

A NIRM system running under accreditation ISO 17025 for the detection of processed by-products of animal origin

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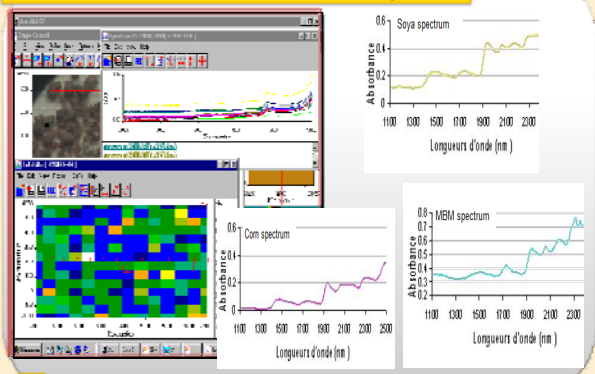
Contaminated ruminant protein enters the feed chain mainly in the form of meat and bone meals (MBM).

Bovine Spongiform Encephalopathy (BSE) CRISIS

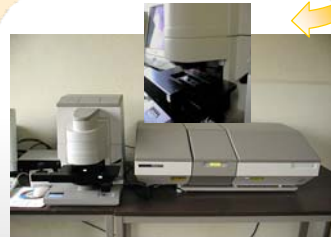


Need of a fast and efficient analytical method for the detection and quantification of meat and bone meal (MBM) contamination in animal feed:
NIRM method.

Collection and visualization of spectra



Studies undertaken in the framework of the activities of different projects have demonstrated the high potential of the NIRM method combined with chemometrics to detect animal particles in feedingstuff at concentrations as low as 0.1 %.

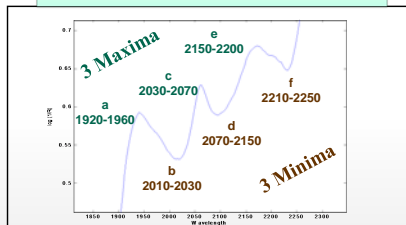


The combination of NIRS and a microscope allows high quality spectra for small (50 – 1000 µm) feed particles. The NIRM method has been proposed as an alternative to the official method, the optical microscopy.

The method is running under accreditation ISO 17025 since 2005 at the CRA-W.

Interpretation of spectra

Based on visual observation



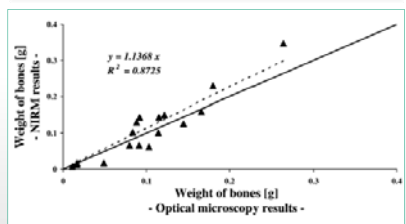
$$[Abs(\text{line segment (b-f)})]_{\text{wav(d)}} > Abs(\text{d})$$

Ch. von Holst, V. Baeten, A. Boix, B. Slowikowski, J. A. Fernández Pierna, S. Tirendi, P. Dardenne. *Anal. Bioanal. Chem.* 392 (2008), pp. 313-317.

Mathematical discrimination

Chemometric models like Partial Least Squares (PLS) and Support Vector Machines (SVM) have been developed to determine the origin of the animal particles.

Accordance with optical microscopy (OM)



NIRM and OM are in a good agreement with a correlation of 0.87 % between weights of bones in the sediment obtained by the two methods.

Baeten V., von Holst C., Garrido A., Vancutsem J., Michotte Renier A. and Dardenne P. (2005). Detection of banned meat and bone meal in feedstuffs by near-infrared microscopic analysis of the dense sediment fraction. *Anal. Bioanal. Chem.*, 382, 149-157.

ISO 17025

Limit of detection (LOD)

Raw fraction

600 particles should be analyzed in order to detect a contamination by MBM at a level of 0.5 % with a probability of 95 %.

3000 particles have to be analyzed in order to detect at least one MBM particle with a probability of 95 % in a 0.1 % adulterated feed.

Time consuming

Sediment fraction

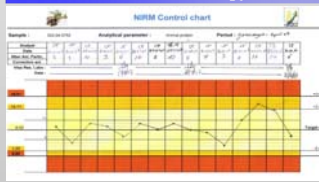
300 particles analyzed: It is possible to detect animal particles at a level inferior to 0.1 % (LOD < 0.1 %)

Gain in analysis time

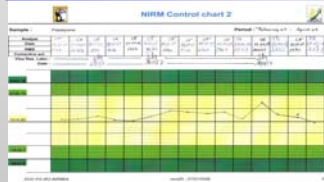
Control chart

- The analysis of an internal standard is done at the beginning of the week
- 60 particles are analyzed
- A counting of the number of particles is performed.

Control Chart- NIR microscopy (Asterix)



Control Chart – NIR microscopy (polystyrene)



Repeatability

A sample spiked at 0.1% with MBM was analysed 10 times.

Code	% sediment (g)	No. animal particles	% bones in the sediment (g)	Weight of bones in this sample (g) (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

Note : (a) No. animal particles = Number of particles clearly identified (by means of their infrared spectrum) as having an animal origin; (b) % bones in the sediment = (No. animal particles/No. analysed particles) * 100 / % of particles in the animal feed ingredient shown to be of animal origin.

Baeten V., Michotte Renier A., Sinnave G., Garrido Varo A., Dardenne P. (2004). In *Proc. Of the 11 International Conference on Near-Infrared Microscopy* (Davies T. and Garrido Varo A.), Cordoba, Spain, 6-11 April 2003.



The NIRM method can be also combined with other analytical methods like optical microscopy (OM) or Real Time Polymerase Chain Reaction (PCR).



These methods can give a confirmation on the presence of animal species of the particles.

Part of this work has been performed within the framework of the EC Project SAFEED-PAP – (FOOD-CT-2006-036221) – entitled “Detection of presence of species-specific processed animal proteins in animal feed”, <http://safeedpap.feedsafety.org/>



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