Discrimination of the class origin of bones present in the sediment fraction of animal by-products using near infrared microscopy

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Introduction

As a result of European legislation [1], there is an urgent need to develop reliable, fast and accurate forensic methods that could identify material at a class level such as fish or terrestrial and to detect animal ingredients in raw materials and compound feeds down to levels of 0.1 %.

Classical microscopy is currently the only official method available to identify ingredients of vegetable and animal origin in a mixture [2]. This method is time consuming and laborious. Its performance, which depends largely on the experience of the analyst, is mainly based on the presence of easily identifiable consituents such as bone fragments. It only allows discrimination between fish and terrestrial bones [3].

Near infrared (NIR) microscopy has been successfully used for the detection of animal particles at low levels in the sediment fraction of compound feeds [4 - 7]. In this study, the potential of NIR microscopy to discriminate the class origin of the animal particles included in the sediment fraction of animal by-products has been investigated.

Material and methods

A spectral library, consisting of more than 5,000 particles, was built with well identified animal by-product samples from fish, cattle, sheep, pigs and poultry.

An auto image microscope connected to a Perkin-Elmer Fourier transform near-infrared (FT-NIR) spectrometer was used. The particles of the sediment fraction were spread on a spectralon plate and presented to the NIR microscope. Using the pointer of the microscope, the infrared beam was focused on each particle and the NIR spectrum (1,112 - 2,500 nm) collected.

The discrimination procedure consisted of two different algorithms: support vector machines (SVM) [8] and partial least squares discriminant analysis (PLS-DA) [9].

Results and discussion

Figure 1 shows the 2nd derivative spectra of the different classes of animal by-products in the region between 1,600 and 1,800 nm. There were visible differences in this region, probably reflecting differences in fatty acid profiles. Though not shown in Figure 1 there were also spectral differences in the vicinity of 2,054 nm; a region which is related to protein absorption [10]. The existence of such marked spectral differences shows that species identification using discriminant analysis should be feasible.

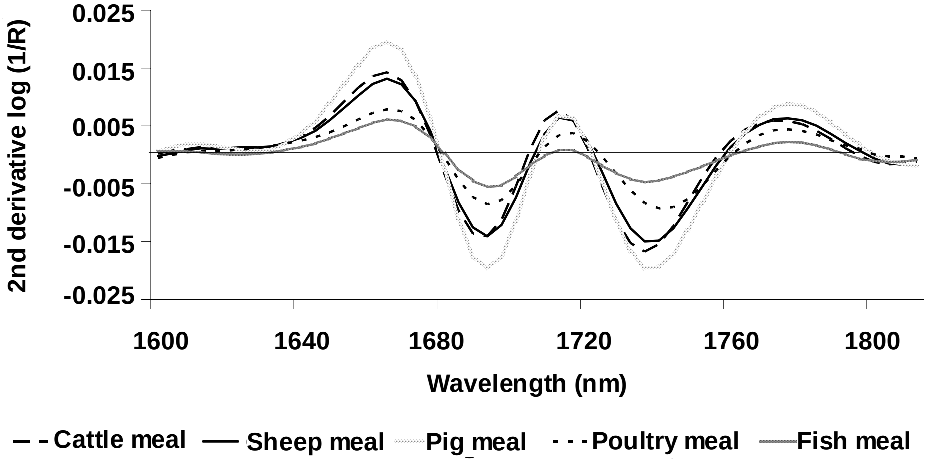

Figure 1. Average second derivative spectrum of different classes of animal by-products**.**

To develop discriminant equations, groups of samples need to be identified with known grouping characteristics. In this paper two different models, for each algorithm, were evaluated: discrimination between fish and terrestrial rendered animal meals; and discrimination among mammalian meat and bone meal derived from farm animals, and poultry animal by-products.

Table 1. Classification rates (%) of the particles spectra from the training and validation stages using SVM as discriminant algorithm. MMBM = mammalian meat and bone meal derived from cattle, sheep and pigs.

Training set			Validation set		
	% classified as...			% classified as...	
Belong to...	Fish	Terrestrial	Belong to...	Fish	Terrestrial
Fish	100	0	Fish	99	1
Terrestrial	0	100	Terrestrial	16	84
	% classified as...			% classified as...	
Belong to...	MMBM	Poultry	Belong to...	MMBM	Poultry
MMBM	98	2	MMBM	67	43
Poultry	7	93	Poultry	36	64

Table 2. Classification rates (%) of the particles spectra from the training and validation stages using PLS-DA as discriminant algorithm. MMBM = mammalian meat and bone meal derived from cattle, sheep and pigs.

Training set			Validation set		
	% classified as...			% classified as...	
Belong to...	Fish	Terrestrial	Belong to...	Fish	Terrestrial
Fish	97	3	Fish	90	10
Terrestrial	1	99	Terrestrial	1	99
	% classified as...			% classified as...	
Belong to...	MMBM	Poultry	Belong to...	MMBM	Poultry
MMBM	100	0	MMBM	100	0
Poultry	0	100	Poultry	10	90

Tables 1 and 2 present the classification rate for the different groups obtained for the particles used in the training and validation stages of the study applying the SVM and PLS-DA models.These tables show that the SVM and PLS-DA procedures produced discriminant models having high correct classification rates in the training stage (93 to 100%).

SVM analysis (Table 1) discriminates better between fish and terrestrial animal particles (100% hits) than it discriminates between mammalian and poultry meals (96% hits).

PLS-DA (Table 2) produces 95% and 100% of correct classification when discriminating between fish and terrestrial animal particles, and between mammalian animals and poultry particles respectively. Furthermore, the results of the external validation of each discriminant method and model (Tables 1 and 2) show that both discriminant models correctly classified most of the fish particles (90 - 99%). It seems that SVM (Table 1) is less efficient to distinguish between fish and terrestrial animals than PLS-DA (84 vs. 99%). When distinguishing between mammalian animals and poultry, PLS-DA (Table 2) produces discriminant models with the highest correct classification rate.

Some of the differences in the classification ability of the two studied discriminant methods may be explained by the fact that the data set used for validation of the PLS-DA models contained samples that were also represented in the training set. This is not the case for the validation set used with SVM. Here the validation dataset is completely independent from the training set with the samples used in the validation stage not being used for model construction.

Conclusions

It seems clear that it is possible to differentiate between broad classes of animal by-product samples using NIR microscopy with a high rate of success.

Further work is necessary to enlarge the libraries with more authenticated pure samples in order to reduce classification errors. Moreover, SVM and PLS discriminant algorithms should be constructed using the same training set and evaluated with the same and independent validation sets. This is an important point in order to be able to compare the results of both discrimination techniques as the model and the validation are influenced strongly by the samples and the manner in which they have been selected.

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