NIRS for animal species identification in animal protein by-products: a viability study

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Introduction

On 3 October 2002 the EU adopted Regulation EC Nº 1774/2002 governing Animal By-Products (ABPs)[1]. On article 21 the regulation seeks to address the possible risk inherent in recycling potential infectivity due to the absence of barrier within species and to exclude the cannibalism, which may be induced by the intra-species recycling.

There is an urgent need for developing methods of analysis which allows to identify the animal specie in ABPs and which could overcome the limitations of the current analytical methods (ie. microscopy, ELISA and PCR).

NIR spectroscopy has demonstrated its ability to recognize ingredients in a feed mixture[2-8] and to distinguish the animal specie in food meat products[9-14.]

The aim of this study is to evaluate the ability of NIR spectroscopy to identify the animal specie in rendered protein by-products.

Material and methods

Samples

A total of 119 rendering meal samples were supplied weekly (4-5 samples/week) by the biggest rendering plant of Andalucía (Spain) over a period of six months. These samples were supplied weekly (4-5 samples/week) by the biggest rendering plant of Andalucía (Spain). Each sample was supplied 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.

NIRS analysis

A Foss NIRSystems model 6500 scanning monochromator (Foss NIRSystem, Silver Spring, MD, USDA) equipped with a transport module, was used to measure reflectance spectra from 400 to 2498 nm, every 2 nm. The analysis was carried out using the rectangular cup (Natural Product Sample Cup IH-0331) with dimensions of 4.7 cm wide, 20 cm long and 4.3 cm depth. The samples

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were analysed as received, without any further grinding. Spectra were recorder with the ISI NIRS 3 software ver. 3.11[14]. Every sample was measured in two replicates and the average of the replicated spectra, obtained as log (1/R), was used in the discrimination analysis. All the discrimination models were performed using the WINISI II software ver. 1.5[15]. Several data pretreatements were used: standard normal variate and detrending (SNVD)[16] for scatter correction, and different derivative math treatments: log (1/R) (0,0,1,1), first derivative (1,4,4,1; 1,10,4,1) and second derivative (2,4,4,1; 2,10,4,1).

Discriminant analysis

The WINISI software version 1.5[15] was used to perform PLS2 discriminant analysis. PLS-DA use dummy variables 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[15] . The statistic used to evaluate the performance of the different PLD-DA models was the classification error or percentage of misclassified samples.

Results and discussion

To develop a PLS discriminant equation, groups of samples must be identified with known grouping characteristics. In this paper two different model types were evaluated: Model type I, to discriminate ruminant and non-ruminant rendered animal meals; and Model type II, to discriminate among three categories of rendered animal meals (pure poultry, pure pork or mixture of cattle with other species).

Model type I: Discrimination between ruminant and non-ruminant rendered animal meals

The set of 119 samples of meat meal available for this study was built with 86 and 25 samples which were produced using ruminant and non-ruminant animals respectively. PLS2 discriminant models were developed with a training set of 78 samples belonging to Class I (Ruminant), makes up of meal mixtures containing different percentages of cattle and other species and 25 samples belonging to Class II (Non-ruminant), makes up of pure pork and pure poultry meals and of one binary mixture of pork/poultry meal.

Several PLS-DA (Type I) models were obtained by using different data pre-treatments and spectral regions. The most accurate discriminant (Type I) model was obtained using first derivative (1,10,4,1) of log (1/R) without scatter correction and the full VIS + NIR (400 to 2500 nm) spectral region. Four cross-validation passes recommended 5 PLS factors and give a standard error of cross validation (SECV) of 0.18. That model was used to classify the training set and one validation set of N=16 samples which were produced during the last two weeks of the study period. Classification results for the training and validation sets are showed in Table 1.

All the samples from the Non-ruminant classes and belonging to the training and validation sets were correctly classified. However, one sample belonging to the Ruminant class was classified wrong as belonging to the Ruminant class. The non-ruminant misclassified sample was always the same in all the models Type I evaluated. This indicates that this sample may have in its composition same amount of cattle meat meal. To confirm this, it was analysed also by PCR, as blind sample, in one external lab (CRAGx, Gembloux, Belgium) and the PCR results confirmed that the sample has also non- ruminant DNA protein. The best PLS-DA Type I model was applied to the validation set of 16 samples. Table 1 shows the classification results for the 16 samples included in the validation set.

Table 1. Classification results for the training and validation sets anD for Model Type I

Training Set Ruminant (n=78); Non-ruminant (n=25)			Validation Set Ruminant (n=8); Non-ruminant (n=8)		
Belong to...	Classified as...		Belong to...	Classified as...	
	Ruminant	Non-ruminant		Ruminant	Non-ruminant
Ruminant	24	1	Ruminant	8	0
Non-ruminant	0	78	Non-ruminant	0	8

Model type II. Discrimination among pure poultry, pure pork and mixtures of cattle with other species.

Table 2 shows the classification results obtained with the best PLS-DA model Type II. In this case, one pork sample belonging to the training set was misclassified .

Table 2. Classification results for the training and validation sets and for Model Type II

Training Set Poultry (n=17); Pork (n=7); Mixture (n=79)				Validation Set Poultry (n=7); Pork (n=1); Mixture (n=8)			
Belong to...	Classified as...			Belong to...	Classified as...		
	Poultry meal	Pork meal	Mixture meal		Poultry meal	Pork meal	Mixture meal
Poultry meal	17	0	0	Poultry meal	7	0	0
Pork meal	0	6	1	Pork meal	0	1	0
Mixture meal	0	0	79	Mixture meal	0	0	8

The pork misclassified sample was the same in all the PLS-DA models type II developed. This sample was also the same which was misclassified by the best PLS-DS model Type I. Despite that the provider register this sample as “pork meal”, the PLS-DA and PCR analyses indicate that the sample may have same amount of ruminant meal.

The best PLS-DA model type II obtained was applied to the validation set consisting of 7 pure poultry, 1 pure pork and 8 mixture meal samples. The classification matrix (Table2) indicates that 100% of samples belonging to each of the three classes are correctly classified.

Conclusions

This preliminary study shows that it is possible to identify the animal specie in animal protein by-products. However, further work is needed to build a sample and spectral data bank which contain samples representing the variability existing into each give rendered animal protein meals. NIRS technology must be considered of great potential for the screening of a large volume of animal meals to fulfil quality control requirements layed down on Regulation EC Nº 1774/2002 governing Animal By-Products (ABPs).

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