THE NEAR INFRARED MICROSCOPIC (NIRM) METHOD: A COMBINATION OF THE ADVANTAGES OF OPTICAL MICROSCOPY AND NEAR-INFRARED SPECTROSCOPY

(WP5)

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1. Introduction

In the previous section, the potential of near-infrared reflectance spectroscopy (NIRS) was discussed. Using this technique, a single spectrum is obtained from the analysis of one sample (e.g. feed ingredient or compound feed). Recent developments have led to combining two instruments – a near-infrared spectrometer and a microscope. With these instruments, spectra of hundreds or thousands particles can be obtained from the analysis of one feed ingredient or one compound feed **[1]**. Near-infrared microscopy (NIRM) could be an essential tool in tackling the BSE problem **[7,2]**.

2. Context

In 1999, Piraux and Dardenne published the first study demonstrating the potential of NIRM for feed authentication **[7]**. The results of this study show that the near-infrared spectra of particles can be used to discriminate between allowed and forbidden feed materials. About 3000 particle spectra –(from 43 animal feed ingredients and 56 vegetal feed ingredients) were used to calibrate the spectrometer. The discriminant equations were successfully tested on samples spiked with meat and bone meal (MBM) at levels between 2 and 10%. Based on these preliminary results, the Walloon Agricultural Research Centre (CRA-W) decided to include this technique in the STRATFEED project to compare it with the reference method and other methods in development. The apparent advantages of

the NIRM method were , primarily, that it is not based on subjective interpretation, it accelerates the analysis and it does not require skilled and experienced analysts.

With the NIRM instrument, a microscope is used to focus the infrared beam on each particle of a sample spread on a sample holder, and the near-infrared spectrum is collected. The result of the sample analysis is a successive collection of hundreds of spectra, each one being the molecular near-infrared signature of a particle from one of the ingredients in the compound feed. The ingredients are identified using the spectral features measured in the near-infrared region (1100–2500 nm) of the electromagnetic spectrum. Figure 7.1. shows particles spread on the sample holder and the spectra of several feed ingredients **[3]**.

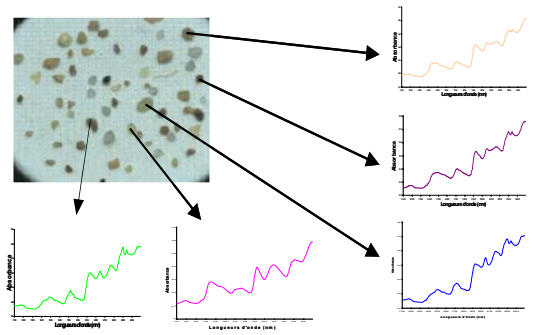
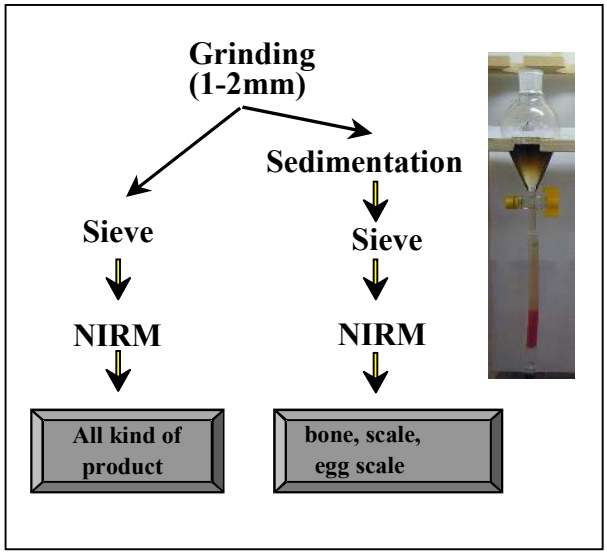


Figure 7.1. Feed particles spread on the sample holder and the NIR spectra resulting from the NIRM analysis.

3. Protocol developed during the project

For the NIRM studies, CRA-W used an Auto Image Microscope connected to a Perkin-Elmer Fourier transform near-infrared spectrometer (FT-NIR). This instrument enables one to collect spectra from a small surface (50 µ x 50 µ). The microscope includes a camera and a viewing system to magnify the visible-light image of the sample so as to highlight and isolate a point of interest. The particles of the sediment samples are spread onto a spectralon plate and placed under the NIR microscope. The JRC laboratory involved in validating the method used a Perkin Elmer FT-IR Spectrum 2000, coupled with an auto-image microscope. The measurement set-up comprised a single-beam Michelson interferometer, with beam splitters covering a wave number of 15,000 to 30 cm^{1}.

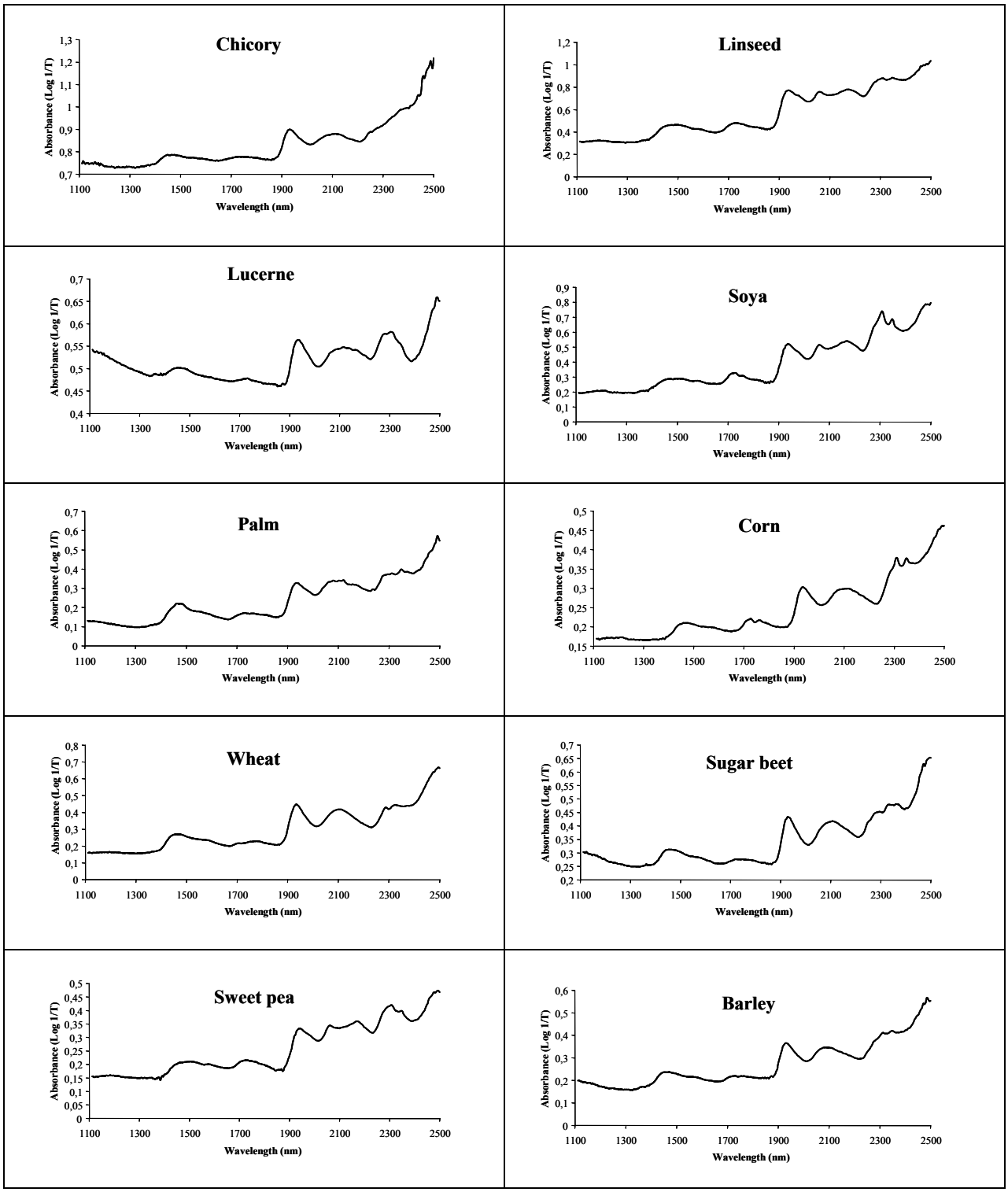
Figure 7.2. The NIRM protocol.



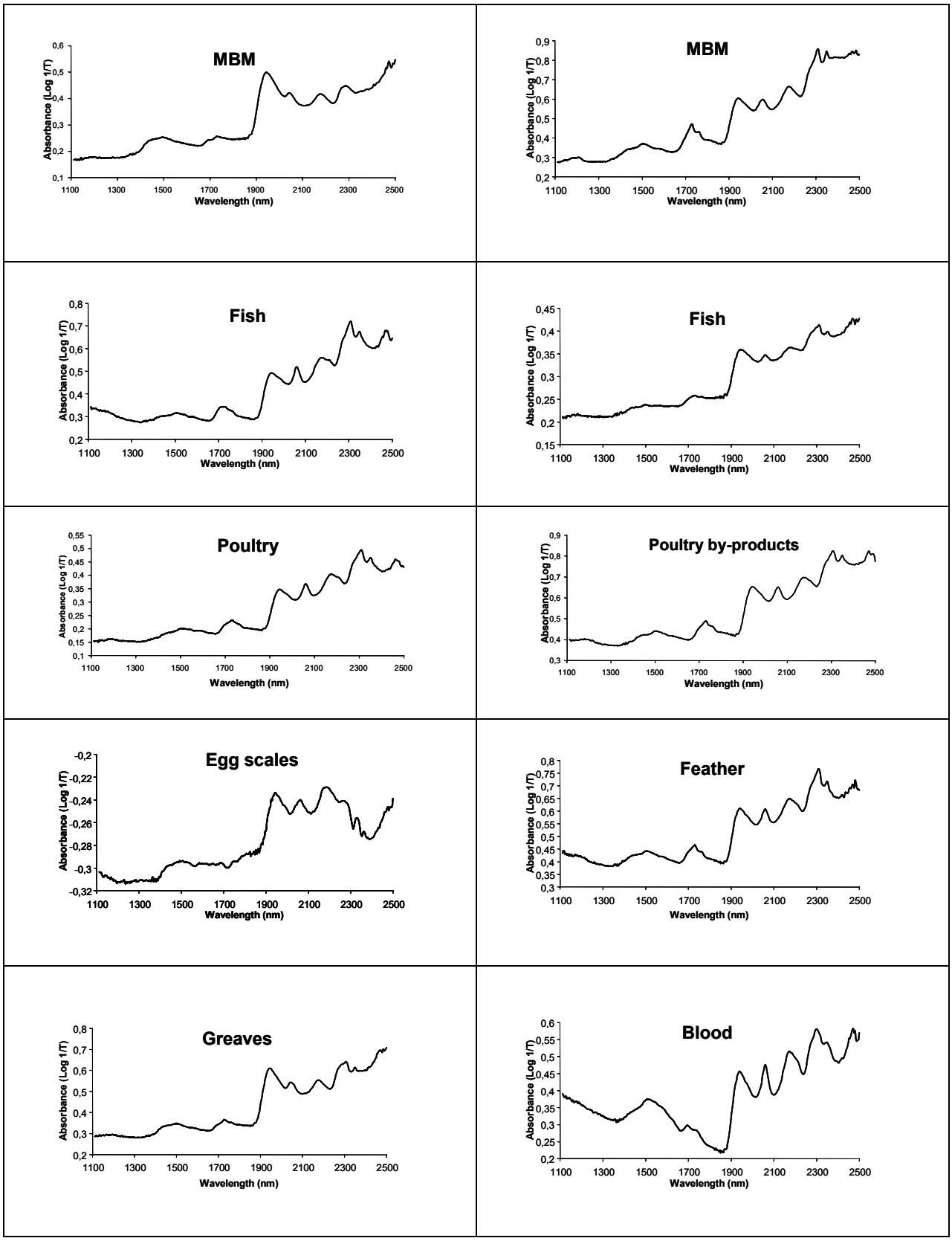
The protocol used in the STRATFEED project was quite simple and very similar to that used in optical microscopy (Figure 7.2.). The compound is first ground to a size of 1 or 2 mm. Then the NIRM analysis can be made on the raw fraction or on the sediment fraction of the sample. Usually, the analysis is done on those particles larger than 250 µm. In the case of the analysis of the raw fraction, detection is based on the presence of different kinds of particles (e.g. bone or muscle) of animal origin. In the analysis of the sediment fraction, detection is based on the presence of specific animal particles (e.g. bone).

A large spectral library was constructed, including more than 20,000 spectra from the raw and sediment fractions of feed ingredients and compound feed. One tenth of animal (e.g. mammalian meal, poultry by-product meal and fish meal), vegetal and mineral feed ingredients, and compound feeds destined for various farmed animals, was analysed. The samples were selected at the CRA-W and the STRATFEED sample bank in order to cover the full diversity of feed ingredients used in formulating feedstuffs. Figures 7.3. and 7.4. present near-infrared spectra of particles from various animal and vegetal feed ingredients, respectively.

Based on the results of the PCA applied on the spectral library, it was decided to define different groups. In the work space defined by all possible feed ingredients used in compound feed, we have subspaces defined by the vegetal and animal feed ingredients analysed. The animal subspace was divided into a group for fish meal and one for terrestrial animal feed ingredients. This latter group was further subdivided into the poultry group and the mammal group, including the feed ingredients of bovine and pig origin. Various supervised methods for achieving the best discrimination between groups were studied. Partial least square (PLS), neural network (NN) and SIMCA procedures were tested **[3]**.



Figures 7.3. Near-infrared spectra of particles from various vegetal feed ingredients.



Figures 7.4. Near-infrared spectra of particles from various animal feed ingredients.

Based on the results achieved with a reduced spectral library, it was decided to use the PLS algorithm to construct discriminant equations. For each mathematical model constructed, the interval limits for the group was calculated. A spectrum outside the limits of both groups defined by the discriminant model is considered as unclassified particle spectra. Table 7.1. shows the equations constructed with the defined groups, and the features of each group. To strengthen confidence in the classification, it was decided to take into account the results of various equations before deciding in which group a particle spectrum should be placed . Between 2 and 4 equations were used to decide on the group membership of a particle spectrum (see Table 7.1.).

Table 7.1. Description of the equations constructed and used to deciding which group a particle spectrum should be placed. **[3]**

EQUATIONS		GROUPS					
N°	Discrimination	Vegetal	Animal	Terrestrial animal	Fish	Poultry	Bovine and Pig
1	Vege / Animal	●	●	●	●	●	●
2	Terrestrial Ani. / Fish			●	●	●	●
3	(Bovine&Pig) / Poultry					●	●
4	Vege / Terrestrial Ani.	●	●	●	●	●	●
5	(Vege&Aniter) / Fish		●		●		
	Number of equations used	2	3	3	4	4	4

Table 7.2. presents the classification results obtained for the particles used in the test stage (i.e., the construction of the discriminant models) of the study. The number of particles, the classification percentage for the various groups, the percentage of the particles not classified (i.e. outside the specified limits of one of the equations) and the percentage of the particles correctly classified (i.e. the number of the particles classified in the correct group over the total number of particles classified in one of the groups) are given in Table 7.2.

Table 7.2. Classification results (in round figures) of the particle spectra obtained during the training stage. **[3]**

	PARTICLE ORIGIN					
GROUP	***Vegetal***	**Animal**	**Terrestrial animal**	**Fish**	**Poultry**	**Bovine and Pig**
Number of particles	**7492**	**2484**	**1585**	**889**	**505**	**1090**
VEGETAL	95.0	**0.0**	**0.1**	**1.5**	**0.0**	**0.1**
ANIMAL	**0.1**	94.3	**95.8**	**92.7**	**98.0**	**93.9**
TERRESTRIAL ANIMAL	**0.0**	**60.1**	95.5	**0.0**	**97.4**	**93.7**
FISH	**0.0**	**32.2**	**0.0**	90.0	**0.0**	**0.0**
POULTRY	**0.0**	**17.4**	**27.2**	**0.0**	84.1	**1.7**
BOVINE AND PIG	**0.0**	**40.1**	**62.8**	**0.0**	**2.8**	90.1
% not classified (a)	***4.9***	***5.7***	***4.1***	***5.8***	***2.0***	***6.0***
% correctly clasified	***99.9***	***100***	***99.9***	***98.4***	***96.8***	***99.3***

Legend: (a) = % not classified in the vegetal group or the animal group (i.e. false positive); % not classified = number of particles not classified over the total number of particles analysed in one of the groups; % correctly classified = number of particles classified in the correct group over the total number of particles classified in one of the groups.

Up to 95% of the vegetal feed ingredient particles were shown to have a vegetal origin, whereas only 8 particles were classified as an animal ingredient. This indicates a 0.1% misclassification for the vegetal group. The 8 misclassified particle spectra (most of them with a lot of noise) came from different vegetal feed ingredients. For the animal particles, about 94.3% of the particles were correctly classified. Less than 0.2% (i.e. 4 particles) of these were classified in the vegetal group (false negative). One of the misclassified particles came from a mammal meal sample, while and three came from three fish meal samples. The spectral in question clearly had the characteristics of the vegetal spectra. The presence of vegetal material in animal meal and, particularly, in fish meal samples is possible, as vegetal ingredients

are present in the digestive system before the processing of the by-products.

The species classification results showed that 95.5% of the analysed particles were in the terrestrial animal (bovine, pig, poultry) group and 90.0% in the fish meal groups Four particles from fish ingredients (= 0.5%) were classified in the group including the terrestrial animal ingredients. No particle was classified in the mammal or poultry meal group. Regarding the mammal ingredients (bovine, pig), 90.1% of the particle spectra were classified in the mammal group. About 1.7%, (19 particles) were classified in the poultry meal group. These spectra came from three samples. No particle was classified in the fish meal group. Of the particles from the poultry meal samples analysed, 84.1% were classified in the correct group. Fourteen particles were classified in the mammal meal group; 12 of them came from the same poultry ingredient. After PCR analysis, it was shown that this sample clearly contained mammal ingredients. None of the mammal or poultry particles was classified in the fish meal group.

Regarding the percentage of particles not classified (i.e. the number of particles not classified in the animal and vegetal meal groups over the total number of particles analysed), between 2% (poultry meal group) and 5.8% (fish meal group) of the particle spectra were outside the specified limits of one of the equations used to decide on the membership of a particle in a vegetal or animal meal group. For each group, more than 96.8% of the particles were classified in the correct group.

4. Testing the NIRM protocol

4.1. NIRM analysis of raw feedstuffs

After the test stage, the mathematical models constructed were tested. This involved analysing numerous raw feed ingredients from various animal origins, raw free MBM compound feed and raw compound feeds spiked at different levels. The samples analysed during the test stage were not included in the test and were analysed using the reference method and one of the alternative methods being developed. The results demonstrated the high potential of the NIRM method to detect animal ingredients.

Figure 7.5. presents some of the classification results for a series of animal feed ingredients. About 351 particle spectra from 12 MBMs were analysed, following the NIRM protocol described in section 7.3. Of these, 315 particle spectra (89.7 %) were classified in the animal group. None of the particle spectra was classified in the vegetal group, showing a classification level of 100%. The remaining particle spectra were unclassified because they were outside the 95% limit of confidence of one or both of the equations used to decide membership of the animal group (see Table 7.1.). **[3]**

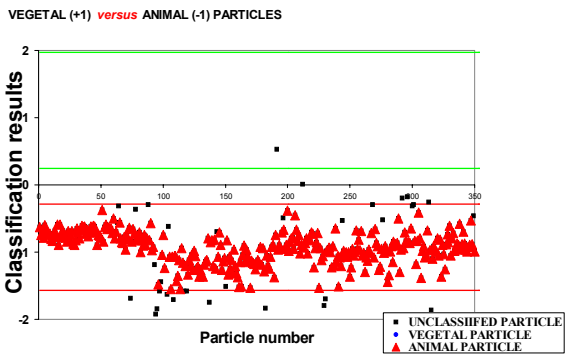


Figure 7.5. Classification results of animal particles analysed during the test stage.

Table 7.3. presents the results of the NIRM analysis of the raw fraction of 21 sample feeds contaminated with animal meal at various concentrations and pure animal meal. The description of this set is given in the chapter on the sample bank and the preparation of the samples during the project. For the analysed samples, Table 7.3 shows the percentage of animal particles detected, an evaluation of the presence of each species of particle, and the number of particles from the raw fraction analysed by NIRM. Between 28 and 800 particles of each sample were analysed blind. The discrimination of spectra was correct. Indeed, pure animal meal spectra were correctly classified, and. contaminated feed was correctly detected. Regarding the discrimination between animal species, the unexpected contaminations accorded with the results obtained using optical microscopy and PCR methods.

Table 7.3. Results of the NIRM analysis of the raw fraction of 21 samples contaminated with animal meal at different concentrations and pure animal meal.

STRATFEED SAMPLES	Animal	Terrestrial animal	Fish	Poultry	Bovine and Pig
101, MBM	**100**	++	--	+/-	++
102, Feed + 0.9% MBM	**1.8**	++	--	--	++
103, Feed 0% (sedimented)	**0**	--	--	--	--
104, Fish	**100**	--	++	--	--
105, MBM	**100**	++	--	--	++
106, Poultry	**100**	++	--	++	--
107, MBM	**100**	++	--	--	++
108, Poultry	**100**	++	++	++	--
109, MBM	**100**	++	--	--	++
110, MBM	**100**	++	--	--	++
111, Feed + 2.5%	**7.5**	++	--	++	++
112, Feed + 6% MBM	**12.3**	**++**	**++**	**++**	**+/-**
113 Feed + 9% MBM + 3% Fish	**8,9**	**++**	**++**	**+/-**	**+/-**
114 Feed + 2.5% MBM	**1.8**	**++**	**--**	**--**	**++**
115 + 3% Fish	**3.8**	**--**	**++**	**--**	**--**
116 + 0.5% Fish	**0.3**	**--**	**+/-**	**--**	**--**
117 MBM	**100**	**++**	**+/-**	**--**	**++**
118 Fish	**100**	**--**	**++**	**--**	**--**
119 Greaves	**99.0**	**++**	**--**	**++**	**++**
120, Feed + 5% MBM + 6.25% Fish	**8,4**	++	--	++	+
121 Poultry	**99.0**	**++**	**+**	**+/-**	**++**

Table 7.4. summarises the results of the NIRM analysis of the raw fraction of the 48 samples included in the GS1 set. The description of this set is given in the chapter on the sample bank and the preparation of the samples during the project. For the analysed samples, Table 7.4. includes the theoretical percentage, the number of particles from the raw fraction analysed using NIRM, the number of particles detected as having an animal origin and the conclusion of the NIRM analysis. Between 141 and 710 particles of each sample were analysed blind. Some 47 samples were detected as positive for the presence of MBM (i.e., a correct results level of about 97.8%). One sample (No. 17) was erroneously detected as negative (i.e., a false negative level of 2.1%). Sample 17 from the GS1 set was a sample spiked at 0.5%. About 200–300 mg were required to perform the NIRM analysis on the raw fraction of compound feed. Thus, the spiked samples needed to have good homogeneity. The detection limit of the NIRM method depends on the homogeneity of the sample and the number of particles analysed. In an additional study, it was shown that the detection of 0.1% of MBM adulteration is possible using the NIRM analysis in the case of at least 3000 particles analysed.

Table 7.4. Results of the NIRM analysis of the raw fraction of the 48 samples in the GS1 set.

N°	%	No. of analysed particles	No. of MBM particles	Conclusion
1	0.5	371	3	*Positive*
2	1	301	1	*Positive*
3	1.5	273	4	*Positive*
4	2	360	4	*Positive*
5	2.5	334	2	*Positive*
6	3	334	6	*Positive*
7	3.5	603	5	*Positive*
8	4	378	6	*Positive*
9	4.5	329	6	*Positive*
10	5	321	8	*Positive*
11	5.5	374	9	*Positive*
12	6	621	25	*Positive*
13	6.5	357	5	*Positive*
14	7	194	8	*Positive*
15	7.5	694	11	*Positive*
16	8	172	9	*Positive*
17	0.5	617	0	*Negative*
18	1	627	7	*Positive*
19	1.5	656	1	*Positive*
20	2	302	2	*Positive*
21	2.5	347	4	*Positive*
22	3	365	7	*Positive*
23	3.5	350	12	*Positive*
24	4	196	4	*Positive*
25	4.5	574	8	*Positive*
26	5	179	6	*Positive*
27	5.5	593	17	*Positive*
28	6	171	4	*Positive*
29	6.5	194	8	*Positive*
30	7	141	6	*Positive*
31	7.5	208	8	*Positive*
32	8	331	32	*Positive*
33	0.5	595	2	*Positive*
34	1	710	4	*Positive*
35	1.5	327	3	*Positive*
36	2	386	2	*Positive*
37	2.5	328	4	*Positive*
38	3	368	6	*Positive*
39	3.5	344	4	*Positive*
40	4	385	5	*Positive*
41	4.5	646	7	*Positive*
42	5	190	3	*Positive*
43	5.5	415	16	*Positive*
44	6	180	8	*Positive*
45	6.5	204	10	*Positive*
46	7	636	15	*Positive*
47	7.5	166	8	*Positive*
48	8	322	11	*Positive*

Note: No.= sample number, % = theoretical percentage of adulteration.

In order to study the ability of the NIRM method to detect MBM in fish meal, a specific study was designed. About 20 samples used in a previous study **[6]** were selected by SAC and sent blind to CRA-W. The sample fish meal set prepared by SAC included 10 pure fish meal samples and 10 samples spiked with MBM at various levels (3, 6 and 9%). The NIRM analysis was performed on the raw fraction of the samples. About 600 particles of each sample were analysed. Table 7.5. summarises the results of the NIRM analysis of the 20 samples. All the spiked samples were shown to be positive. A correlation of 88.6% between the theoretical percentage and the percentage calculated by NIRM was observed. These results show that the NIRM method can be used at least as a semi-quantitative method. Two out of 10 of the free MBM samples were shown to have been spiked at a low level with MBM. Respectively, 1 and 3 particles were classified as having a terrestrial origin for samples F4 and F11, respectively. Both samples were analysed using PCR, which revealed the presence of a trace of terrestrial animal DNA. This study demonstrated the potential of NIRM for detecting MBM in fish meal. Taking into account that detecting 3% of MBM in fish meal is possible, it can be said that detecting 0.15% of MBM in 5% of fish meal is theoretically possible.

Table 7.5. Results of the NIRM analysis of the raw fraction of 20 fish meal samples.

N°	Fish detection[a]	MBM detection[b]	Theoretical MBM %[c]	NIRM MBM %[d]
F1	++	++	3	1.2
F2	++	++	9	7.9
F3	++	++	6	3.9
F4	++	+-	none	0.2
F5	++	--	none	0
F6	++	--	none	0
F7	++	--	none	0
F8	++	++	9	10.4
F9	++	--	none	0
F10	++	++	9	7.1
F11	++	+-	none	0.5
F12	++	++	6	1.7
F13	++	--	none	0
F14	++	++	3	3.9
F15	++	++	6	8.2
F16	++	--	none	0
F17	++	++	3	2.7
F18	++	++	3	2.1
F19	++	--	none	0
F20	++	--	none	0

Note : a = NIRM result for detecting fish material in the sample, b = NIRM result for detecting terrestrial animal in the sample, c = theoretical percentage of MBM in the sample, d = percentage of MBM calculated by NIRM.

4.2. NIRM analysis of the sediment fraction of feedstuffs

The NIRM method was also tested for detecting MBM in the sediment fraction of compound feeds during the test stage. For this, an initial tentative study was made using samples spiked at 0.05, 0.1, 0.5 and 1% with MBM. All the samples were detected as positive. The repeatability of the method was also studied. A sample spiked at 0.1% with MBM was analysed 10 times. Table 7.6. presents the results of the repeatability study. The tables also shows 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 conclusion of the NIRM analysis. All the replicates were detected as positive for the presence of bones in the sample. The standard deviation of the percentage of bone particles in the sediment was about 0.16. **[5]**

Table 7.6. Results of the repeatability study (Baeten *et al.*, 2004)

Code	% sediment (a)	No. analysed particles	No. animal particles (a)	% bones in the sediment (b)	Weight of 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*

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.

The sediment fraction of the 48 samples of the GS1 set and the 24 matrices used to prepare these samples were analysed using NIRM. All the spiked samples were detected as positive, whereas the free MBM compound was classified as negative. In this study, some of the samples were analysed using optical

microscopy (OM) by ROLT. The OM results were expressed in the same way as the NIRM results; i.e. number of animal particles vs. number of particles analysed. All the samples were shown to be positive and the correlation between the weight of bones in the sediment obtained using OM and NIRM was higher than 0.85%. In addition, the t-test showed that there was no significant difference between these results. The results allow us to conclude that the NIRM method is an adaptable method for detecting and determining the content of bones in the sediment fraction (Baeten, von Holst, Vancutsem, Garrido, Michotte Renier & Dardenne, unpubl. results).

In the development of a method, a crucial aspect is its transferability from the developing laboratory to another laboratory. In view of this, and within the framework of the STRATFEED project, it was decided to analyse blind at the Joint Research Centre (JRC) of the European Commission a set of MBM-free and spiked samples from the GS1 sample set. The samples were analysed in an order defined by CRA-W, whereby the free and spiked samples were randomly alternated. The NIRM method developed at CRA-W was successfully transferred to the JRC at a false negative value less than 5%. A high correlation between the CRA-W and JRC results was observed (Baeten, von Holst, Tirendi, Fissiaux & Dardenne, unpubl. results)

Figure 7.6. shows microscopic images and infrared spectra of particles of the sedimented fraction. The microscopic images are produced by the ARIES-DSS, developed under the STRATFEED project by RIKILT to help the microscopist detect and identify animal protein in feedstuffs.

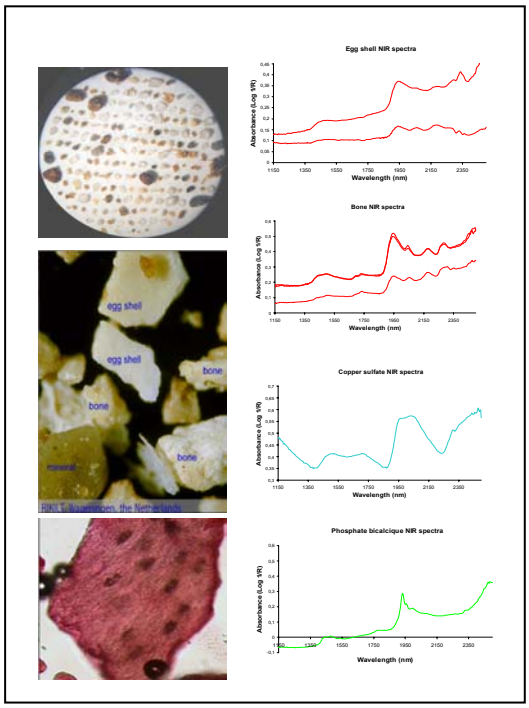


Figure 7.6. Microscopic images and infrared spectra of particles of the sedimented fraction (Baeten* et al.*, 2004)

5. Pro and cons of the NIRM method

Several features of the NIRM method can be highlighted:

5.1. NIRM analyses particles

The principle of NIRM analysis is similar to the accepted reference method for detecting MBM in compound feed. Indeed, classical microscopy analysis is based on the identification of particles of animal origin by microscopic inspection. For this, several slides from fractions prepared using defined procedures and various magnifications were analysed. The NIRM analysis also involves the microscopic analysis of the particles of a compound feed, but the visible light analysed by the eyes of the microscopists replaced by infrared light detectors. Conversely, NIR spectral features and the discriminant

equations act as a substitute for the expertise of the microscopist.

The microscopic analysis of feed particles postulates that each particle comes from a single feed ingredient. In the work undertaken since 1998, tens of thousands of particles have been analysed while developing the NIRM method. Sometimes, particles made of conglomerates of small particles (< 250 µm) were observed using optical microscopy. This was mainly the case with mineral and vitamin complements that had a specific infrared signature **[5]**. One feature of NIRM analysis is that all the particles from the various constituents of animal meal (e.g. muscle, bones, hairs) are analysed in a single analysis. However, the fact that NIRM analysis is based on the analysis of particles limits its application to particles larger than 50 µm (this limitation depends on the NIR microscope used and some configuration allowing a spectra of 5 µm particles to be collected). In addition, this method is not suitable for slurries and liquids that do not include particles. In these cases, only molecular biology techniques such as PCR and ELISA could be used, provided that the sample includes the targeted DNA fragments or protein, respectively.

5.2. NIRM is based on near-infrared information

The near-infrared spectra are constituted by the overtone (mainly first and second overtones) and combination bands of fundamental bands occurring in the mid-infrared region of the electromagnetic spectrum. The bands in the infrared region derive from the absorption of the infrared light by the organic bands, causing a change in their dipole moments. Broadly, all the molecules absorb part of the energy in the near-infrared region and contribute to absorbance at each wavelength. By definition, the absorbance at a determined wavelength is the result of the combination of the absorbance of a wide range of organic molecules. However, the interpretation of near-infrared spectra is difficult. The spectra of the particles from feed ingredients are influenced mainly by the type and the protein, fat, sugar and water content of the material. For NIRM analysis of feed particles, the spectra are the molecular signatures of the feed ingredients included in the compound feed. The results of the analysis of the samples produced at various temperatures show that this did not affect the identification of the particles using the NIRM method.

Another important issue regarding methods developed for control laboratories is the need to have methods that, in the case of fraud, can be used as legal evidence in a court of justice. The NIRM technique can be considered as an initial method that has to be confirmed by another method (e.g. PCR) that can be used as legal evidence. Clearly, macro NIRS cannot be used as evidence. But in the case of NIRM, the specificity is more easily assessed, the spectral signature being that of a single particle. As noted at the start of this chapter, the differences between vegetal and animal, for instance, are clear and easily distinguishable visually. It should be noted that the spectroscopic method coupled with a microscope is one of the methods used in forensic laboratories to determine the type of explosive used in terrorist attacks or to detect the presence of illegal drugs.

5.3. NIRM does not require expertise

One of the main advantages of the NIRM method compared with the optical microscopic method is that expertise is not necessary to discriminate between the origins of particles. Indeed, optical microscopy requires expert analysts to detect and identify microscopic features of animal origin. Although the discrimination of bones and egg shells is relatively easy and requires training for a few days, the

detection and recognition of other ingredients of animal origin require more experience in order to avoid confusion with ingredients of vegetal origin. For instance, the structure of hair of animal origin that can reveal the presence of animal ingredients can be confused with 'hair' of vegetal origin. On the other hand, the detection of particles of animal origin and the classification of these particles into species groups is made using the NIRM method through the use of mathematical equations. Thus, the identification and the classification do not require the intervention of the analyst. Moreover, the analytical results obtained by various analysts from the same samples will not be affected by the expertise of the analyst.

An additional advantage of the NIRM method is that a single analysis could enable a wide range of feed ingredients to be detected. Indeed, the spectra of particles from a compound feed can be used to identify particles of animal origin as well as those from the vegetal or mineral world **[4]**. This requires using, in the discrimination procedure, the correct equations to determine the vegetal or mineral origin of the particles. This feature allows the method to be adapted rapidly when the legislation changes. Indeed, changes in the list of forbidden or accepted products could mean that only the construction of a new equation, using the existing training database, was necessary.

5.4. NIRM is a non-destructive method

The NIRM method has the decisive advantage of being a non-destructive method. After analysis using this method, the particles classified as animal origin can be selected and analysed using other techniques to get additional information on the origin of the particle.

6. Conclusion

This section demonstrates the significant potential of NIRM for detecting MBM in feedstuffs. The combination of NIRS and a microscope allows high quality spectra from small (50-1000 μm) feed particles to be collected, the analysis of these spectra unaffected by the expertise the analyst. The analysis can be performed by a technician after brief training on the transfer method

The results presented in this chapter also indicate that the method is efficient for the specific detection of animal meal, and for discriminating between fish meal, mammal (pigs and cattle) meal and poultry meal. In addition, as the method is non- destructive, samples can be kept for analysis using another technique.

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