Classification of cattle feed particles by FT-NIR-microscopy and artificial neural network (ANN) data treatment

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I. Introduction

Bovine spongiform encephalopathy (BSE) is a fatal degenerative disease that affects central nervous system. To date, scientific community widely agrees with the prion hypothesis to explain transmissible spongiform encephalopathies (TSE). A prion is a native protein that becomes pathological by contact with other pathological ones. It spreads through central nervous system by chain reaction.

The most common explanation of the BSE epidemic that started in the early 80's is the recycling of scrapie sheep carcass into cattle feed. Some have suggested an exposure to rare sporadic BSE or the apparition of a new strain of scrapie that was particularly infectious for cattle. But both hypotheses do not fit totally with epidemiological data.

The mad cow crisis and socio-economical consequences gave rise to a strict legislation about cattle feedingstuffs. The control of the regulations application calls for accurate, precise and reliable methods at legislator's disposal. Detection of animal meat and bone meal (MBM) is usually performed by classical microscopy, which is a slow and tedious method that highly depends on a skilled and experienced analyst. In this study, FT-NIR-microscopy has been used for MBM detection and quantification.

II. Material and method

A. Material

FT-NIR-microscope

The spectrometer must allow the collection of spectrum of every single flour particle. We use a Fourier transform interferometer coupled with an optical microscope. This material works as follows: the beam generated by the interferometer goes to the microscope, which is equipped with a video camera that allows to visualize the sample and to localize the particles we want to analyze. The infrared rays are focused into particles to analyze and a detector located in the microscope measures the reflected beam. Then an inverse Fourier transform is applied. In this study, a Spectrum Identicheck FT-NIR System coupled to an AutoIMAGE system from Perkin Elmer was used (see Figure 1).

Figure 1. : Interferometer coupled to an optical microscope.

Particles identification

Figure 2 shows image (visible range) of the sample carrier on which are particles of milled feedingstuff and spectra of some of these particles. Most of the feedingstuffs samples were provided by Ministry of Small Enterprises, Traders and Agriculture (DG4, Row Materials Control).

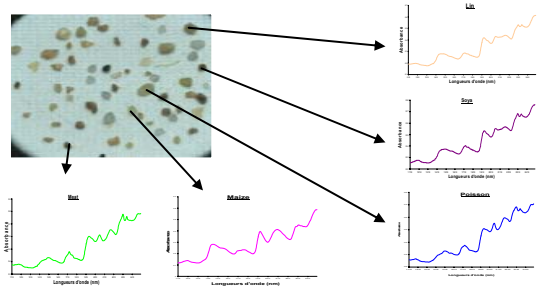

Figure 2. : Image and spectra of feedingstuff particles

B. Method

The method consists in building a sample spectral library by scanning any kind of flour particles that are used in animal feedingstuff industry. Using this spectral library we created an ANN equation able to determine if particles are allowed or forbidden. These equations were tested on independent sets of particles. In addition, a calibration was developed in order to correlate number and surface of MBM particles to actual percentage.

III. Results

Qualitative analysis

The 1792 first spectra (circles and squares) have been taken from known particles. The value –1 has been attributed to allowed particles while the value 1 was attributed to forbidden ones. The ANN program from WinISI constructed an equation based on these spectra. In a second step, the equation was tested with an unknown sample. Results are displayed on the right side of Figure 3 (triangles).

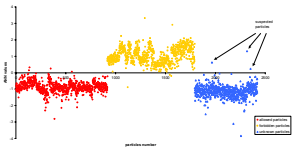

Figure 3. : Results of ANN calibration

Quantitative analysis

Using the ANN equation generated as explained above, we analyzed samples containing MBM (from 2 to 10 %) and we obtained a linear regression between MBM percentage and the relative MBM surface. This validation model with independent samples has shown that the precision obtained was certainly as good as the one obtained with traditional microscopy which is at present time the reference.

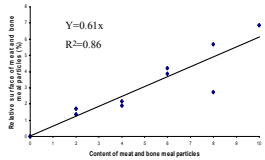

Figure 4. : Results of quantitative analysis

IV. Prospect

The first stage of the study demonstrated the high potential of FT-NIR microscopy for the identification and the quantification of MBM addition in feedingstuffs. In addition to ANN, Stepwise Linear Discriminant Analysis (SLDA) is investigated to construct discriminant equations for the identification of all the particles included in feedingstuffs. Figure 5.a presents the results of SLDA (using the absorbance at 10 wavelengths). The dimensions are the two first dimensions extracted from the discriminant functions constructed for each particles group. The filled and unfilled marks correspond to the calibration and validation samples respectively. Figure 5.b presents the results for the animal particles according to the second and sixth dimensions.

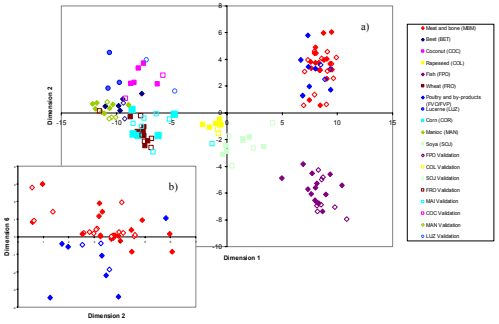

Figure 5. : SLDA discrimination

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