

# Development of a Near Infrared (NIR) Hyperspectral Imaging to detect animal species in compound feeds

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One of the main concerns of the CRA-W is the development and the validation of new methods for a rapid, precise and reliable detection of meat and bone meal (MBM) in feedstuffs. For this reason, we proposed the use of the Near infrared microscopy (NIRM) and the Near Infrared hyperspectral imaging methods as promising alternative methods having clear advantages (speed, flexibility, easy to use...).

NIR imaging technique consists in the analysis of several hundreds of particles being the result of the grinding of compound feedstuffs. The major advantages are that the recognition is independent on the expertise of the analyst and that it is possible to automate all procedures and to analyse more samples per unit of time than classical microscopy.

A complete strategy has been developed in order to discriminate feed particles according to the species. This strategy comprises a complete experimental design, improvements in the acquisition step (sample preparation and sample stage and software) as well as a complete chemometric and statistic procedure. Chemometric classification strategies have been applied to automate the method, and reduce the need for constant expert analysis of the data. Multivariate classification methods as Support Vector Machines (SVM) or Partial Least Squares Discriminant Analysis (PLS-DA) have been used in the procedure for the classification of feed particles as the different species using the NIR hyperspectral images.

## Hyperspectral NIR Imaging system



One of the near infrared systems used at the CRA-W is a MatrixNIR Chemical Imaging System (Malvern Instruments, Analytical Imaging, Columbia, MD, USA). Two coupled liquid crystal tuneable filters (LCTF) allow go through sequentially the reflected energy at a defined wavelength range. The LCTF have to be adjusted in order to collect the energy in the 900 nm to 1700 nm spectral range (resolution of 10 nm). After the LCTF, the reflected energy goes to an infrared focal plane arrays of size of 240 x 320 corresponding to 76 800 individual infrared detector elements (or pixels). For each pixel, the compilation of the absorbances at each wavelength gives a spectrum.

In order to increase the speed of sample collection, some improvements have been performed in both the instrument itself and the software (Burgermetrix, Riga, Latvia). The main improvement consists on the construction of a new sample support allowing to measure until 12 samples in a continuous way without human intervention.



## Data

The materials analysed were selected to span the diversity of animal materials mainly used for the formulation of compound feeds. These samples were selected from the sample bank existing at the CRA-W, and from the European sample bank constructed in the framework of the CRL-AP (Community Reference Laboratory for Animal Proteins). All the samples have been certified by the Polymerase Chain Reaction (PCR) technique, in order to confirm the specie.

In total more than 74000 spectra have been collected from the animal meals; each spectrum spans the 900-1700 nm range, in 10 nm increments.

Species	Details	Number of Samples	Number of Spectra
Fish	MBM	38	26600
Chicken	MBM, blood, feather,	28	19600
Pig	MBM, blood, plasma, hémoglobine, hydrolysed proteins	22	16800
Beef	MBM (treatment at 133 C & 145 C) and blood	9	6300
Sheep	MBM (treatment at 133 C & 145 C)	6	4200
Horse	MBM	1	700

MBM = Meat and bone meal

## Exploratory analysis

In a first step, PCA has been performed in order to make an exploratory analysis of the data. The results are shown in Figures 1 and 2 where different groups for the different species are evident showing a great diversity of the database. When studying the data, samples coming from blood are easily distinguished from not blood samples (Figures 3 and 4), then they can be removed from the database.

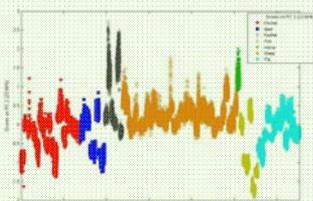


Figure 1: Scores on PC2 vs. sample

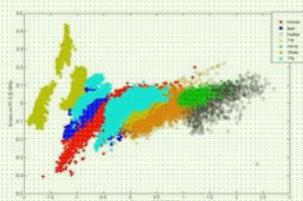


Figure 2: PC2 vs. PC5

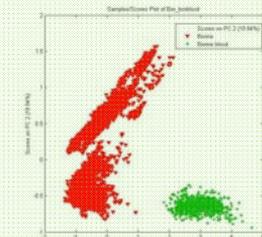


Figure 3: PC1 vs. PC2 for bovine samples

## Conclusion

The ability of the hyperspectral NIR imaging for the detection of animal products in the feed has been proved.

All the chemometric methods have shown good results for the discrimination between species. SVM gives in general better results.

## Discrimination models

Two different Chemometric algorithms have been tested to construct models to discriminate/classify the origin of feed particles according to the species: Partial Least Squares (PLS) and Support Vector Machines (SVM). For both of them, a dummy variable was attributed to represent each group (for instance for the model fish vs. the rest, 1 represents the fish spectra and -1 the rest), and was considered as reference value (y) during the derivation of the PLS and SVM classifiers. Each classification method was trained independently, with the best model of each type selected for the comparison. The results for prediction on an independent test set are shown in the following table in terms of percentage of correct classification.

Table 1: Results using PLS-DA and SVM in terms of % of correct classification

	PLS-DA		SVM	
	Considered specie	Other	Considered specie	Other
Fish	0,9281	0,0030	0,9966	0,0021
Other	0,1674	0,8326	0,0161	0,9839
Beef	0,8823	0,1374	0,8810	0,1492
Other	0,3019	0,6981	0,0093	0,9907
Horse	1	0	0,9911	0,0123
Other	0,0125	0,9875	0,0062	0,9938
Sheep	0,6673	0,3327	0,7965	0,2035
Other	0,0083	0,9917	0,0066	0,9934
Pig	0,8612	0,1388	0,7966	0,2031
Other	0,2693	0,7307	0,0193	0,9807
Chicken	0,8005	0,1995	0,8409	0,1591
Other	0,2743	0,7257	0,0176	0,9824

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