Chapter 8
Near Infrared microscopy (NIRM)

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NEAR INFRARED MICROSCOPY (NIRM)

SUMMARY

The evolution of the different rules about forbidden and authorized raw materials in feed formulation requires developing and continuously adapting the methods used for the detection of illicit ingredients. Different studies carried out during the last years have permitted the development of techniques like the NIR microscopy (NIRM) based on spectroscopy. This methodology combines the optical microscopy and the spectroscopic benefits. Moreover, it allows the analysis of a larger number of samples per unit of time than with optical microscopy and the recognition does not depend on the expertise of the analyst. Based on studies performed in the framework of the SAFEED-PAP project, the aim of this chapter is to give the readers an overview of the NIRM technique applied to the discrimination of the species origin of animal protein by-products (APBs) in feeds.

Keywords: Near infrared spectroscopy, microscopy, species origin, feed, avian, mammalian, fish meal

8.1. INTRODUCTION

The detection of meat and bone meal (MBM) in compound feed became a crucial issue since MBM has been suspected as the main cause of the spread of Bovine Spongiform Encephalopathy (BSE). One of the consequences of BSE that appeared in the beginning of the nineties was the European Commission Decision No 94/381/EC of the 27th of June 1994 (amended several times) that stated the prohibition on the use of mammalian proteins in ruminant feedingstuffs. After the come-back of the BSE crisis by the end of 2000, the European Commission decided to enlarge this prohibition to other animal origin ingredients in the feed of any animal destined to human consumption. So, by the European Council Decision number 2000/766/EC of the 4th of December 2000, the animal proteins (meat and bone meal, blood meal and derivatives, hydrolyzed proteins, hoof meal, poultry by-product meal, fish meal, dicalcic phosphate and gelatins) were totally forbidden for the feeding of this category of animals, which has been subsequently transferred into a permanent ban by Commission Regulation (EC) No 1234/2003.. Nevertheless some products like fish meal are allowed for non ruminant. These decisions will be prolonged for an undetermined period.

The evolution of the different rules about forbidden and authorized raw materials in feed formulation requires developing and continuously adapting the methods used for the detection of illicit ingredients. Optical microscopy is the only official method in the European Union for the detection of MBM in compound feed and is largely accepted at international level and routinely used in control laboratories. Controls are based on the microscopic identification of illicit ingredients and more particularly by searching for bone particles in the sediment fraction of the analyzed feed. However, the necessity of reliable and faster methods led to the development of alternative approaches, like methods based on spectroscopy (e.g. NIR spectroscopy). For this reason, different studies have been carried out during recent years (STRATFEED project and SAFEED-PAP project) on the development of spectroscopic-based techniques like the NIR microscopy (NIRM) to analyse compound feeds. This technique consists of the analysis of several hundreds of particles being the result of the grinding of compound feedstuffs. The major advantages of this technique are that the recognition is based on the measured NIR spectrum and does therefore not depend on the expertise of the analyst as when applying classical microscopy. With NIRM it is possible to automate all procedures and to analyse more samples per unit of time than with the optical microscopy (official method). NIRM combines the optical microscopy benefits (detection based on the presence of animal particles and not affected by the rendering process applied to the MBM) and the spectroscopic benefits (detection based on the specific chemical composition of animal tissues).

Different studies have been done in recent years to demonstrate the potential of NIRM for feed authentication (Baeten et al. 2000A, Baeten et al. 2004, De La Roza et al. 2007, Fernández-Ibañez et al. 2008). The first work was published by Piraux and Dardenne in 1999 showing that it was possible to recognize animal particles in a ground compound feedstuff with an error of 0.64% (Piraux and Dardenne 1999). Other studies have also demonstrated (Stratfeed, Baeten et al. 2001, De La Roza et al. 2007) the high potential of NIRM to detect MBM at level as low as 0.5 %. In order to decrease the limit of detection for the detection of banned MBM in feedstuffs by NIRM analysis, the use of a sedimentation step similar to the one used in the European official microscopy method was proposed (Baeten et al. 2003). Based on this sediment fraction, Baeten et al. proposed some multivariate discriminant models constructed with more than 8000 spectra coming from different feed ingredients of animal origin (including bovine, pig, sheep and poultry) and some MBM-free compound feeds. Approximately 97.5% of the animal particles were classified in the defined interval of animal origin class with misclassification of 0.064% (Baeten et al. 2005A). With a protocol focused on the sediment part of the sample, NIRM can be used to detect MBM in feed at level as low as 0.05%. Similar studies have been conducted in order to investigate the ability of the NIRM method to

discriminate bone particles of fish origin and those of land-animal origin in sediment fraction with important success rates (De La Haba et al. 2007).

The aim of this chapter is to give the readers an overview of the NIRM technique applied to the discrimination of the species origin of animal protein by-products (APBs) feeds. For this, important points of the defined protocol are explained as sample preparation or spectral acquisition.

Two examples studied in the framework of the SAFEED-PAP project are shown here, namely the selection of markers for the discrimination of avian and mammalian particles and the discrimination of fish and terrestrial particles. In this work, some possible NIR markers and their chemical interpretation are detected and justified. In order to prove the feasibility of such markers as such as well as their stability, different tests have been performed.

8.2. INSTRUMENTATION

8.2.1 NIR microscopy

A NIR microscope consists of an Auto Image Microscope connected to a Fourier Transform Near-Infrared Spectrometer (FT-NIR) (Figure 8.1). This instrument enables the collection of spectra from small surfaces (50µ x 50µ). The microscope includes a camera and a viewing system to magnify the visible light image of the sample, as well as to highlight and isolate a point of interest. The particles are spread on a spectralon plate and presented to the NIR microscope.

Figure 8.1 - NIR microscope

This instrument works as follows: the beam generated by the interferometer goes to the microscope, which is equipped with a video camera that allows visualisation of the sample and localization of the particles to be analyzed. The infrared rays are focused into the particles to be analyzed and a detector, located in the microscope, measures the reflected beam. Then using the microscope pointer, the infrared beam is focused on each particle and the NIR spectrum is collected (4000 – 7800 cm-1). A resolution of 8 cm^{-1} and a total of 10 co-added scans are used. Spectra are obtained after background correction.

A complete protocol for the NIRM for the measurement of compound feed has been established. This protocol is valid for both, the sediment fraction as obtained after applying the optical microscopic method and the whole sample (not sedimented) and gives clear information about sample preparation, particle selection and spectra acquisition.

8.2.2 Sample preparation for the NIRM

Whole sample

After mixing the sample with the help of a slice, 4 sub-samples of about 100 mg are taken. The sub-samples are combined and placed in the sieve of 250 µm, which is then placed over an agitator for 2 minutes. The particles (>250 µm) are then taken from the sieve with the help of a stainless steel spoon, placed and spread on the sample holder (e.g. Spectralon). A minimum of 50-150 particles should be spread out in the sample holder in a thin layer. These steps are repeated till at least 600 separate particles have been measured.

Sediment fraction

The sediment, prepared applying the corresponding protocol from classical microscopy (European commission 2009), has to be placed in the sieve of 250 µm and mixed manually for a few seconds. As for the whole sample, the particles (>250 µm) are then taken from the sieve with the help of a stainless steel spoon, and then are placed in the sample holder (e.g. Spectralon). A minimum of 50-150 particles should be spread in the sample holder in a thin layer. If no animal particles are detected, then a minimum of 300 particles should be analysed, as long as the obtained number of particles coming from the sedimentation allows this analysis. However, if animal particles are found, the analysis has to continue until the detection of at least 3 animal particles or the complete analysis of the sediment.

Particle selection and spectra acquisition

As previously explained, the NIRM instrument is equipped with a video camera that allows visualization of the sample and the localization of the particles to analyse. The use of

the microscope pointer allows the infrared beam being focused on the centre of each particle individually (marking the particles). The infrared rays are focused into particles to analyse and a detector located in the microscope measures the reflected beam. Then an inverse Fourier transform is applied and the NIR spectrum is collected. Spectra can be considered as fingerprints that are based on the chemical composition of the analysed particles.

8.3. SELECTION OF MARKERS FOR THE DISCRIMINATION OF THE SPECIES ORIGIN OF ANIMAL INGREDIENTS BY NIR-MICROSCOPY

NIR spectra might appear rather complicated for non- NIR users. Therefore it is important to show that when using spectra not only it is possible to develop robust multivariate predictive models for the detection of the animal species, but also that there is a fundamental chemical basis in the spectra. Markers for the discrimination of the species origin of animal ingredients by NIRM can be defined as relevant NIR bands, in which the absorptions can be associated with specific compounds that are useful for discrimination purposes between animal species. The study described here consists in finding the relevant bands on pure animal origin meal samples and have been restricted to the discrimination between two pairs of classes: (1) avian *versus* mammalian and (2) fish *versus* terrestrial.

8.3.1 NIR markers for the discrimination between Avian and Mammalian particles

The average spectrum of the avian class was constructed from 320 spectra of particles corresponding to 9 different samples of pure avian meals, while for the mammalian class the average spectra was constructed from 632 spectra of particles corresponding to 20 different samples of several kind of mammalian pure meals (blood samples were not considered). The average spectra were pre-treated with a combination of a first derivative treatment (1,5,5,1 being the first digit the number of the derivative, the second the gap over which the derivative is calculated, the third the number of data points in a running average or smoothing, and the fourth the second smoothing) (Shenk and Westerhaus, 1995) and a scatter correction treatment (Standard Normal Variate + Detrend) (Barnes et al., 1989), in order to extract the more relevant differences between the two classes, avoiding the redundant information (Figure 8.2).

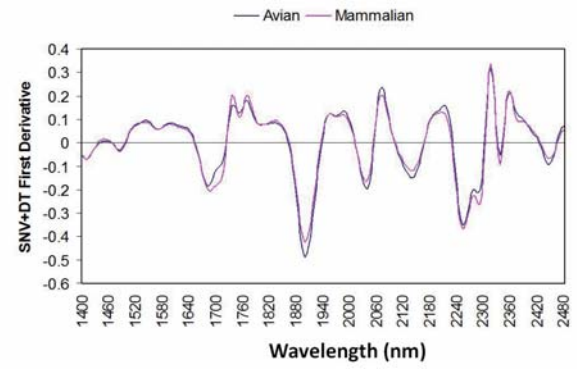
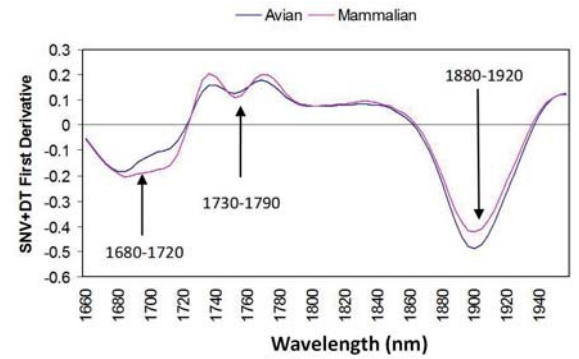
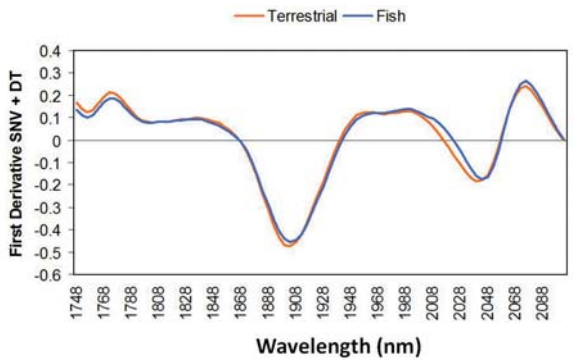


Figure 8.2. Pre-treatment average NIR-m spectra of avian and mammalian classes.

In the Figure 8.3, it is shown in close up the same spectra of the Figure 8.2, but divided in three ranges, in order to appreciate more clearly which are the more relevant NIR spectra bands for the discrimination of these two groups.





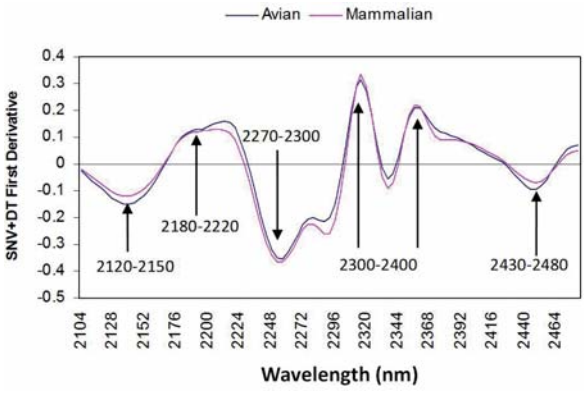


Figure 8.3. Zoom of Figure 8.2 in three spectral ranges

The most relevant NIR bands or regions identified after visual interpretation of the average pre-treated spectra of avian and mammalian, were revised in the scientific bibliography (Murray 1987; Murray and Williams, 1987; Sato et al., 1991; Devaux et al., 1993; Hourant et al., 2000; Garrido et al., 2004) to know with which chemical groups or compound absorptions were associated. As can be appreciated in Table 8.1, the most relevant NIR bands, that even allow to visually observe differences between the two classes (mammalian and avian), are characteristics of protein (amino-groups) and fat (fatty acids) absorptions.

Table 8.1 List of relevant NIR bands to discriminate between avian and mammalian samples and their chemical interpretation. ovt: overtone; asym: asymmetric; sym: symmetric; str: stretching; def: scissoring; MUFA: Mono Unsaturated Fatty Acids; PUFA: Poly Unsaturated Fatty Acids.

NIR band (nm)	Interpretation
1680-1720	Aromatic Compound 1st ovt CH_3 asym str
1730-1790	Oil , 1st ovt CH_3 asym str MUFA
1880-1920	Protein (Amino groups)
1940-1960	Water-OH, RCOOH, RCOOR, CONH2
2020-2040	Protein (Amino groups)
2060-2090	Protein (Amino groups)
2120-2150	Oil (=CHstr + C=Cstr, PUFAs)
2180-2220	Protein and oil (C=C, PUFAs)
2270-2300	Protein (Amino groups)
2300-2400	Oil (CH_2 asym str + CH_2 def)
2430-2480	Oil (CH3 asym str + C-Ostr

In fact, Garrido et al. (2004) on a review of the literature about the NIR analyses of fats and oils conclude the following:

1. All fatty acids (FAs) display peaks of varying intensity at 1700 nm due to C-H stretching vibration. They also show a weak second overtone near 1200 nm and bands at 2200 and 2500 nm, which are combinations involving C-H stretching with other vibrational modes.
2. The methyl C-H stretching bands lie near 1690, 1730 and 2260 nm. The methylene C-H bands lie in the vicinity of 1740, 1770, 2300 and 2340 nm.
3. The FAs with *cis* double bonds have combination absorption bands at 2150 and 2190 nm, a weak first overtone at 1680nm, and second overtones at 1180 nm. Absorptions at 2150 and 2190 nm increase with greater unsaturation.

All the peaks mentioned in statements 1 to 3 are in the regions where more clear differences can be observed between avian and mammalian meals, as can be observed in Figures 8.2 and 8.3 and Table 8.1. Furthermore, simultaneously Hourant et al. (2000) and Baeten et al (2000B), in extensive studies of the spectral interpretation of fats and oils using their own NIRS transmission spectra, demonstrated, among others, that the absorbance near 2148 nm was positively correlated with the total amount of PUFA (polyunsaturated fatty acids) while the absorbance in the region 1720-1760 nm were positively correlated with the total amount of MUFA (monounsaturated fatty acids). It seems evident that the differences observed in the micro-NIR spectra of mammalian and poultry meals may be partially explained by differences in the fatty acids profile, and more clearly by the differences between MUFA and PUFA content.

In order to confirm the markers obtained another test has been performed. The F-values from the F-Fisher test (Fernández Pierna et al 2005) are also useful indicators of relevant wavelengths. This criterion describes the ratio of between-class variance/within-class variance that is helpful to decide which original variables have an important discriminating power.

For each variable *j*:

$$FC_j = H_j / E_j$$

where $H_j = \sum_{i=1}^{K} n_i (\bar{x}_{ij} - \bar{x}_{.j})^2$ is the between-class variance

and $E_j = \sum_{i=1}^{K} (n_i - 1) S_j^2$ is the within-class variance,

where n_i is the number of objects in class i, $\bar{x}_{ij}$ is the mean absorbance of the objects belonging to class i at the *j*-th wavelength, $\bar{x}_{.j}$ is the mean absorbance of the objects belonging to all classes at the *j*-th wavelength and S_{ij} is the

standard deviation of the absorbance of the objects belonging to class i at the *j*-th wavelength.

The Fisher test was applied to 2448 spectra of avian and mammalian samples and the results are shown in Figure 8.4. As in Figure 8.3, the results confirm that the most relevant NIR bands to observe differences between the two classes (mammalian and avian) are the ones, which pioneers NIRS books (Williams y Norris, 1987, Osborne and Fearn, 1986) associated mostly with protein (amino groups) and water-OH overtones, and in much less extension also with the bands characteristic to fat (MUFA and PUFA).

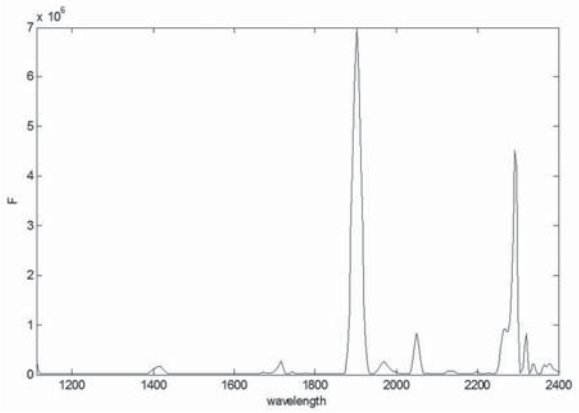


Figure 8.4. F values obtained for each wavelength for the mammalian vs. avian data after SNV, detrend and first derivative

8.3.2. NIR markers for the discrimination between Fish and Terrestrial particles

In this case, the average spectrum of the fish class was constructed from 540 spectra of particles corresponding to 18 different samples of pure fish meal. The average spectra of the terrestrial class have been constructed from 919 spectra of particles corresponding to 29 different samples of several kinds of pure terrestrial meal, including avian, porcine, bovine and ovine. The average spectra were pre-treated with a combination of a first derivative treatment (1,5,5,1) and a scatter correction treatment (SNV+DT), in order to extract the more relevant differences between the two classes, avoiding the redundant information (Figure 8.5).

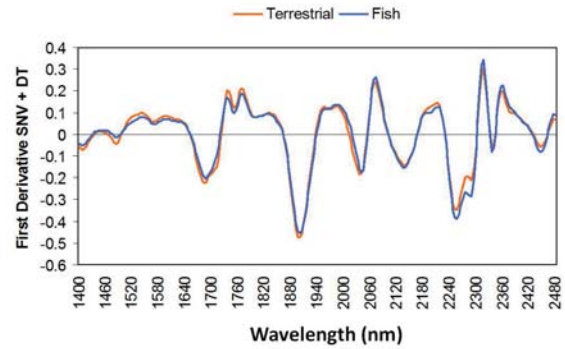
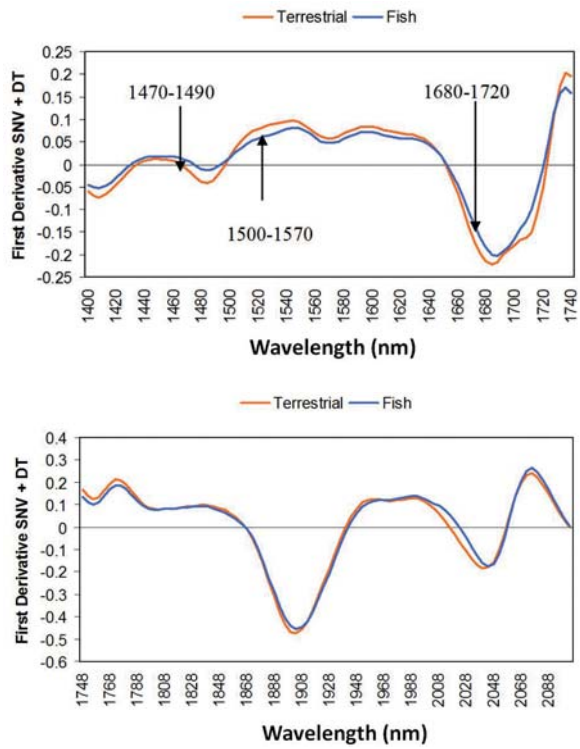


Figure 8.5. Pre-treatment average NIR-m spectra of fish and terrestrial classes

In the Figure 8.6, is showed a close up of the same spectra of the Figure 8.5, but divided in three ranges, in order to better appreciate which are the more relevant NIR spectra bands for the discrimination of fish and terrestrial.

It can be appreciated that some of the relevant bands identified for the discrimination between avian and mammalian are also important to distinguish between fish and terrestrial. Nevertheless, it can be observed that there are two relevant bands in the initial zone, and that the most important differences between the two classes (fish *vs* terrestrial) can be observed at the end of the spectra, between 2100 and 2500nm.



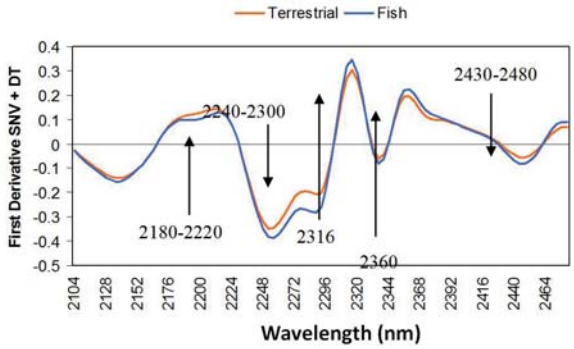


Figure 8.6. Zoom of Figure 8.5 in three spectral ranges.

The list of the most relevant markers to distinguish between fish and terrestrial meals, together with the chemical groups associated by different authors (Osborne and Fearn, 1986; Murray 1987; Murray and Williams, 1987; Sato et al., 1991; Devaux et al., 1993; Hourant et al., 2000; Garrido et al., 2004) to the absorption in this region are showed in Table 8.2.

Table 8.2. List of relevant NIR bands to discriminate between fish and terrestrial samples and their chemical interpretation. ovt: overtone; asym: asymmetric; sym: symmetric; str: stretching; def: scissoring; PUFA: Poly Unsaturated Fatty Acids.

NIR band (nm)	Interpretation
1470-1490	N-H str. First overtone
1500-1570	Protein (Amino-groups)
1680-1720	Aromatic Compound 1st ovt CH_3 asym str
2180-2220	Protein and oil (C=C, PUFAs)
2240-2300	Protein (Amino-groups)
2316	Oil (CH_2 asym str + CH_2 def)
2360	Oil (CH_2 asym str + CH_2 def) and Protein
2430-2480	Oil (CH3 asym str + C-Ostr

As mentioned in the case of avian and mammalian classes, the most relevant NIR bands when observing differences between the fish and terrestrial are characteristics of protein (amino-groups) and fat (fatty acids) absorptions.

In Figure 8.6 can be appreciated that the fatty-acid and amino-acid profiles of fish samples are quite different compared to the profile of terrestrial samples. Particularly, it can be stressed the differences in unsaturated fatty acid composition, that is highly related with the absorption band located at 2180-2220 nm, in which we can observe important differences between the average spectra of the two classes: thus the fish class in this region shows two smoothed peaks that are not observed in the terrestrial class. The important differences in the amino-acid profile between fish and terrestrial can be translated into the important spectra differences observed around the band 2240-2300, associated with these functional group absorptions.

The results obtained when applying the Fisher Test (Figure 8.7) confirms the previous results and also revealed the importance of the region 1900-2000 nm. That is a traditional region for OH- absorption in water (near 1930 nm), but also for $CONH_2$ and $CONH_2R$ (near 1960-1970) (Osborne and Fearn, 1986).

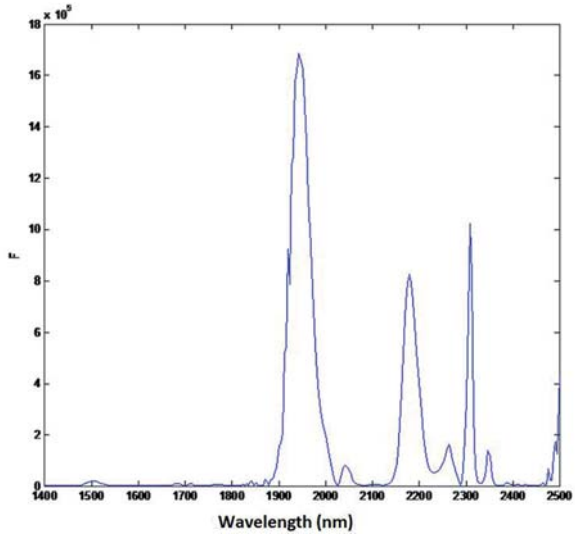


Figure 8.7 F values obtained for each wavelength for the fish vs. terrestrial data after SNV, detrend and first derivative.

8.4. TEST OF THE STABILITY OF THE SELECTED MARKERS AND CHEMICAL INTERPRETATION OF THE SELECTED NIR BANDS

As previously explained the study has revealed specific wavelengths, located in the Near Infrared Region of the electromagnetic spectrum, that are suitable for the discrimination of avian against mammalian and fish meal against terrestrial animal meals. The interpretation of the specific wavelengths together with mathematical data pre-treatments hinted at several major factors responsible for the separation as the unsaturated fatty acids. Based on these results, some experiments have been conducted to confirm and/or to support by traditional wet chemistry that finding,

on a set of animal meal samples. Due to the results obtained when using the Fisher test, which highlighted the importance of the OH associated to the water region as one of the basis of the discrimination, some experiments were also performed in order to evaluate the impact of the water content in the spectra.

8.4.1. Fat analysis

The analytical method applied for determining the fatty acid profile of MBM samples consists of (1) sample extraction (2) transesterification to transform the fat into fatty acid methyl esters and (3) determination of fatty acid profile by gas chromatography equipped to a flame ionisation detector (FID). Identification is made by comparison of the retention times of the test sample with those of the reference standards. A set of samples consisting of eight terrestrial animal meals from different species and eight different fishmeals were analysed to determine the fatty acid profile. The samples were extracted and analysed in duplicates. This study was supported by the spectroscopic analysis of the samples containing the fat and the defatted samples.

Important differences have been found when comparing the fatty acid profile of a fishmeal sample and a terrestrial animal meal. Fishmeals contain polyunsaturated fatty acid (i.e. C20:5, C22:5, C22:6) not present in terrestrial animal meals. On the other hand, terrestrial animal meals are richer in short and monounsaturated fatty acids (Figure 8.8).

This study has been complemented with the NIR microscopic analysis of the samples before and after being defatted. The spectra were collected by selecting visually individual particles up to 300 per sample. Some differences can be observed in the NIR band of 5550-6000 cm^{-1} between terrestrial meals and fishmeal with fat, and between terrestrial with and without fat and fish with and without fat. To support these results, chemometric analyses of the obtained spectra have been carried out. Principal Component Analysis (PCA) has been applied to the spectra obtained from the samples with and without fat. Fisher test has been applied to the data set to better understand which bands are the ones that better indicate the differences between the two classes, terrestrial and fish. The comparison between the untreated and the defatted samples indicated that the fat is not the sole factor for the differentiation of the animal classes.

8.4.2. Water content experiments

The spectroscopic marker needs to be stable and lead to reproducible spectra being independent of the effects of moisture. Therefore the effect on the NIR spectrum of moisture and water in the sample was studied. Even if moisture doesn't affect the marker directly, overlapping absorption bands coming from the water in the sample could cause spectral interferences.

To this end, an experimental scheme was devised to measure the NIR spectra of a range of different animal meal samples

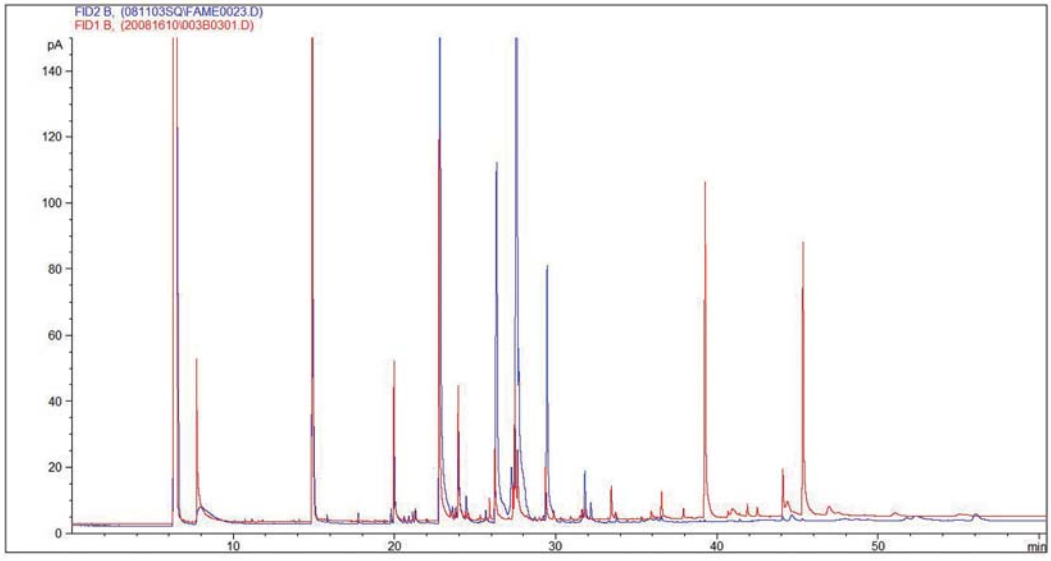


Figure 8.8 Typical GC-FID chromatograms obtained from a fishmeal sample (in red) and a terrestrial animal meal (in blue).

(Table 8.3). The samples were dried (± 2 g) and re-measured. Drying was carried out overnight at room temperature under vacuum (< 10 mbar). Oven heating the sample to dry it was avoided in order to be sure that the fatty acids in the sample would not be changed by the action of the heat. Vacuum drying was chosen as a gentle and non aggressive method. The initial water content was measured (in duplicate, n=2) by the Karl-Fisher method.

Table 8.3 Samples used in the study to evaluate the water impact on the NIRM spectra. The initial water content was determined by Karl-Fischer analysis (% H_2O).
NM = Not Measured.

Sample composition	Sample reference	% H_2O
Avian MBM	DQ / 06 / 1172	6.55
Avian MBM	DQ / 07 / 0099 – 01	4.44
Bovine MBM	DQ / 06 / 0959 - 17	NM
Bovine meat meal 137 ºC	DQ / 07 / 0134 – 03	4.41
Fish meal	DQ / 05 / 0653 – 10	8.97
Fish meal	DQ / 07 / 1089 – 14	6.94
Lamb meal	DQ / 06 / 0959 – 07	5.46
Ovine meat meal 141 ºC	DQ / 07 / 0134 – 06	1.79
Porcine MBM	DQ / 06 / 0959 – 13	4.69
Porcine protein	DQ / 07 / 0094 – 05	3.70

Chemometric analysis of the obtained spectra has been performed. Principal Component Analysis (PCA), Partial Least Squares Discriminant Analysis (PLS-DA) and the Fisher test have been applied before and after water extraction in order to study their influence demonstrating that the peak around 1960 nm revealed by the F-Test may be associated to the differences in protein and non-protein nitrogen compounds existing in the animal meals studied (ie. amines formed by decarboxylation of amino acids).

8.5. CONCLUSIONS AND PERSPECTIVES

Near-infrared microscopy is an objective, rapid, sensitive and highly-selective technique for detecting meat and bone meal in compound feed. The different studies performed during the last years have demonstrated the powerful characteristics of NIRM together with visual rules or/and chemometrics for the detection of meat and bone meal in both the whole and the sediment fractions of animal feedingstuff samples. Moreover, based on spectra obtained using the NIRM, relevant NIR bands or markers for the discrimination between animal protein by-product particles can be defined. These markers are mostly chemically supported by the existing differences in the content of fatty acids and maybe also due to differences to other nitrogen compounds existing as well as OH overtones present in the different types of APBs. Recently, von Holst et al (2008) reported the NIRM results of a transferability study between two independent laboratories in which decision rules based on the visual observation of the spectrum to discriminate the animal origin are applied. The success of this transfer between instruments will be crucial for further research activities to exploit the discrimination power of NIRM for the detection of traces of animal origin in animal feedingstuffs at higher taxonomic level. The potential of the NIR microscopy method for the quantification of MBM in feed should be considered also to support the eventual introduction of a tolerance level in the feed ban. However, the main limitation of NIRM is that it is slow because of the sequential collection of particle spectra. To overcome this problem, it has been suggested to work only on the sediment fraction, as in the optical microscopy method (reference method) (Baeten et al. 2005A). This approach boosts the method and reduces the problem of sampling with heterogeneous samples; however the detection is based only on the presence of bones in the sediment fraction.

Therefore, new alternatives to NIRM have been developed in the last years. One of the most encouraging methods is the technology called near infrared (NIR) hyperspectral imaging (Baeten and Dardenne 2005B, Fernández Pierna et al. 2009). This technology allows the spatial and spectral (and therefore chemical) information characterising the samples to be obtained at the same time and in minutes. Some studies have shown the combination of NIR imaging spectroscopy and some chemometric classification techniques could allow a regulatory laboratory to certify and quantify the presence of MBM in compound feed (Fernández Pierna et al. 2004). Recently, the NIR hyperspectral imaging has been validated for detecting PAPs in feeds in line with the International Standard ISO 17025. However, further research is needed before NIR imaging can be recommended as a routine method to be used for official laboratories to inspect with a high accuracy the huge variability of APBs and compound feeds currently put into circulation in the European and World markets.

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