

$$= \sqrt{\sum_{i=1}^{N}\sum_{j=1}^{n}(Y_{ij} - \overline{Y}_i)^2/n(N-1)}$$

where N is the number of sub-samples.

Since the expression of STD (error variance) follows a χ^2 distribution, a limit was used

$$\mathrm{STD}_{\mathrm{limit}} = 1.036 \times \sqrt{\sum_{K=1}^{K=m}\mathrm{STD}_K^2/m} = 1.036 \times \sqrt{\overline{\mathrm{STD}^2}}$$

where m is the number of samples.

The $\mathrm{STD}_{\mathrm{limit}}$ values were used to obtain $\mathrm{RMS}_{\mathrm{cut\ off}}$. Thus, any spectra in a sample that were above this limit were eliminated, and recalculations were performed until all the values found were below the $\mathrm{RMS}_{\mathrm{cut\ off}}$.

2.5.3. Principal component analysis

Principal component analysis (PCA) was used to develop a study of the spectral population, before developing the predictive models. This analysis eliminates redundant information resulting from high correlations between absorbancies in different regions of the spectra. Therefore, a linear combination of variables is defined, in a benchmark system in which the axes are linearly independent. Thus, the initial information is synthesised, by reducing the number of variables, explaining the same variability practically.

Once the PCA was carried out on each sample presentation, the centre of the spectral populations was determined in order to detect anomalies and samples with the characteristic behaviour. These anomalies could have a detrimental effect on the quality of the calibration models (Pizarro, Esteban-Díez, Nistal, & González-Sáiz, 2004).

After the centre of each population was calculated, the distance of each sample from the centre was subsequently determined. The Mahalanobis distance (H) (Otto, 1999)

was used, and the limit established for a sample to be considered anomalous was $H > 3$ (Shenk & Westerhaus, 1996).

2.5.4. Calibration and validation

The collective employed in each sample presentation ($N = 100$ samples) was divided into two groups. The first one was constituted by 80 samples (calibration set) and they were used to develop the predictive models. The second one (validation set) consisted of 20 samples and they were used to validate the models. These samples were randomly selected, taking two of each of the five treatments (A, B, C, D and E) in each of the two periods described in Sections 2.1 and 2.2.

First, calibration models were produced using modified partial least squared (MPLS) regression with internal cross validation (six cross validation groups) to avoid overfitting (Shenk & Westerhaus, 1995a, 1995b). For each of the four sample presentations, 20 equations were determined for each parameter, as a result of the different derivative and scattered radiation correction treatments applied to the spectra (Fig. 2).

Once the best MPLS models had been selected for each parameter in each sample presentation, with a specific combination of treatments, regression models were applied to the principal components (PCR) using the same combination in order to compare the two calibration strategies.

The criteria used to select the best models were few standard errors and a high regression coefficient of determination, both in calibration (SEC, R^2) and in cross validation (SECV, r^2) (Workman, 2001). Furthermore, in order to evaluate the predictive ability of the calibration models, the residual predictive deviation (RPD) was used. The RPD is the relationship between the standard deviation (SD) of the population's reference values and the standard error of cross validation (SECV). If the RPD > 3, the predictive ability of the model could be considered very good (Williams & Sobering, 1996).

In calibration, the software identifies chemical anomalies (T), that is to say, samples in which the reference values differ from those predicted by NIRS. These anomalies are identified using the student's T statistic (quotient between the difference between the reference and the predicted value and the SEC). Samples with a value $T > 2.5$ were considered chemically anomalous.

The behaviour of the external validation group used in the different models was evaluated using the standard error of prediction (SEP) and the coefficient of determination in external validation (R^2_{EV}) (Westerhaus, Workman, Reeves, & Mark, 2004).

3. Results and discussion

3.1. Reference values

The reference values employed for developing the predictive models were obtained from chemical analyses carried out at the Regional Laboratory of the Department of Agriculture and Fishing (Andalusia's Regional Government) (Section 2.4).

Table 2 shows the average values, standard deviation and the range of reference values for the three constituents analysed (fat, moisture and protein), for both the calibration and validation sets.

When the minced sausage meat was stuffed into artificial guts to complete the process of fermentation and ripening over 21 days in order to obtain the traditional dry-cured sausage, the mean values for fat, moisture and protein changed approximately to 31%, 35% and 27%, respectively. Therefore, the average values for fat and protein in dry material should be approximately 48% and 42%, respectively. Bearing in mind the average values of these parameters, according to the Spanish legislation governing quality categories based on analytical composition, they would be classified as extra quality (BOE 21/03/1980). However, the experimental group, as a whole, spans a wide range,

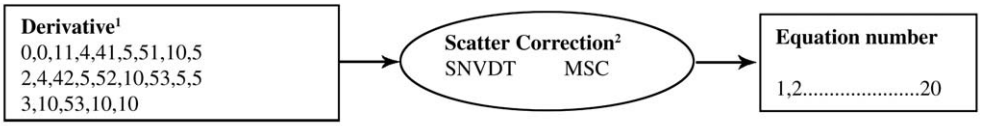

Fig. 2. Flow chart of derivative treatments and scatter corrections applied to the spectra.

Table 2
Reference values of the calibration and validation sets

Constituent	Calibration set				Validation set			
	N	Mean	SD	Range	N	Mean	SD	Range
Fat (%)	80	20.27	7.31	8–31.7	20	20.29	7.44	9.5–30.9
Moisture (%)	80	58.94	5.3	50.2–68.4	20	58.89	5.48	51–67.1
Protein (%)	80	16.7	2	12.7–20.5	20	16.9	2.28	13.9–21.6

representing a great deal of the variability found in the manufacturing of these sausages in the Spanish meat industry.

3.2. Root mean squared (RMS) values

The procedure described in Section 2.5.2 was used to obtain a cut-off value for the RMS statistic ($RMS_{cut\ off}$). Table 3 shows the $RMS_{cut\ off}$ values obtained in the different sample presentations, together with the percentage of spectra eliminated in each of them by surpassing the said limit ($RMS_{cut\ off}$).

The Up-view analysis mode establishes very slight differences between $RMS_{cut\ off}$ values; however, major differences were observed between the two types of samples with the Down-view mode. Therefore, this mode has greater sensitivity in the acquisition of spectra with regard to product variation.

On the other hand, lower $RMS_{cut\ off}$ values were obtained using the Down-view analysis mode on both types of products. So, this analysis mode gives greater similarity between the spectra of a single sample (lower $RMS_{cut\ off}$ values). Once the spectra that were over the $RMS_{cut\ off}$ value were eliminated, the average was obtained of the rest of the spectra in each sample in order to obtain a representative spectrum of the samples. As seen in Table 3, the percentage of spectra eliminated in each sample presentation was between 21.4% (MD) and 23.5% (HU). Bearing in mind that, in each sample presentation, a total of 500 spectra were obtained, over 100 were eliminated from each of them. Despite this, no sample was completely eliminated, since at least one of them had two spectra readings.

3.3. Spectra interpretation

Fig. 3 shows the average spectra of the four sample presentations.

All the spectra were trimmed at the ends (400–515 nm; 1650–1700 nm) because of the low repeatability in these areas. The four presentations follow similar patterns, although a grouping into product type (minced and homogenised) can be discerned, with visual differences between the two. This grouping is more noticeable for minced samples, with minor differences between the average spectra analysed with both modes of analysis (MD and MU).

A visual study of spectra revealed that there were fundamentally four important absorption bands in the four sample presentations. In the visible band, there was one particularly noticeable band, between 560 and 585 nm. Within this range, absorptions are found related to muscle pigments (Cozzolino, Barlocco, Vadell, Ballesteros, & Gallieta, 2003; Cozzolino & Murray, 2004). Hence, this band can be explained by the blue region of the spectrum due to heme proteins, oxymyoglobin and myoglobin (Cozzolino & Murray, 2002; Mitsumoto, Maeda, Mitsuhashi, & Ozawa, 1991).

Table 3
$RMS_{cut\ off}$ and percentage of eliminated spectra (>$RMS_{cut\ off}$) in the sample presentations studied

	Up-view mode		Down-view mode	
	$RMS_{cut\ off}$	%Spectra > $RMS_{cut\ off}$	$RMS_{cut\ off}$	%Spectra > $RMS_{cut\ off}$
Minced samples	25,500	21.6	20,000	21.4
Homogenized samples	26,500	23.5	10,500	222.2

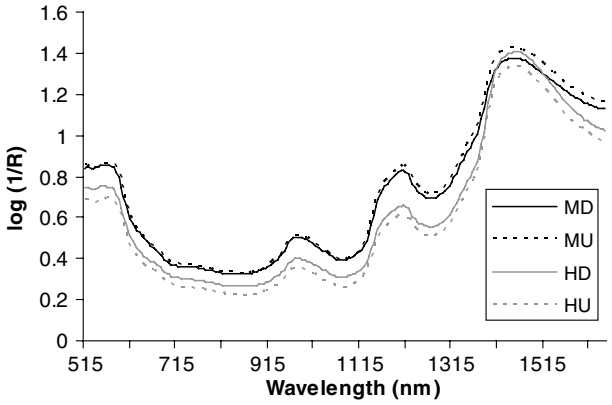

Fig. 3. Mean spectra of four sample presentations studied: Minced Down-view (MD), Minced Up-view (MU), Homogenized Down-view (HD) and Homogenized Up-view (HU).

Within the 965–1020 nm range, an absorption band in the transition area of the visible to near infrared zone is detected. This band is most likely due to water (Osborne, Fearn, & Hindle, 1993), related to the third overtone (Cozzolino & Murray, 2004).

In the NIR region, an absorption band 1195–1230 nm is observed, with a peak at 1215 nm. At wavelengths close to 1200 nm, the C—H bonds, which are a fundamental constituent of fatty acid molecules, absorb strongly (Willians & Norris, 1987). Specifically, Shenk, Workman, and Westerhaus (2001) link the place, where the greatest intensity is achieved in this band (1215 nm) with the structure of CH_2. These authors relate the absorbancies of 1195 nm and 1225 nm to CH_3 and CH_2, respectively. So, around 1200 nm, significant information is found related to fat (CH stretch second overtone). The final important band was observed at around 1450 nm. Shenk et al. (2001) relate this region to combination bands of the bond C—H (CH_2 at 1440 nm and aromatic structure at 1446 nm), OH stretch first overtone and C=O stretch third overtone (1450 nm), and NH stretch first overtone (urea at 1460 nm and $CONH_2$ at 1463 nm). In meat products, this band has been linked with water due to the OH bond (Cozzolino & Murray, 2004; Hoving-Bolink et al., 2004; Leroy et al., 2003; Liu & Chen, 2001; Realini, Duckett, & Windham, 2004).

3.4. Principal component analysis

Principal component analysis (PCA) was applied to the over group of each presentation. The Mahalanobis distance was calculated from the centroid of each sample,

defined by its population. By applying the limit discussed in Section 2.5.3 ($H > 3$), the possible appearance of spectral outliers was detected. An anomalous sample only occurred in one sample presentation (Minced Up-view, MU) although, in minced samples analysed in the Down-view mode, two displayed a value very close to the limit ($H = 2.98$). This same situation also occurred in a homogenised sample analysed in the Down-view mode ($H = 2.93$). Togersen, Isaksson, Nilsen, Bakker, and Hildrum (1999) did not discover any apparent outliers in the PCA they conducted on meat samples (fresh ground pork and beef).

The number of principal components (PCs) recommended by the software varied between 9 (minced samples, Down and Up-view) and 10 (homogenised samples, Down and Up-view). In all cases, the variance explained by the PCs selected was found to be close to 100% (from 99.5% for MU to 99.7% for HD and HU). Fig. 4 shows the evolution of the variance explained by the PCA as PCs were added.

Cozzolino and Murray (2004), when carrying out a PCA on animal meat muscle samples (100 of beef and 140 of lamb), obtained similar variance accumulated as the PCs were added. For instance, with the first three PCs, Cozzolino and Murray (2004) obtained 95% of variance explained. In this study, the first three PCs explained an accumulated variance of between 93.2% (HD) and 96.3% (MD).

3.5. Prediction equations

3.5.1. Characterisation of predictive models

The treatments described in Section 2.5.4, were then applied in order to calculate the equations in each sample presentation.

The greatest number of samples excluded in the calibration development process because of chemical anomalies (T) occurred in the MU samples. This number is determined by subtracting the number of samples selected for each predictive model and the number of spectral anomalies (H) from the initial calibration group (80). This is due to the greater heterogeneity of the minced samples, and the different strategies that the instrument used to store information, depending on which analysis mode was used (Down or Up-view).

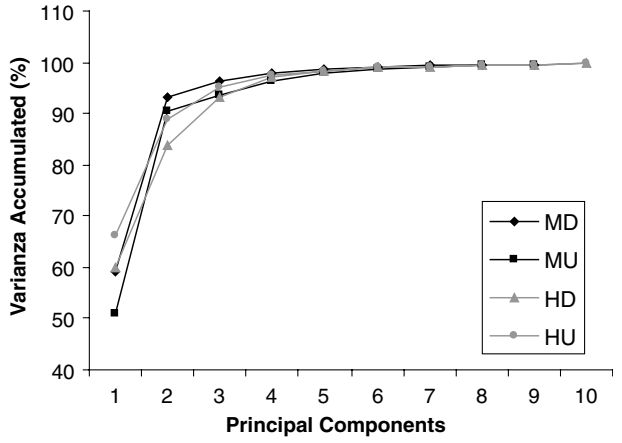

Fig. 4. Accumulated variance of each sample presentation with the addition of the principal components.

Table 4 shows the characteristics of the equations selected for each constitution, along with the number of samples that make up each model (N), the number of factors or regression terms, type of regression (PCR or MPLS regression) and mathematical treatment applied to the signal (derivatives and scatter correction).

There was no homogeneity observed with regard the number of factors that made up the models – varying between 2 and 9 – or in the derivative treatment used on the signal. The regression method used for all the equations selected was minimum partial least squares (MPLS), except for the constituent fat in minced samples (Down and Up-view), in which principal component regression achieved better results.

The scatter correction treatment selected was SNVDT, except for fat in MU, where MSC treatment achieved better behaviour.

Chan et al. (2002) obtained PLS predictive models of fat, moisture and protein from whole pork loin samples with a Perten DA-7000 spectrometer in the Up-view mode. Their best calibration models were a 10,13,12-factor PLS model for fat, moisture and protein, respectively, with first and second derivative pre-processing.

In this study, the PLS factors with the Up-view mode ranged from 2 to 4 PLS; therefore the complexity of the models was lower. On the other hand, in the selected models, greater variation was found with regard to types of derivative treatments (none, first, second and third) when compared to those applied by Chan et al. (2002), who selected first and second derivatives.

Table 4
Characterisation of selected equations for each sample presentation (Minced Down-view MD, Minced Up-view MU, Homogenised Down-view HD, and Homogenised Up-view HU) and constituent

	MD	MU	HD	HU
Fat				
N	77	74	77	77
Factors	7	3	3	4
Regression[a]	PCR	PCR	MPLS	MPLS
Derivative	2,4,4	3,10,5	2,4,4	0,0,1
Scatter correction[b]	SNVDT	MSC	SNVDT	SNVDT
Moisture				
N	77	75	79	78
Factors	6	2	9	3
Regression[a]	MPLS	MPLS	MPLS	MPLS
Derivative	2,4,4	2,5,5	0,0,1	3,5,5
Scatter Correction[b]	SNVDT	SNVDT	SNVDT	SNVDT
Protein				
N	78	75	77	79
Factors	5	4	2	2
Regression[a]	MPLS	MPLS	MPLS	MPLS
Derivative	1,4,4	1,5,5	2,4,4	2,5,5
Scatter correction[b]	SNVDT	SNVDT	SNVDT	SNVDT

[a] PCR: principal component regression; MPLS: modified partial least squared.

[b] SNVDT: standard normal variate and de-trending; MSC: multiplicative scatter correction.

Anderson and Walker (2003) also applied second derivatives in order to obtain on-line analysis PLS models for ground beef with nominal 50% and 15% fat contents. These authors also used a Perten DA-7000 spectrometer, with special position, similar to the Down-view mode.

Fumière et al. (2000) used similar spectral treatments (scatter corrections and derivatives) to those used in this study in their attempt to authenticate cut pieces of chicken meat, using a Perten DA-7000 in the Up-view mode. In this case, their best models were obtained with first and second derivatives, along with SNV and SNVDT scatter corrections (legs with skin 1,5,3-SNV; skinless breasts 2,1,1-SNVDT; carcasses with skin 1,5,3-SNV).

3.5.2. Evaluation of predictive models

3.5.2.1. General. Table 5 shows the statistics of the equations designed to predict fat, moisture and protein, both in calibration and validation (cross and external), for the four sample presentations.

3.5.2.2. Differences between constituents (fat, moisture and protein) and products (homogenized and minced) on the predictive models. With regard to the nutritional parameters fat and moisture, the coefficients of determination (R^2) selected for both calibration and validation were over 0.9 in all the equations. Shenk and Westerhaus (1996) reported that any equations with values achieved that were equal to or higher than this value were extremely accurate. So far, from the other statistics used to evaluate the models (SEC, SECV, RPD and SEP), we can see that there are differences between the homogenised and minced sample presentations. The homogenised samples presented models with lower typical errors (SEC, SECV and SEP), and higher RPD values, and are therefore more robust models. Consequently, greater heterogeneity is the product entailed a loss of accuracy in the equations; the highest typical error or prediction was obtained for the minced samples analysed in the Up-view mode (SEP = 1.41%).

This loss of accuracy in the prediction models for fat in minced product versus homogenised product was also observed for moisture, as we can see in Table 5. The great typical error obtained for this constituent was SEP = 1.01%, lower than that for fat (SEP = 1.41%). This comparison is made in absolute values, since the predictive range of fat is higher than that for moisture (fat 8.2–31.7% vs. moisture 50.2–68.4%); hence these prediction errors will be of a similar magnitude if the model's predictive range is taken into account.

The statistics obtained in the different sample presentations for the nutritional compound protein did not reveal any differentiation between homogenised and minced samples, as they did for fat and moisture. In calibration and cross validation, lower values were found for the typical errors, SEC = 0.51–0.57%, SECV = 0.53–0.65%, of the three predicted constituents. However, once again, it is important to underline that this assessment of the typical errors was carried out in terms of absolute values, since the predictive range in protein is considerably lower than that for moisture, and especially for fat. Furthermore, lower typical errors of prediction in external validation (SEP) were obtained for the parameter moisture (SEP = 0.76–0.77%) At the same time, the coefficients of determination obtained for protein in the four sample presentations (both in calibration and cross and external validation) were the lowest of the three constituents. The predictive capacity of the models selected for protein might be lower than those of fat and moisture. This evaluation gains greater force when observing the RPD statistic,

Table 5
Statistics of selected equations in calibration (standard error of calibration, SEC; coefficient of determination in calibration, R^2), cross validation (standard error of cross validation, SECV; coefficient of determination in cross validation, r^2; residual predictive deviation, RPD) and external validation (standard error of prediction, SEP; coefficient of determination in external validation, R^2_{EV})

	Range (%)	SD	Calibration		Validation				
			SEC (%)	R^2	Cross			External	
					SECV (%)	r^2	RPD	SEP(%)	R^2_{EV}
Fat (%)									
MD	8–31.7	7.36	1.28	0.97	1.35	0.97	5.45	1.38	0.97
MU	8–31.7	7.34	1.16	0.98	1.17	0.97	6.27	1.41	0.97
HD	8–31.7	7.29	0.8	0.99	0.87	0.99	8.38	0.94	0.98
HU	8–31.7	7.26	0.81	0.99	0.89	0.99	8.16	1.18	0.98
Moisture (%)									
MD	50.2–68.4	5.37	0.74	0.98	0.95	0.97	5.65	1.01	0.97
MU	50.2–68.4	5.32	0.95	0.97	0.99	0.96	5.37	1	0.97
HD	50.2–68.4	5.31	0.71	0.98	0.84	0.98	6.32	0.77	0.98
HU	50.2–68.4	5.27	0.73	0.98	0.79	0.98	6.59	0.76c	0.98
Protein (%)									
MD	13.6–20.5	1.97	0.54	0.93	0.61	0.9	3.23	0.83	0.87
MU	13.7–20.3	1.9	0.56	0.91	0.65	0.88	2.92	0.84	0.87
HD	13.6–20.5	1.98	0.51	0.93	0.53	0.93	3.74	0.87	0.86
HU	13.6–20.5	1.96	0.57	0.92	0.6	0.91	3.27	0.87	0.86

which gave the lowest values for protein. Even the equation selected for protein for the minced samples analysed in the Up-view mode (MU), yielded a value that was just below (RPD = 2.92) the limit established by Williams and Sobering (1996) (RPD > 3) for a model to be classified as having very good predictive characteristics. Anderson and Walker (2003) obtained predictions for fat using ground beef in a sample presentation similar to the Minced Down-view (MD) used in this study, as well as a similar variation range (Anderson et al., 7.2–24.4% vs MD, 8–31.7%). They obtained values of $r^2 = 0.96$ and SECV = 1% (cross validation), and $R^2_{EV} = 0.83$ and SEP = 2.15% (external validation). Comparing these values with those shown in Table 5 for the MD sample presentation, a great similarity is observed between the values for r^2; Anderson and Walker (2003) obtained lower values for SECV (Anderson et al., SECV = 1% vs. MD, SECV = 1.35%). However, the values obtained in the present study in external validation (SEP), were considerably lower (Anderson et al., SEP = 2.15% vs. MD, SEP = 1.38%). This loss of accuracy could be due to the fact that Anderson and Walker (2003) designed a specific analysis device adapted to a transportation line, and did not use the natural position of the equipment.

Chan et al. (2002) included predictions of fat, moisture and protein in their study of pork quality characteristics, using the Up-view mode. The values obtained in cross validation (r^2) were 0.76, 0.8 and 0.69, and SECV, 0.62%, 0.58% and 0.43%, respectively, for fat, moisture and protein. In the same order, in external validation, they obtained R^2_{EV} of 0.61, 0.69 and 0.7, and SEP of 0.62%, 0.63% and 0.42%. Table 4 (Up-view modes: MU, HU) shows how, in both cross validation (r^2) and external validation (R^2_{EV}), better correlations were obtained between predicted and observed values, in the three constituents, than those obtained by Chan et al. (2002). However, the values obtained by these authors for SECV and SEP were lower than those obtained in this current study with MU and HU for the three constituents, and closer to those obtained for the homogenised product (HU). These differences are partly rooted in the fact that Chan et al. (2002) used pork loin samples, a more homogeneous product than that used in this study, but were mainly due to the lower range of variation and standard deviation of their reference values, especially for moisture (range 67.1–79%, SD = 1.37%) and fat (range 19.3–24.3%, SD = 0.8%), as we can see Table 5.

Kang, Park, and Choy (2001) obtained predictions for fat, moisture and protein in ground pork sausages using a NIR spectrophotometer with a greater scanning range (400–2500 nm). Their reference values were very similar to those described here. The results obtained in the equations were also similar to the minced sample presentations (MD, MU), although less accurate. This can be quantified by comparing the values for MD and MU (Table 5) in external validation with those obtained by Kang et al. (2001); they obtained R^2_{Ev} of 0.88, 0.93 and 0.54 for fat, moisture and protein, respectively, and SEP of 1.53%, 1.15% and 1.12% in the same order.

3.5.2.3. Differences between modes of analyses (Down-view and Up-view) on the predictive models. To appreciate similarities and differences between both modes of analyses (Down and Up-view modes) for the three nutritional parameters predicted, the SEC (calibration stage), SECV (cross validation stage) and SEP (external validation stage) are inspected in Table 5.

The differences between the two modes of analysis for the constituent fat in homogenised samples were barely noticeable in calibration ($SEC_{HD} = 0.8\%$ vs. $SEC_{HU} = 0.81\%$) and cross validation ($SECV_{HD} = 0.87\%$ vs. $SECV_{HU} = 0.89\%$). On the other hand, the difference in accuracy of the models in external validation is taken into account, and the Down-view analysis mode gave lower prediction errors ($SEP_{HD} = 0.94\%$ vs. $SEP_{HU} = 1.18\%$). In the predictions of fat in the minced product, the Up-view mode gave better values in calibration ($SEC_{MD} = 1.28\%$ vs. $SEC_{MU} = 1.16\%$) and cross validation ($SECV_{MD} = 1.35\%$ vs. $SECV_{MU} = 1.17\%$). However, the behaviours of the prediction models in external validation were similar for both modes of analysis ($SEP_{MD} = 1.38\%$ vs. $SEP_{MU} = 1.41\%$).

For the parameter moisture, the values observed were notably different between the two modes of analysis for any statistic in the homogenised product (Table 5). In the minced product, the Down-view model selected for moisture particularly stood out, yielding better values in calibration ($SEC_{MD} = 0.74\%$ vs. $SEC_{MU} = 0.95\%$), but barely noticeable differences in cross ($SECV_{MD} = 0.95\%$ vs. $SECV_{MU} = 0.99\%$) and external validation ($SEP_{MD} = 1.01\%$ vs. $SEP_{MU} = 1\%$).

For protein, in both the homogenised and minced products, a slight improvement of the model used with Down-view was observed in calibration ($SEC_{HD} = 0.51\%$ vs. $SEC_{HU} = 0.57\%$; $SEC_{MD} = 0.54\%$ vs. $SEC_{MU} = 0.56\%$) and cross validation ($SECV_{HD} = 0.53\%$ vs. $SECV_{HU} = 0.6\%$; $SECV_{MD} = 0.61\%$ vs. $SECV_{MU} = 0.65\%$), although identical values were acquired in external validation (SEP = 0.87%) for the homogenised product, and practically identical values for the minced product ($SEP_{MD} = 0.83\%$ vs. $SEP_{MU} = 0.84\%$).

Thus, for the homogenised samples, when dealing with fat predictions, it seems slightly more advisable to use the Down-view rather than the Up-view mode of analysis, given the differences in behaviour of the external prediction models (evaluated by SEP). These differences are not really noticeable in moisture and protein predictions for the homogenised product, or for the three constituents in the minced product. Hence, in this case, it would be more advisable to use the mode that allows less handling of the product as well as greater simplicity of analysis. Given these criteria, it would be sensible to use the Up-view mode of analysis, since it would only be necessary to place fresh meat mass on the circular glass plate (Fig. 1B) in order to analyse it, without having to put it into an analysis device (capsule) as with the Down-view mode.

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