Looking at mixtures with near infrared microscopy

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Introduction

Analytical methods that allow the identification/quantification of ingredients in animal feedstuffs are an essential part of an integrated food safety policy.[1] Optical microscopy is the official method approved by the EU for the detection of constituents of animal origin in feeds.[2] This has the disadvantages of being time-consuming and needing an expert microscopist.

Different authors have investigated the ability of macro near infrared (NIR) spectroscopy to obtain information about the composition and possible contamination of feedstuffs.[3,4] In recent years, NIR microscopy (NIRM) has emerged as an alternative to other methods (optical microscopy, PCR, etc). Its potential advantages include objectivity, sensitivity and high selectivity. It combines the analytical advantages of microscopy and spectroscopy techniques.[4,5] The application of NIRM is usually based on the collection of microspectra from individual particles contained in a sample.[3] When working with animal feeds, where there is substantial sample heterogeneity, several hundreds or thousands of particles must be analysed. The result of an analysis is a large collection of spectra of different particles from the sample, with each single spectrum being the molecular near infrared signature of one particle, the average spectrum becoming the fingerprint of the sample.[6,7] The practice of measuring individual particles is tedious to implement. An alternative is to measure small areas of sample. The question then arises as to how mixtures of ingredients behave when measured in this way. Will an individual measured spectra correspond to just one of the ingredients in the mixture? Is quantification of the proportions possible?

The objective of this study has been to explore these questions, and investigate chemometric strategies for predicting the proportions in the mixture.

Materials and methods

Samples

A sample set was produced by physically mixing pure samples in the laboratory. Four pure samples, one each of barley, maize, soya, and meat and bone meal (MBM), were used to make three 50/50 mixtures, each being of MBM and one of the others. The samples were ground to

a particle size of 1 mm prior to mixing. The seven samples—four "pure" ingredients and three mixtures - were each measured by NIRM as described below.

NIRM analysis

An Auto Image Microscope connected to a Perkin-Elmer Spectrum One FT-NIR spectrometer was used. The sample was spread on a sample holder as a continuous film, an area in the centre of the sample was selected and focused on. For each sub-sample (one for the pure samples, 10 for the mixtures) presented to the instrument, 234 spectra, each being the average of 70 scans, were measured using fields of view of size 50 µm × 50 µm arranged in a 13 × 18 grid. This method avoids any subjective selection of individual particles, while still representing the inherent variability in the sample. As in previous works in NIRM,[3] spectra were obtained as the ratio between raw spectra and a Spectralon reference, and the spectral information was stored as log (1/*R*), recorded at 4 nm intervals over the range 1112–2500 nm, after conversion from cm^{-1} using Spectrum v. 5.01 software.

Data treatment

The log (1/*R*) spectra were transformed to first derivative, trimmed to a range of 1500–2448 nm and scatter-corrected by SNV. A range of exploratory data analyses was carried out in MATLAB,

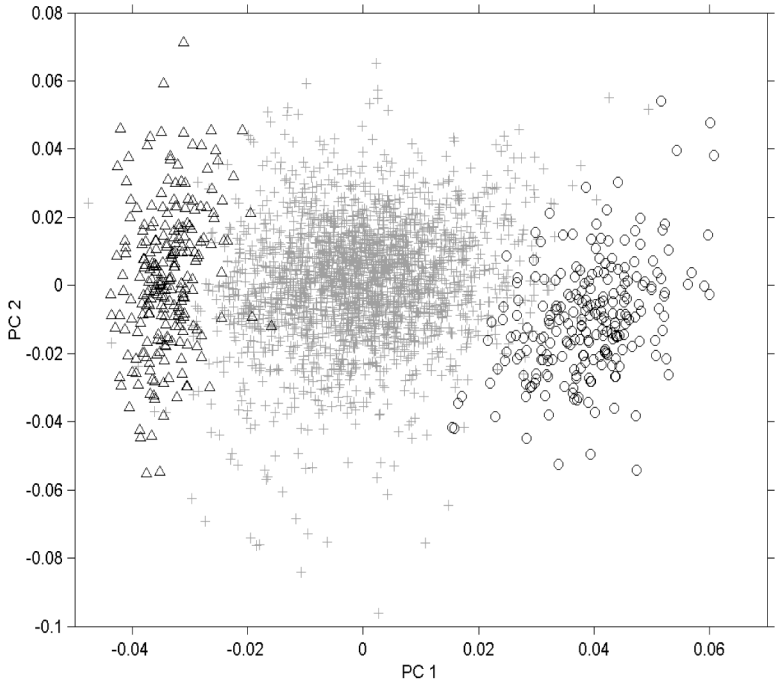

Figure 1. PC score plot of spectra from pure barley (Δ), pure MBM (o) and a 50/50 mixture (+).

mostly looking at one mixture at a time, but also investigating them jointly. To obtain the results shown here for the barley-MBM mixture, the spectral range was cut to 1640–1800 nm, a principal component (PC) analysis was carried out on the 468 pooled spectra for the two pure samples, and the first five PCs were input to a canonical variate analysis. The spectra from the barley-MBM mixture were then projected onto the resulting PCs and the canonical variate (CV) score calculated for each spectrum. The scores on this *CV* were used to identify representative mixture spectra for the construction of Figure 2.

Results and discussion

The results for the barley-MBM mixture are typical and only these will be reported. Figure 1 shows the spectra for the two pure samples and the mixture plotted in the space of the first two PCs derived from the pure samples.

The mixture almost fills the space between the pure samples, suggesting that the spectra collected from the very small areas correspond to mixtures with widely varying proportions. Figure 2 shows three mixture spectra, together with all of the spectra from the pure samples.

Spectra similar to the intermediate one of the three are the most common; the two that resemble pure ingredients are quite rare. Plotting all of the spectra fills the gap between the two pure sets, just as it does in the PC-space. The impression that the cloud of grey points in Figure 1 is roughly symmetric and centered on a point roughly half-way between the two sets of pure spectra

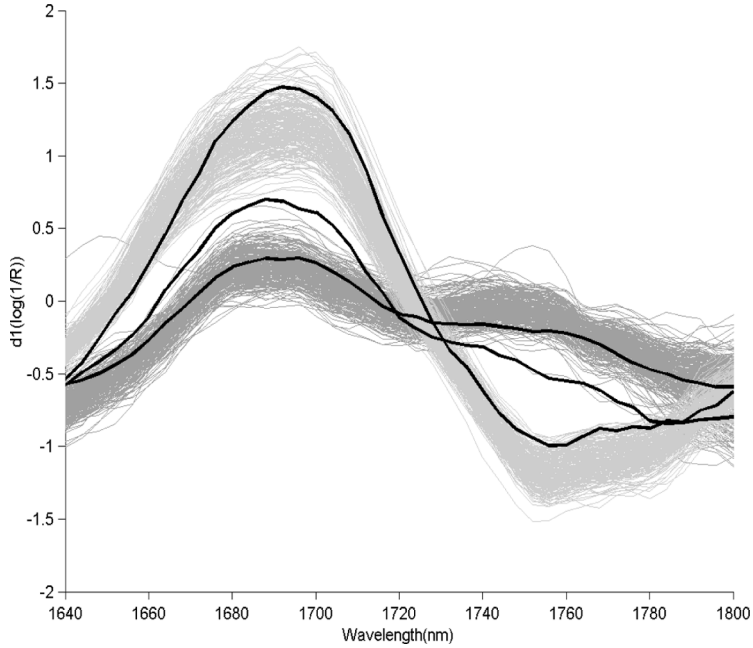

Figure 2. Spectra of pure barley (dark grey), pure MBM (light grey) and three spectra from the 50/50 mixture (black).

was confirmed by projecting the PC scores onto the canonical variate and examining a histogram of the resulting distribution. If the canonical variate scores were used to predict the proportion of meat and bone meal in the area represented by each individual small-area spectrum, the resulting proportions would range from 0% to 100% MBM, with a fairly symmetric distribution and an average close to the correct 50%.

Conclusions

The conclusion from these two plots, and from the distribution of the *CV* scores for the mixture spectra, is that even on this scale the spectrometer is seeing a mixture in most of the areas sampled. This has implications for any attempt to use this type of sample presentation to detect contamination with MBM: it is probably not good enough just to look for pure MBM spectra and the chemometrics will need to be more sophisticated. An encouraging finding is that the barley-MBM system appears to be roughly linear in the 1640–1800 nm region, so that a quantitative calibration would be feasible. Much work remains to be done to extend this system to mixtures of larger numbers of ingredients, and hence solve the general problem.

Acknowledgements

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