Analysis of the sediment fraction of feed by near-infrared microscopy (NIRM)

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Introduction

With the emergence of the BSE crisis we assist to the development of a wide range of analytical methods to detect meat and bone meal in feed ingredients and compound feeds.[1] Indeed, contaminated feeds are commonly accepted as the main transmission of the mad cow disease in the European bovine herds. Briefly, there are the methods based on optical microscopy, molecular biology (e.g. PCR and ELISA) and spectroscopic analysis of the sample. Classical microscopy analysis of the feed is the reference method used throughout Europe to control the correct implementation of the ban of meat and bone meal. Firstly, it was applied for the ruminants and then extended to all farmed animals. With this method, the detection of meat and bone meal is done by microscopic observations, after adequate sample preparations including the use of staining reagents, of specific features of particles coming from different sieved and/or sediment fractions. The test is mainly based on the detection of bones by visual observation of the particles resulting from the sedimentation of the sample.

The main spectroscopic methods proposed to control the illegal addition or to detect the contamination of feeding stuffs are based on the near-infrared spectroscopic technique.[2,3] With these methods, the infrared spectra from the raw sample (near-infrared spectroscopy) or from the particles making up the sample (near-infrared microscopy) are used to detect samples including meat and bone meal material. In previous studies,[4,5] it has been demonstrated the high potential of near-infrared microscopy method to detect meat and bone meal at level as low as 0.5%. To decrease the limit of detection and to increase the speed of the near-infrared microscopy analysis, the addition of a sedimentation step before the near-infrared microscopic analysis has been investigated.

Materials and methods

The methodology applied in the NIRM analysis of particles from the sediment is the same that the one used to analyse raw material. This methodology has been described in previous papers (3,5). In this study, an Auto Image Microscope connected to a Perkin-Elmer Fourier transform near-infrared spectrometer (FT-NIR) was used. This instrument allows collecting spectra from small surface (50µ x 50µ). The microscope includes a camera and a viewing system to magnify the visible-light image of the sample to observe, to point out and to isolate a point of interest. The particles of the sediment fraction is spread on a spectralon

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plate and presented to the NIR microscope. Using the pointer of the microscope, the infrared beam is focused on each particle and the near-infrared spectrum (1112-2500 nm) is collected. For this study, a resolution of 4 cm, a gain of WW and a number of co-added scan of 10 were used. The spectra were obtained after the ratio between the raw spectra and the background consisting on the measurement of the spectralon plate. The software from Perkin Elmer was used to collect and store the spectra.

Results and discussion

In order to calibrate the near-infrared microscope for the detection of bones in the sedimented fraction, a series of feed ingredients from animal, vegetal and mineral origin as well as MBM free compound feed were sedimented according to the procedure described in the UE microscopic guideline and were analysed by near-infrared microscopy (NIRM).Figure 1 presents several microscopic images and NIR spectra of particles coming from the sedimented fraction of a compound feed. The animal particles spectra shows bands with maximum near 1946, 2044, 2176 and 2288 nm.

To test the method developed at CRA, a spiked sample at 0,1 % with MBM was analysed ten times. Table 1 presents the results. The number of analysed particles, the number of particles detected as having an animal origin, the percentage of bone particles in the sediment and the weight of bones in the sediment are included. All the replicates were detected as positive regarding the presence of bones in the sample. The standard deviation of the percentage of bone particles in the sediment (SD = 0.16) underlines the quality of the method proposed taking into account the difficulty to get a perfect homogeneous spiked sample at this low level.

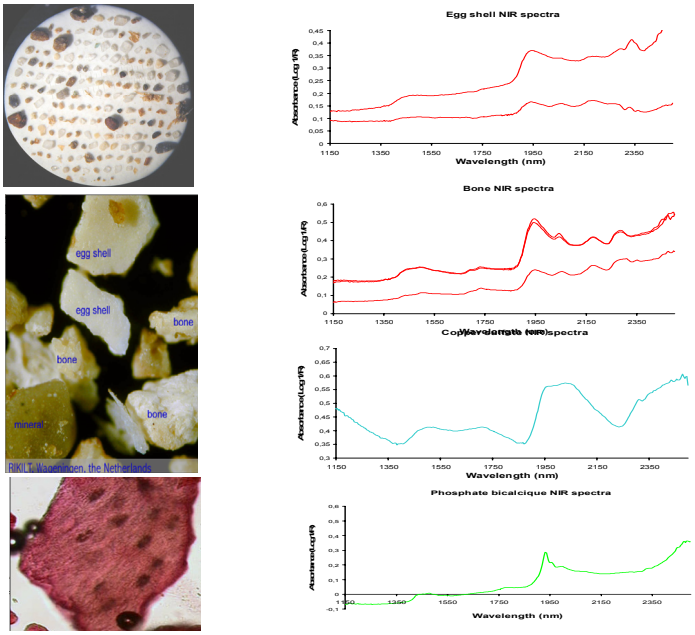

Figure 1. Microscopic images and infrared spectra of different particles of the sedimented fraction. (Part of the microscopic images are issued from the Decision Support System ARIES develop in the framework of the STRATFEED project by RIKILT (Dr. Leo van Raamsdonck) and aiming to assist the microscopist in the detection and identification of animal protein in feeding stuff)

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Table 1. Analytical results of the 10 NIRM analysis of a sample spiked at 0.1 % of meat and bone meal.

Code	% sediment (a)	Nb analysed particles	Nb analysed particles (b)	% bones in sediment ©	Weight bones in the sample (d) mg	Conclusion
a	2,73	333	2	0,63	1,73	Positive
b	2,57	329	3	0,96	2,47	Positive
c	2,59	340	2	0,62	1,61	Positive
d	2,96	330	2	0,64	1,89	Positive
e	2,81	305	2	0,69	1,94	Positive
f	2,66	292	2	0,72	1,92	Positive
g	2,55	342	1	0,31	0,79	Positive
h	2,49	329	2	0,64	1,59	Positive
i	2,72	279	2	0,75	2,05	Positive
j	2,37	378	2	0,56	1,32	Positive

Legend: (a) % sediment = (Weight sediment/Weight sample * 100
(b) Nb animal particles = no. of particles clearly identified (by means of their infrared spectrum as having an animal origin
(c) % bones in the sediment = (Nb animal particles/Nb analysed particles) * 100/% of particles of the animal feed ingredient detected as animal origin
(d) Bones in the sample = Weight sediment* % bones in the sediment (c)

In the framework of the STRATFEED project (www.stratfeed.cra.wallonie.be), a total of 24 MBM free compound feeds and 48 spiked samples at a level ranging from 0.5 to 8 % were analysed using the NIRM method. The samples were prepared by UCO and analysed in blind by CRA. All the free MBM compound feeds were detected as negative samples and all the adulterated samples were detected as positive regarding the presence of meat and bone meal. Figure 2 displays the analytical results of a series of the blind samples spiked with the same MBM.

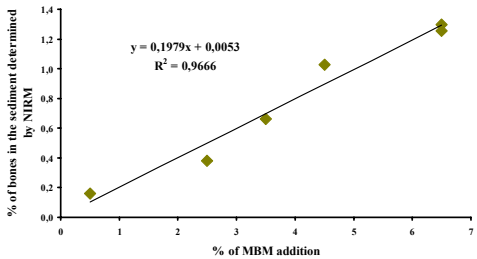

Figure 2. NIRM results of the analysis of a series of blind samples.

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Conclusion

The analysis of the sedimented fraction by near-infrared microscopy is an attractive and powerful method to detect low level of meat and bone meal in feeding stuffs. The main features of this method are:

- the analysis of particles from the sample. This is similar to the classical microscopy (UE reference method).
- the limit of detection (LOD < 0,1 %).
- the use of infrared detectors and discriminant equations instead of the eyes and the expertise of the analyst as within the classical microscopic method.

Acknowledgements

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