# Pork meat mixes characterization through near infrared spectroscopy. Freezing / thawing effect on the models

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## Introduction

In Spain, the swine industry is characterised by the production of the Iberian pig, and exclusive strain from the Iberian peninsula. Production areas are characterised by a rearing system distinguished from others for the perfect adaptation of animals to the environment in which they live/feed freely. Fresh meat and elaborated products from these animals are, by far, the most appreciated in Spain, being considered as holders of the highest quality in relation to their inherent characteristics. Consequently, these products reach the highest market prices among the meat produces, increasing their demand as the standard of living rise.

This fact is currently bringing about frauds by which non pure Iberian pig products are sold as such. These counterfeit produces can attain very high prices, although their quality is far behind that of the genuine Iberian pig ones.

Furthermore, the current Spanish Standards of Quality regarding cured products from Iberian pig meat, only regulate entire cured products as forward and rear hams and loin sticks, which are, doubtless, the most valuables, but does not take into account those other products as the Iberian pig sausages which are increasingly demanded. This reality allow cheaters to act much more freely, dumping in the market, as genuine, products which are made of variable mixtures of Iberian pig meat with standard pig meat.

In this regard, a method able to detect different types of meats in mixtures would be of great interest.[1] In this work, near infrared (NIR) spectroscopy is used as a tool to spot different mixtures of pork meats. This is carried out by the development of multivariate analysis models fitted to samples with different known meat mixtures.

Therefore, the objective of this study was the application of the NIR methodology to the detection of meat mixtures in fresh pork sausages, applying classification models such as the discriminant analysis, to identify the origin of meat. A secondary purpose was to study the effect of samples freezing/ thawing on the effectiveness of the models previously obtained.

## Materials and methods

Raw material used in this study consisted in lean meat of Iberian pigs (I) and lean meat of standard pig (S). To this last one, a 10% of fat from the same origin was added. All the meat was



Near Infrared Spectroscopy: Proceedings of the 11th International Conference.

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received from the slaughter house, frozen, and was maintained so until samples setting up. To carry out this study three different trials were set, as follows:

- Trial 1: Five treatments consisting in different combinations of meat from I and S: a (100% I), b (75 %I /25 % S ), c (50 % I/50 % S ), d (25 % I/75 % S ) and e (100% S). The same preparation was repeated once after 8 days.
- Trial 1: Five treatments consisting in different combinations of meat from I and S: a (100% I), b (75% I /25% S ), c (50% I / 50% S ), d (25% I/75% S ) and e (100% S). The same preparation was repeated once after eight days.
- Trial 2: Same design as above, but repeated five times every eight days.
- Trial 3: Only two treatments: a (100 % I) and e (100 % S). Same procedure was repeated four times every eight days.

## Sample set up and conservation

Meat of both origins was thawed at room temperature, right after, it was grinded separately. Ground meat was then mixed according with the treatment designs. Later, meat pastes were braked manually and mechanically. At the same time the standard additives used in the preparation o traditional sausages, were added and then the pastes were let in rest for 24 hours.

Then samples were taken in the first trial per treatment and period, resulting in 100 samples. In the second trial, three samples per treatment and period were taken, resulting in 75 samples. In the third trial, several samples were taken from both treatments, a and e, as to carry out four different processes of freezing/thawing.

All samples were frozen and maintained at –20°C: in the 1st trial, samples in plastic bags were vacuumed and kept frozen during one month until used. In the 2nd trial samples in non-vacuumed plastic bags were kept frozen for one month until used, and in the 3rd trial were kept frozen during eight months in non-vacuumed plastic bags.

## Spectroscopic analysis

Samples were scanned on a Perten DA-7000 equipment. This instrument covers the vis/NIR range from 400–1700 nm. Spectra acquisition was carried out in a rotating capsule, which allows scan samples from several points. Before taking spectra, samples were thawed at room temperature and homogenised. Five spectra were taken from each sample, resulting in 500 spectra in trial 1, 375 in trial 2 and 40 in trial 3. In trial 3 samples underwent up to four consecutive processes of freezing/thawing.

## Statistic analysis

All Statistical treatment were carry out with WinISI II v. 1.05 program.

For quantitative models,[2,–4] a total of 80 samples were used for calibration purposes and 20 for prediction. Modified partial least square regression (MPLS) technique was used. Equations were developed for fat, moisture and protein content, using different derivative treatments and systems of correction of scatter radiation.

To select the best equations the followings statistics were taken into account:

- $R^2$ y $r^2$: Coefficients of determination in calibration and cross-validation
- *SEC* (standard error of calibration), Equation (1)

$$SEC = \sqrt{\frac{\sum_{i=1}^{N}(y_i - \hat{y}_i)^2}{N - p - 1}} \quad (1)$$

- *SECV* and *SEP* (standard error of cross-validation and of prediction), Equation (2)

$$SECV \cong SEP = \sqrt{\frac{\sum_{i=1}^{N}(y_i - \hat{y}_i - bias)^2}{N - 1}} \quad (2)$$

$N$ : number of samples on the calibration, cross validation or prediction group
$p$ : number of regression factors
$y_i$ : value of the constituent to calibrate from sample i
$\hat{y}_i$ : predicted value by the calibration equation for sample i

In the development of qualitative models, discriminant functions were determined by using minimum partial squares (PLS) as terms of the regression equations. Several runs of cross-validation were carried out and, as in the case of quantitative models, different derivative treatments and system of correcting scatter radiation were used on log (1/*R*) signal.[5,6]

## Results and discussion

### Quantitative models development

In the development of these models, the different treatments for correcting the scatter radiation and the smoothing by derivation of the spectral data, have been done on basis to the predictive equations obtained previously.

Table 1 shows the reference values obtained by standard chemical analyses of fat, moisture and protein content, used in the development of the calibrations.

**Table 1. Chemical values for fat, moisture and protein content.**

| Constituent | Min. | Max. | Mean | *SD* |
|---|---|---|---|---|
| Fat | 8 | 31.7 | 20.26 | 7.3 |
| Moisture | 50.2 | 68.4 | 58.94 | 5.3 |
| Protein | 12.7 | 20.5 | 16.66 | 1.99 |

The differences found in these parameters are rather due to the diverse genetics and feeding habits of the animals (Iberian pigs and standard pigs).

Table 2, shows the different equations obtained for each constituents in both calibration (80 samples) and prediction (20 samples) phases as well as the mathematical treatment taken into account in each one and the number of PLS factors retained.

**Table 2. Calibration and Prediction parameters for fat, moisture and protein content.**

| Constituent | Mathematical Treatment | Fact. PLS | Calibration | | | | Validation | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | *N* | $r^2$ | *SEC* | *SECV* | *RSQ* | *SEP* | *Bias* |
| Fat | 0,0,1,1 SND-DT | 6 | 78 | 0.98 | 0.87 | 1 | 0.98 | 1.18 | –0.19 |
| Moisture | | 7 | 78 | 0.97 | 0.76 | 0.85 | 0.98 | 0.74 | 0.11 |
| Protein | | 2 | 76 | 0.93 | 0.51 | 0.53 | 0.86 | 0.88 | 0.25 |
| Fat | 1,4,4,1 SNV-DT | 4 | 76 | 0.98 | 0.83 | 0.91 | 0.97 | 1.01 | 0.07 |
| Moisture | | 8 | 78 | 0.97 | 0.73 | 0.86 | 0.98 | 0.82 | 0.15 |
| Protein | | 2 | 77 | 0.92 | 0.53 | 0.55 | 0.85 | 0.89 | 0.21 |
| Fat | 1,5,5,1 MSC St | 6 | 79 | 0.98 | 0.87 | 1.01 | 0.97 | 1.19 | –0.17 |
| Moisture | | 8 | 78 | 0.98 | 0.71 | 0.84 | 0.98 | 0.84 | 0.18 |
| Protein | | 2 | 77 | 0.92 | 0.53 | 0.56 | 0.85 | 0.9 | 0.21 |
| **Fat** | **2,4,4,1 SNV-DT** | **3** | **75** | **0.99** | **0.81** | **0.86** | **0.98** | **0.99** | **0.04** |
| Moisture | | 6 | 77 | 0.98 | 0.65 | 0.83 | 0.97 | 0.87 | 0.08 |
| **Protein** | | **2** | **76** | **0.93** | **0.5** | **0.52** | **0.86** | **0.88** | **0.21** |
| Fat | 2,5,5,1 MSC St | 3 | 75 | 0.99 | 0.81 | 0.87 | 0.97 | 0.98 | 0.06 |
| Moisture | | 6 | 77 | 0.97 | 0.67 | 0.84 | 0.98 | 0.8 | 0.11 |
| Protein | | 2 | 77 | 0.93 | 0.52 | 0.54 | 0.85 | 0.88 | 0.23 |
| Fat | **2,10,5,1 SNV-DT** | 4 | 76 | 0.99 | 0.81 | 0.9 | 0.97 | 1.06 | 0.08 |
| **Moisture** | | **3** | **75** | **0.98** | **0.77** | **0.83** | **0.98** | **0.7** | **0.05** |
| Protein | | 1 | 76 | 0.93 | 0.53 | 0.54 | 0.84 | 0.93 | 0.22 |

The selected equations (those in bold) have been obtained by obtaining the second derivative of the spectral data and correction of the scatter radiation SNV-DT.

In general, all the equations fitted fairly, mostly for fat and moisture showing a very high determination coefficients ( > 0.9 ).[3] For protein, $R^2$ values, in all cases, were lower than those of the other parameters, and the SEP values showed higher bias. This could be due to the no exact relationship between the NIRS measurements and the reference method (% N × 6.25).

## Qualitative models development

As in the development of quantitative models, the treatment used in the discriminant analysis are based on selections carried out in previous experiments, carried out with a similar group of samples.[7]

Table 3 shows the different mathematical treatments utilised for the discriminant models obtained to tell between the five treatments (A, B, C, D, E) as well as the classification matrix attained with the selected model.

**Table 3. Models obtained with MPLS regressions and Classification of selected model.**

| Mathematical treatment | *N* | PLS factors | **SECV** | Classification error (%) | Classification matrix | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | **A** | **B** | **C** | **D** | **E** |
| | | | | | **15** | 1 | 0 | 0 | 0 |
| 1,4,4,1 SNV-DT | 75 | 9 | 0.26 | 9.33 | 0 | **13** | 0 | 0 | 0 |
| **2,5,5,1 SNV-DT** | **75** | **12** | **0.26** | **8** | 0 | 1 | **14** | 2 | 0 |
| 2,5,5,1 MSC St | 75 | 12 | 0.26 | 9.33 | 0 | 0 | 1 | **13** | 1 |
| 2,10,5,1 SNV-DT | 75 | 11 | 0.27 | 14.67 | 0 | 0 | 0 | 0 | **14** |

It can be noticed that the three first models of the table are very similar, varying only in the number of PLS factors, showing the least classification error (8–9.3 %).

It is worth to mention that the error committed in the sample classification, in all cases, was due to the classification of samples in neighbouring categories rather than in far-off ones.

## Effect of the freezing/thawing processes of the samples on the qualitative and quantitative models. An approach

Figure 1 shows the average spectrum for treatment A and E after the first and fourth thawing processes.

It can be noticed that all the spectra follow a very similar pattern, particularly in the absorbance peaks and valleys, although in the visible region, bigger differences can be observed on the spectra corresponding to the fourth thawing process. This fact was already detected by the simple visual observation of samples in which some weird colours were perceived, which did increase as the freezing/thawing processes went on.

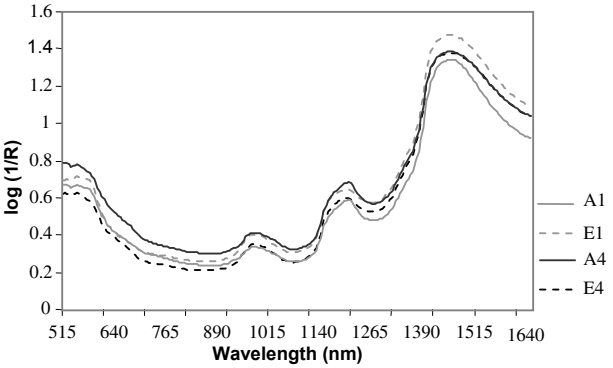


**Figure 1. Average spectrum of the A and E treatments after the first and fourth thawing processes.**

In the NIR region, however, those spectra presenting wider differences in absorbance, were those of the first freezing/thawing treatment on the wave length range of 1450–1470 nm related to the first overtone of the O–H bond (water) and N–H (proteins), so these differences might be caused by the variations on the water and protein content produced during the freezing/thawing process.

When these samples are included in the quantitative models developed in point 1, for fat, moisture and protein prediction, the results show a limited variation for the tree parameters predictions, although a general trend of decrease in fat content and increase in moisture and protein content can be observed (Table 4), except for moisture in treatment E, 4$^{th}$ process of freezing/thawing.

This small variation can be substantiated when the standard errors of each constituent: $SEP_{fat}$ = 0.48; $SEP_{moisture}$ = 0.52; $SEP_{protein}$ = 0.21, are compared with the standard errors obtained in the laboratory chemical determinations: $SEL_{fat}$=0,64; $SEL_{moisture}$=0,68; $SEL_{protein}$=0,76 which are always higher than those obtained with the models.

**Table 4. NIR predicted values for fat, moisture and protein content of samples corresponding to treatments A and E after different processes of freezing /thawing**

| Samples | Fat | Moisture | Protein |
|---|---|---|---|
| A1 | 22.91 | 57.95 | 15.72 |
| A2 | 22.53 | 58.33 | 15.88 |
| A3 | 21.83 | 58.89 | 16.12 |
| A4 | 21.53 | 59.13 | 16.34 |
| E1 | 11.4 | 67.04 | 18.72 |
| E2 | 11.09 | 67.76 | 18.84 |
| E3 | 10.85 | 68.24 | 18.9 |
| E4 | 10.9 | 67.82 | 18.92 |

1,...,4: Freezing/Thawing processes

**Table 5. Assignments of samples corresponding to treatments A and E after four processes of freezing /thawing**

| Validation (Freezing/Thawing Processes) | | | | |
|---|---|---|---|---|
| **A** | **B** | **C** | **D** | **E** |
| A1 | | | | E1 |
| A2 | | | E2 | |
| A3 | | | | E3 |
| A4 | | | | E4 |
| 1,...,4: Freezing/Thawing processes | | | | |

Following the same procedure, classification models obtained in point 2, were validated with the spectra acquired after each freezing/thawing process.

Table 5 shows the sample allocations on the different treatment groups.

Among all the models, that one in which the second derivative was applied and corrected the scatter radiation (MSC standard) shows the smallest error, classifying in D only one spectra of treatment E of the 2nd thawing process

## Conclusions

The results of this work show the usefulness of NIR analysis for:

Predicting fat and moisture content with high accuracy and protein with fair precision in pork meat sausages; Detecting presence of standard pork meat on Iberian pig sausages; Freezing/Thawing of samples does not significantly affect prediction or classification models.

## Acknowledgements

Thanks: Dr. Juan García and laboratory staff Antonio López, Isabel Leiva and Antonia Herruzo, for their skilful cooperation.

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