# NIR for animal species identification in rendered fats: a viability study

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## Introduction

Much of the perishable animal by-products produced are handled by renders and converted into beneficial products used in the feed and oleo-chemical industries. Consequently the BSE outbreak in the UK, the use of certain specified bovine offals and tissues have been banned,[1] however rendered fats have been omitted from the ban.

Recent EU Regulation EC Nº 1774/2002 governing animal processed by-products (ABPs)[2] address the possible risk inherent in recycling potential BSE infectivity due to the absence of barrier within species recycling. These Regulations are opening discussions about a possible ban of ruminant fats in feedingstuffs and about the importance of differentiate fat from various animal species.

Most of the current existing methods to identify ruminant tissue in feeds, are based on analysis of protein (inmunological methods, ELISA, electrophoretic methods, etc) or DNA (DNA hybridisation, PCR amplification). Those methods are costly, time consuming and would be not of use in rendered animal fats, unless they contain same useful residual protein.

The NIRS literature highlights a variety of pattern recognition models developed with purposes of classification, authentication and or discrimination of different types of fats and oils.[3]

The purpose of this study is to carry out a viability study for the evaluation of NIRS technology on its ability to identify the animal specie in rendered fats.

## Material and methods

### Samples

A total of 58 rendered fats were supplied weekly (four to five samples per week) by the biggest rendering plant in Andalucía (Spain) over a period of four months. Each sample was provided with an identity form containing the following information: sample number, date and time of sample processing, raw materials used (percentage of meat of each animal specie), sterilization conditions (temperature, pressure and time), and the name of the person in charge of the plant quality control.

### Instrumentation and software

Liquid fat samples were analysed by folded transmission in a Foss-NIRSystems 6500 monochromator equipped with a spinning module. It was used with a transflectance cam-lock cup (pathlenght of 0.1 mm) with a gold reflectance surface. Spectra were collected using the ISI NIRS 3

software Ver. 3.11 (Infrasoft International, Port Matilda, PA, USA).[4] Every sample was measured in two replicates and the average of the replicated spectra, obtained as log (1/*R*), was then used for chemometric data treatment. PLS discriminat analysis was performed using the WINISI II software Ver. 1.05 (Infrasoft International, Port Matilda, PA, USA).[5] Several data pre-treatements were applied before developing the discrimination models. They were the standard normal variate and detrending (SNVDT) for scatter correction[6] and different derivative math treatments: log (1/*R*) (0,0,1,1), first derivative (1,5,5,1; 1,10,5,1) and second derivative (2,5,5,1; 2,10,5,1).[4]

### Chemometric analysis

The WINISI software version 1.5[5] was used to perform PLS2 discriminant analysis. PLS-DA use dummy variables (0 or 1) to develop the discriminant equations. The calibration method applied to this procedure is PLS2. Cross-validation is conducted as in normal PLS to test the accuracy of the discriminations. A predicted value of 2.0 is a perfect identification, 1.0 is no identification and 1.5 indicates the classification could go either way.[5] In this paper, a spectra file for pure poultry meal ($n$ = 15), a spectra file for pure pork ($n$ = 3) and a file for a mixture ($n$ = 20) of several species were used as training groups for the PLS discriminant models. The PLS-DA models developed were further tested with a validation set ( $n$ = 20) consisting of $n$ = 5 pure poultry fats, $n$ = 5 pure pork fats and $n$ = 10 mixture of fats. The statistic used to evaluate the performance of the different PLS-DA models was the classification error or percentage of samples wrongly classified.

## Results and discussion

Table 1 shows the results classification statistics for the training a validation sets and for each of the data pre-treatment evaluated. As in PLS regression models the WINISI software uses cross validation to evaluate the performance of the discriminant models on the training set.

As can be seen from Table 1, all the derivatives produce a reduction in the *SECV* and an increase in the percentage of samples correctly classified as compared to the PLS-DA models developed using log 1/*R*. First derivative produces the models with minimum *SECV*s (0.32) and the maximum number of hits (17 out of 20 rendered fats).

**Table 1. Performance of the PLS-DA models developed after SNVDT scatter corrections and different derivatives.**

| Mathematical treatments | Training set $n$=38 | | Validation set $n$=20 |
|---|---|---|---|
| | PLS terms | *SECV* | % of samples correctly classified |
| 2,10,5,1 | 2 | 0.3436 | 75 |
| 1,10,5,1 | 3 | 0.3263 | 85 |
| 2,5,5,1 | 2 | 0.3349 | 75 |
| 1,5,5,1 | 3 | 0.3242 | 85 |
| 0,0,1,1 | 3 | 0.3486 | 55 |

**Table 2. Classification results for the training and validation sets. Math model used SNVDT + 1,5,5,1.**

| Belong to.. | Training set *n* = 38 | | | Validation set *n* = 20 | | |
|---|---|---|---|---|---|---|
| | classified as.. | | | classified as.. | | |
| | Poultry fat | Pork fat | Mixture fat | Poultry fat | Pork fat | Mixture fat |
| Poultry Fat | 12 | 0 | 3 | **2** | **0** | **3** |
| Pork Fat | 0 | 3 | 0 | **0** | **5** | **0** |
| Mixture Fat | 2 | 0 | 18 | **0** | **0** | **10** |

The model producing the lowest SECV was tested for its ability to classified samples belonging to the training and validation sets respectively (Table 2)

For the training set, three samples referenced as poultry fat were classified as mixture fat. Two samples out of 20 mixture fat samples were classified as pure poultry fats. The identification form for these samples inform that they are binary mixtures (poultry: pork) with a high percentage of poultry meal (70–80%). The three pork fat samples were correctly classified.

In the present viability study it was decided to test the performance of the best PLS-DA model on an real external validation set. For that reason, and despite that the training set has still a low number of samples, it was considered as external validation set twenty samples which arrive to the NIR laboratory during the last four weeks of the sample collection period.

As can be seen on Table 2, all the pure pork and mixture samples of the validation set were correctly classified.

Three pure poultry samples out of five were classified as mixtures. It is unknown whether these certainly pure poultry samples if they may have been cross contamination with ruminant meals. Protocols to avoid cross contamination of the reference fat samples during production and handling need to be established.

## Conclusions

According to the classification results obtained on the validation set, the best PLS-DA model obtained correctly classified 85% of rendered fat samples according to the animal specie from which they were produced. Further work is in process to produce new models having more samples of each category and produced by different render producers in order to obtain robust models which can be of use for the authentication of any type of rendered fat produced in Europe.

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