

Usefulness of near infrared reflectance (NIR) spectroscopy and chemometrics to discriminate between fishmeal, meat meal and soya meal samples

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Abstract

D. Cozzolino, E. Restaino, A. La Manna, E. Fernandez, and A. Fassio. 2009. Usefulness of near infrared reflectance (NIR) spectroscopy and chemometrics to discriminate between fishmeal, meat meal and soya meal samples. Cien. Inv. Agr. 36(2):209-214. Near infrared reflectance (NIR) spectroscopy was used in combination with chemometrics to discriminate between fishmeal, meat meal and soya meal samples. Samples were obtained from commercial feed mills and scanned in the NIR region (1100 - 2500 nm) in a monochromator instrument in reflectance mode. Principal component analysis (PCA) and linear discriminant analysis were used to classify samples based on their NIR spectra. Full cross-validation was used in the development of classification models. Partial least squares-discriminant analysis (PLS-DA) correctly classified 85.7% of the fishmeal samples and 100% of the meat meal and soya meal samples. These results demonstrate the usefulness of NIR spectra combined with chemometrics as an objective and rapid method to classify fishmeal, meat meal and soya meal samples. NIR spectroscopic methods can be easily implemented in food mills and may be most useful for initial screening at early stages in the food production chain, enabling more costly methods to be used selectively for suspected specimens.

Key words: Fishmeal, *Glycine max*, meat meal, near infrared, principal component analysis, spectroscopy, soya meal.

Introduction

Quantitative determination of moisture, protein, fat (oil) and other minor chemical components accounts for the majority of applications of near infrared (NIR) spectroscopy in routine food and foodstuff analysis (Osborne *et al.*, 1993; Aufrere *et al.*, 1996; Fontaine *et al.*, 2001; Cozzolino and Murray, 2004; Perez-Marín

et al., 2004). However, the raw materials used in the manufacturing of compound feeds are variable both in composition and in nutritional quality due to multiple factors such as changes in temperature, infection with molds, fraud, or adulteration (Moya *et al.*, 1994). The practical and economic repercussions of this variability are very important in the compound feed manufacturing industry, where a uniform product with both consistent composition and quality must be produced from inherently variable raw materials and by-products (Moya *et al.*, 1994; Cozzolino and Murray, 2004).

In an industrial setting, either qualitative or quantitative analytical control is essential in order to assess raw materials, products and by-products and to optimize the manufacturing process itself (Blanco and Villaroya, 2002). In addition to chemical composition, important aspects of this assessment might relate to the process history of a product (e.g., fresh meat as opposed to frozen meat) or its geographic origin (e.g., Italian olive oil must be produced with olives grown only in Italy) (Downey, 1996; Arhurst and Dennis, 1996). Foods or raw ingredients that are most likely to be targets of adulteration include those that are of high value or that are subject to the effects of environmental conditions (e.g., rain and sun exposure) during their growth or harvesting (Murray *et al.*, 2001; Gizzi *et al.*, 2003). The practice of adulteration occurs for two main reasons: firstly, it can be profitable, and secondly, some adulterants can be easily mixed and are subsequently difficult to detect (Murray *et al.*, 2001). In such cases, visual examination of the NIR spectra cannot discriminate between authentic and adulterated product (Downey, 1996). However, the application of multivariate data analysis techniques like principal component analysis (PCA) and discriminant analysis (e.g., discriminant partial least squares, linear discriminant analysis) might make it possible to unravel and interpret the optical properties of the sample, permitting classification without the use of chemical information (Downey, 1994 and 1996; Cordella *et al.*, 2002).

The objective of this study was to explore the usefulness of near infrared reflectance (NIR) spectroscopy combined with chemometrics to discriminate between fishmeal, meat meal and soya meal samples in the feed mill industry.

Materials and methods

Samples

Seven samples of commercial fishmeal, ten samples of meat meal and fifteen samples of soya meal were collected from different industrial manufacturing plants. The samples were consid-

ered to be authentic as defined by the suppliers. Samples were oven-dried at 100°C for 24 h and ground in a Wiley forage mill to pass a 1-mm screen (Arthur H. Thomas, Philadelphia, PA, USA) prior to NIR analysis.

Near infrared reflectance (NIR) analysis

Samples were analyzed in a NIRSystems 6500 scanning spectrophotometer (NIRSystems, Silver Spring, MD, USA) in reflectance mode (1100 – 2500 nm), using the sample transport module in a 1/4 rectangular cup, 60 mm x 120 mm, (NIRSystems part number 0IH – 0379, NIRSystems, MD, USA). Reflectance data were stored as $\log(1/R)$ (R = reflectance) at 2-nm intervals (700 data points). Each sample was scanned once (no repeated spectral measurements were made) and was not rotated during spectrum collection. Computer operation and spectral data collection were performed using ISI version 3.1 software (InfraSoft International, Port Matilda, PA, USA).

Multivariate data analysis

Spectra were exported from the ISI software in ASCII format into the Unscrambler software (version 7.5, CAMO ASA, Norway) for chemometric analysis. Principal component analysis (PCA) of the spectra was performed using raw data and after pre-processing using the second derivative to reduce baseline variation and enhance the spectral features (Naes *et al.*, 2002). Discrimination models were developed using the partial least squares discriminant (PLS-DA) regression technique (Otto, 1999; Naes *et al.*, 2002). In this technique, each sample in the calibration set is assigned a dummy variable as a reference value. The discrimination model is then developed by regression of the spectral data against the assigned reference value (dummy variable). The PLS-DA models were developed using a non-metric dummy variable (set to 1 = fishmeal; 2 = meat meal and 3 = soya meal). Samples were classified according to meal type based on a cut-off value of 0.5.

Full cross-validation (CV) (using the leave-one-out procedure) was used in development of the PCA and PLS-DA models (Naes *et al.*, 2002). To evaluate the accuracy of the models thus generated and their ability to classify samples, we calculated the coefficient of regression (R) and the root mean square of the standard error in cross-validation.

Results and discussion

Figure 1 shows the mean NIR spectra of fishmeal, meat meal and soya meal after standard normal variate and de-trend (SNVD) transformation. The mean NIR spectra of fishmeal and meat meal samples were very similar. Absorption bands in the NIR region were observed at around 1200 nm, 1480 nm (O-H stretch second

overtone, associated with water), 1700 nm (C-H first overtone, associated with lipids and oil), and between 1900 nm (O-H stretch second overtone) and 2300 nm (C-H combination tones, associated with water, amino acids and fatty acids) (Murray, 1986). The 2300 nm band acts as a reference point whose wavelength does not change across the spectra of a set of similar foods (Murray, 1986). Bands at 2058 nm and 2174 nm are related to the peptide absorption of the amide group. The mean NIR spectrum of the soya meal samples showed similar absorption bands to those described for fishmeal and meat meal. However, the absorption band at 1700 nm is not present in soya meal. The standard deviation of the NIR spectra (not shown) showed that wavelengths associated with water (variable moisture content) and oil are the most important in explaining the variation among the samples.

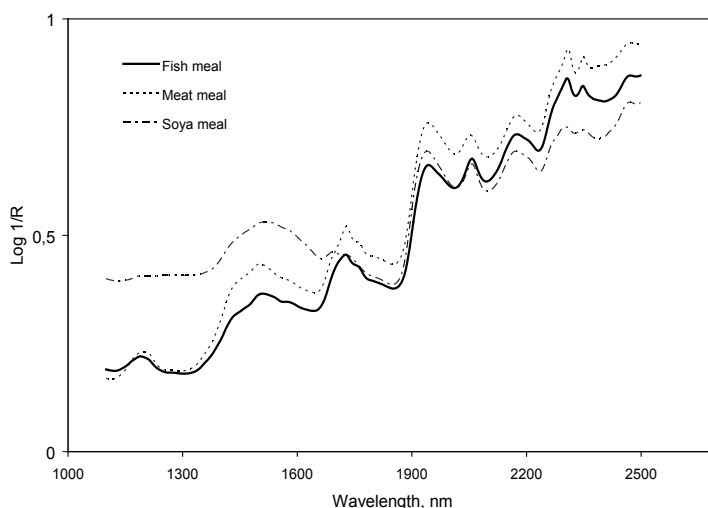


Figure 1. Mean near-infrared spectra of fish meal, meat meal and soya meal samples.

PCA was performed on the raw spectra to examine qualitative differences between the three types of meal samples analyzed. Figure 2 shows the score plot of the first two principal components (PCs) derived from the raw NIR spectra. A separation is found between groups of samples of different origin. The eigenvectors were analyzed in order to explain the differences in the PCA plot. Figure 3 shows the eigenvectors

for the first three PCs. PC1 explains 86% of the total variance among the samples, and the largest eigenvector magnitudes were found at around 1600 nm (C-H first overtone), at 1900 nm (O-H stretch first overtone, associated with water content), and between 2100 and 2300 nm (C-H combination tones, associated with either unsaturated fatty acids or aromatic amino acids) (Murray, 1986; Miller, 2001). PC2 explains

12% of the variation, and the largest eigenvector magnitudes were found at around 1450 nm and 1920 nm, both related to O-H overtones associated with water content (Murray, 1986).

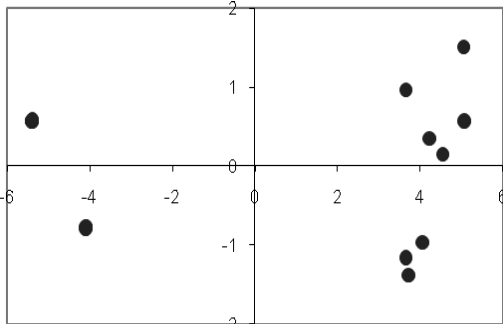


Figure 2. Score plot of the first two principal components of the NIR spectra of fishmeal, meat meal and soya meal samples.

PCA analysis (not shown). PLS-DA correctly classified 85.7% of the fishmeal samples and 100% of the meat meal and soya meal samples. The fishmeal samples that were incorrectly classified had very low crude protein content, similar to the meat meal samples. The ability of the NIR-based model to classify the meal samples is based on the vibrational responses of chemical bonds in the near infrared region (O-H, N-H and C-H). It is probable that greater variability between the sample types in those chemical entities (e.g., protein or oil content) that respond in these regions of the spectrum leads to better accuracy of the model. This explains the strong separation obtained between samples of animal origin (fishmeal and meat meal) versus samples of plant origin. Our results also suggest that other characteristics (e.g., amino acids and fatty acids) may explain the discrimination among samples.

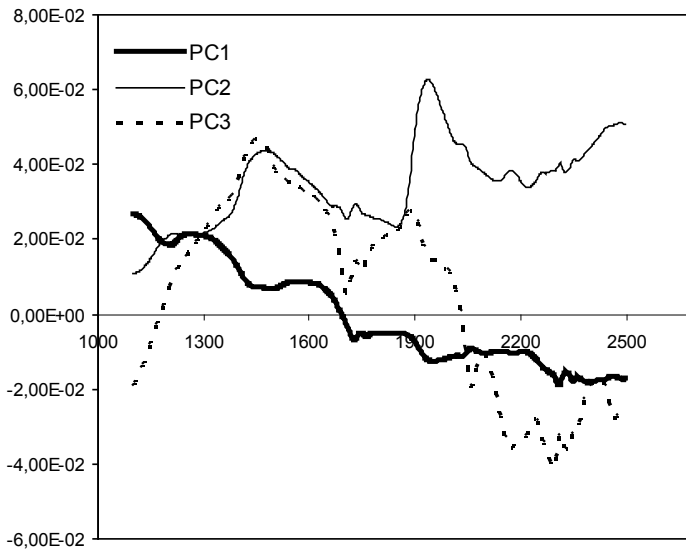


Figure 3. Eigenvectors of the first three principal components of the NIR spectra of fishmeal, meat meal and soya meal samples.

Table 1 shows the calibration statistics and classification results from PLS-DA regression models developed using the raw spectra. These PLS-DA models accounted for 96% and 92% of the variability in calibration and validation, respectively. PLS loadings of the calibration models for discrimination between sample types were similar to the eigenvectors described for the

As suggested by other authors, NIR spectroscopy might be used as a first line of defense against accidental contamination (e.g., cross-contamination) and fraudulent practices or for screening of the raw material (Murray *et al.*, 2001; Gizzi *et al.*, 2003). NIR spectroscopic methods can be easily implemented in food mills. These methods may be most useful for initial screening at early stag-

Table 1. Discriminant partial least squares calibration statistics, validation statistics and classification results of fishmeal, meat meal and soya meal samples using near infrared reflectance spectroscopy.

	R ¹	RMSECV ¹	PLS t ¹	Slope ¹
Calibration (n = 16)	0.96	0.13	6	
Validation (n = 16)	0.92	0.20		0.88
		Correct classification, %		
		FM ¹ (n = 7)	MM ¹ (n = 10)	SM ¹ (n = 15)
Classification	FM	85.7	14.3	0.0
	MM	0.0	100.0	0.0
	SM	0.0	0.0	100.0

¹FM: fishmeal; MM: meat meal; SM: soya meal; R: coefficient of regression; RMSECV: root mean square of the standard error in cross-validation; PLS t: number of terms used to develop the calibration models; n: number of samples.

es in the food production chain, enabling more costly methods to be used selectively for further examination of doubtful specimens. To be able to control and optimize the processing of fishmeal, it is important to measure and analyze the chemical composition of the raw material. Additional qualitative analysis may be possible using NIR spectroscopy and should be incorporated into routine NIR analyses in the feed industry.

Although the work reported here constitutes a preliminary study and requires further develop-

ment, we have shown that more than 85% of the samples were classified correctly into three groups according to sample type by NIR spectroscopy. These results suggest that feed mills can use NIR spectroscopy for the identification and authentication of the products that they produce.

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Resumen

D. Cozzolino, E. Restaino, A. La Manna, E. Fernandez y A. Fassio. 2009. Uso de la espectrofotometría de reflectancia en el infrarrojo cercano (NIR) y la quimiometría para discriminar entre muestras de harinas de pescado, carne y soja. Cien. Inv. Agr. 36(2):209-214. El objetivo de este trabajo fue investigar el uso de la espectrofotometría de reflectancia en el infrarrojo cercano (NIR) en combinación con la quimiometría para discriminar muestras de harinas de pescado, carne y soja. Muestras provenientes de molinos racioneros comerciales fueron leídas en un equipo monocromador NIRS (NIRSystems, Silver Spring, USA) en el rango de longitudes de onda de 400 a 2500 nm, en reflectancia. Análisis de componentes principales (APC) y de discriminantes utilizando la técnica de los cuadrados mínimos parciales (PLS-DA) fueron usados para clasificar las muestras de acuerdo a su origen. El método de la validación cruzada fue utilizado para validar los modelos. El 85,7% de las muestras de harina de pescado y el 100 % de las muestras de carne y soja fueron correctamente clasificados usando el método PLS-DA. Los resultados obtenidos en este estudio demuestran el potencial uso de la reflectancia en el infrarrojo cercano combinada con la quimiometría como un método rápido y de bajo costo para clasificar muestras de harina de pescado, carne y soja.

Palabras clave: Análisis de componentes principales, espectrofotometría de reflectancia, *Glycine max*, infrarrojo cercano, harina de pescado, harina de soja.

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