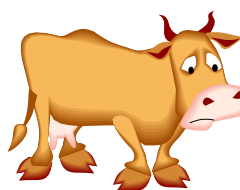


## STATE OF THE ART AND OBJECTIF

Since 1998, the Department Quality of Agricultural Products from the Agricultural Research Center of Gembloux has acquired a microscope coupled with a FT-NIR-spectroscope. The main objective was the development and the validation of a new method for a rapid, precise and reliable detection of meat and bone meal (MBM) in feedingstuffs. According to the promising results obtained in the detection and the quantification of MBM, it was decided to extend the study to the use of FT-NIR-microscopy in the complete screening (quantitative and qualitative) of feedingstuffs.

For the record, the FT-NIR-microscopy method consists in the analysis of several hundreds of particles being the result of the grinding of a compound feedingstuffs. The spectro-microscopic instrument includes a microscope equipped with a camera and a viewing system that magnifies the light image of the sample and allows to collect spectra from extremely small particles. The great advantages of this technique are that the recognition is not dependent 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 the classical microscopy.

The presented project contributes to the implantation of the European Commission Decision 94/381/EC of the 27th of June 1994. It states the prohibition of the use of proteins from all mammalian tissues in feedingstuffs destined for ruminants. This research will also be decisive in the support of the "Proposal for a European Parliament and Council Directive amending Directive 79/373/EC on the marketing of compound feedingstuffs". In this document the Commission underlines the advantages of the labelling provisions of compound feedingstuffs for production animals in order to facilitate the trace back of feed materials.



## MATERIAL

**Sample :**  
A spectral library has been developed with all the raw materials commonly used in feedingstuff manufacture and the forbidden ingredients. It makes up about 1000 MBM particles spectra and 3600 others. More precisely, these flour samples spectra were divided into 17 data sets (fish, coco & palm schiffers, beet, blood & egg, poultry, meat & bone meal, lucerne, potato, manioc, sunflowers, pea, wheat, barely, corn, soya, flax and rape). For each flour sample, 30 spectra were taken. The mean of these spectra was used in this work. The Ministry of Small Enterprises, Traders and Agriculture (DG4, raw material control) provided most of these samples.

### FT-NIR-microscope :

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 with an AutoIMAGE system from Perkin Elmer was used.

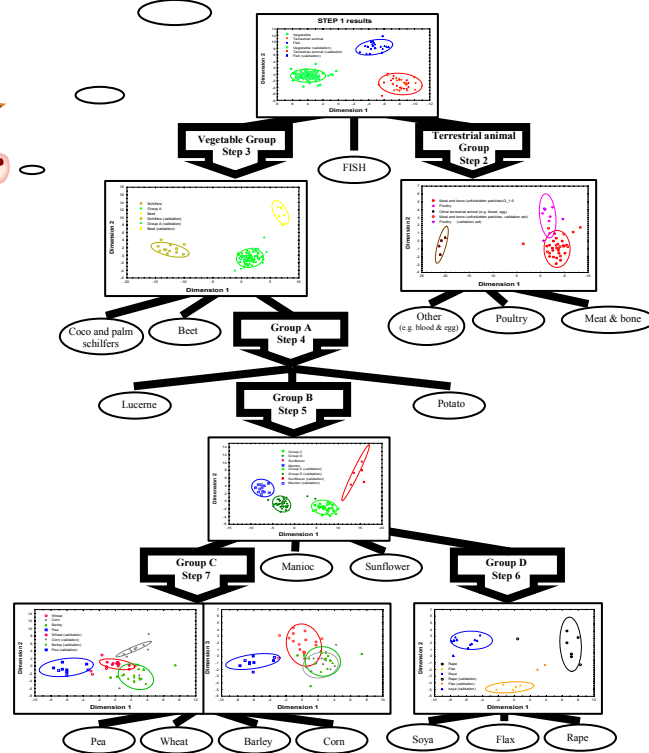
## RESULTS

In the first stage of the work, we decided to use a non-parametric method to discriminate between the allowed particles graph and forbidden particles graph (i.e. MBM particles). An artificial neural network was used and a predictive discriminant model was constructed to classify the particles into two groups according to their absorbance from 1112 nm to 2500 nm. Moreover, the potential of the FT-NIR-microscopy technique in the qualification of feedingstuffs was studied (ref.: Piraux and Dardenne, presented at the NIR 1999 symposium, Verona, Italy).

The following stage of the study concerns the design of an arborescent structure for the successive classification of the particles of a feedingstuff. The objective was to establish series of discriminant functions able to discriminate particles from different origins and to study the wavelengths used to that purpose. Stepwise Linear Discriminant Approach (SLDA) was used to construct the different discriminant equations. The first step consists in the assignment of an arbitrary number to each particle spectrum according to its origin. Then the SLDA procedure is applied to distinguish different groups. This step allows the extraction of the most relevant wavelengths for the discrimination of groups and to define the sources that are easier to discriminate. The procedure was applied until each group corresponds to a single particle source.

Figure 1 presents the arborescent structure by applying SLDA procedure to the absorbance measured in the near-infrared region. The figure shows the different steps with the results of the canonical analysis carried out on the different discriminant functions (one for each particle source).

Two third of the sample spectra were used to compute the discriminant function (filled marks) and the last third was used to validate them (unfilled marks). At the end of the calibration stage, the ellipse of 95 % confidence region was computed using the Mahalanobis distance calculated for each calibration spectrum from the group centroid.



**Figure 1.: Arborescent structure by applying SLDA procedure to the absorbance measurements in the near-infrared region.**

The arborescent structure includes 7 steps. First, the samples were separated into three groups according to their vegetable, terrestrial animal or non-terrestrial animal origin. Then in the second step, the terrestrial animal group was separated into three groups i.e. meat and bone meal, poultry and other terrestrial animal particles (e.g. blood, egg). Following the vegetable branch, the step 3 concerns the discrimination of the beet and palm & coco schiffers from the rest of the vegetable particles (group A). Step 4 discriminates lucerne and potato from the rest (group B). Group B is separated in four groups at step 5: two groups corresponding to the single source (i.e. manioc and sunflower) while others correspond respectively to group C and group D. The particles included in group C are those corresponding to pea, wheat, barley and corn source. These sources are classified in the step 7 of the arborescent structure. Step 6 concerns the discrimination of the group D including the particles of soya, flax and rape samples.

## CONCLUSION AND PERSPECTIVES

The first results of the present study show the possibility to use the FT-NIR- microscopy technique for the full screening of feedingstuffs. The following stage of the study will include the use of a higher number of spectra to make up the successive discriminant functions, the introduction of additional discriminant functions, the introduction of additional feed compounds and their validation by including compounds from different sources.