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Usefulness of Near-Infrared Reflectance (NIR) Spectroscopy and Chemometrics To Discriminate Fishmeal Batches Made with Different Fish Species

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Near-infrared reflectance (NIR) spectroscopy combined with chemometrics was used to identify and authenticate fishmeal batches made with different fish species. Samples from a commercial fishmeal factory ($n = 60$) were scanned in the NIR region (1100−2500 nm) in a monochromator instrument in reflectance. Principal component analysis (PCA), dummy partial least-squares regression (DPLS), and linear discriminant analysis (LDA) based on PCA scores were used to identify the origin of fishmeal produced using different fish species. Cross-validation was used as validation method when classification models were developed. DPLS correctly classified 80 and 82% of the fishmeal samples. LDA calibration models correctly classified >80% of fishmeal samples according to fish species The results demonstrated the usefulness of NIR spectra combined with chemometrics as an objective and rapid method for the authentication and identification of fish species used to manufacture the fishmeal.

KEYWORDS: NIR spectroscopy; fishmeal; identification; fish byproducts; principal component analysis; partial least squares; linear discriminant analysis

INTRODUCTION

The lack of simple, reliable, and nondestructive methods for the determination of composition in both fish and fish byproducts has been one of the main obstacles for the development of quality control in the fish industry (*1*, *2*). It is well-known that the use of rapid methods to measure the composition of foods and agricultural commodities increases efficiency and decreases the costs of quality control protocols by allowing management to use the analytical data in decision-making (*3*). Quantitative determination of moisture, protein, and fat (oil) accounts for the majority of applications of near-infrared reflectance (NIR) spectroscopy in routine food and foodstuff analysis (*3*−*8*). However, the raw materials used in manufacturing of compound feeds are variable both in composition and in nutritional quality, due to multiple factors (e.g., temperature, molds, fraud, adulteration) (*5*, *9*, *11*, *12*). The practical and economic repercussions of this variability are very important in the feed compound manufacturing industry, where a uniform product of both consistent composition and quality is to be produced from inherently variable raw materials and byproducts (*5*, *9*, *11*, *12*). In an industrial environment, analytical control either qualitative or quantitative is essential in order to assess raw materials, products, and byproducts as well as to optimize the manufacturing process itself (*10*). Conventional methods involve time-consuming, laborious, and costly procedures, including dissection, chemical analysis, microscopy analysis, and DNA testing (*11*, *13*, *16*). Chemical composition is not the only aspect that describes food quality. In addition to conventional methods, the process history of a product (e.g., fresh meat opposed to frozen meat) and its geographic origin (e.g., as Italian olive oil produced only with olives grown in Italy) are examples of factors that might be related to or affect the quality of raw material and foods (*11*, *12*, *14*, *15*). Foods or raw ingredients that are most likely to be targets for adulteration include those which are of high value or are subject to the effects of environmental conditions (e.g., rain, sun exposure) during their growth or harvesting (*11*, *16*). The practice of adulteration arises for two main reasons: first, it can be profitable; and, second, adulterants can be easily mixed and are subsequently difficult to detect (*9*, *13*, *16*). To counter this problem, manufacturers subject their raw material and byproducts to a series of quality controls, which include high-performance liquid chromatography (HPLC), thin-layer chromatography (TLC), enzymatic tests, and physical tests, to establish their authenticity and hence guarantee the quality

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of the products manufactured to guarantee their safety for the consumers (*11*, *12*, *14*−*16*). Attributes include bioavailability, provenance, organoleptic scores, or classification of materials for authentication. It is well-known that visual examination of the NIR spectra cannot discriminate between authentic and adulterated product (*17*). Therefore, the application of multivariate data analysis techniques such as principal component analysis (PCA) or discriminant analysis [e.g., discriminant partial least squares (DPLS)] offers the possibility of unraveling and interpreting the optical properties of the sample and allowing a classification without the use of chemical information (*17*, *18*). As reported by other authors, there is a decline in the total landings from marine fisheries, which implies an increasing demand to utilize more fish for human consumption (*19*). It is well-known that ~50−60% of the world catch is used for human consumption, whereas the most important byproducts processed are fishmeal and fish oil (*19*, *20*). Therefore, there is an increasing demand from the industry for rapid techniques to ensure the origin of the raw material used and the foods produced.

The objective of this study was to explore the usefulness of NIR spectroscopy as a rapid tool to identify the species of fish used to make fishmeal under industrial conditions.

MATERIALS AND METHODS

Samples. Fishmeal samples ($n = 60$) from an industrial manufacturing plant (UFP, Tullos, Aberdeen, Scotland, U.K.) were collected from October 1996 to August 1997. They contained different fish species such as mackerel (*Scomber scombrus*), herring (*Clupea harengus*), salmon (*Salmon salar*), and blue whiting (*Micromesistius poutassau*) fish species. Most of this material is white fishmeal produced as a byproduct of filleting fish for human consumption (*20*).

Chemical Analysis. Samples were analyzed by conventional wet chemistry as follows. Moisture content was determined by oven-drying the sample at 105 °C for 4 h, and oil was extracted by Soxhlet using petroleum ether (bp 40−60 °C) (*21*). Total volatile nitrogen (TVN) was measured by alkaline distillation and titration using 0.1 M NaOH until the indicator turns from purple to green (*21*). Temperature was measured in the fresh material using a digital thermocouple. All determinations were done in duplicate, and chemical composition was expressed as grams per kilogram on a dry weight basis. Details of the chemical analysis were reported elsewhere (*20*).

NIR Analysis. The spectra of fishmeal samples were collected using a NIR scanning spectrophotometer NIRSystems 5000 (NIRSystems, Silver Spring, MD) in reflectance mode (1100−2500 nm). Fishmeal samples were placed in the sample transport module in a rectangular (1/4) quartz cup (NIRSystems part 0IH-0379, NIRSystems). Fishmeal samples were scanned immediately after they were made to avoid losses of both nitrogen and moisture that normally occur during storage. Reflectance data were stored as $\log(1/R)$ (R = reflectance) at 2 nm intervals (701 spectral data points). Samples were scanned once (no repeated spectral measurements were made) and were not rotated when spectra collection was made. Two pairs of lead sulfide detectors collected the reflectance spectra. Reflectance energy readings were referenced to corresponding readings from an internal ceramic disk provided by the instrument manufacturer. The spectrum of each sample was the average of 32 successive scans. Computer operation and spectral data collection were manipulated using ISI version 3.1 software (InfraSoft International, Port Matilda, PA).

Multivariate Analysis. Spectra were exported from the ISIsoftware in ASCII format into The Unscrambler software (version 7.5, Camo ASA, Oslo, Norway) for chemometric analysis. Principal component analysis (PCA) of the spectra was performed using raw and, after preprocessing, the second derivative to reduce baseline variation and enhance the spectral features (*17*, *18*). Second derivative was performed using Savitzky−Golay derivation and smoothing (10 point and 2nd-order filtering operation). PCA was used to derive the first 10 principal components from the spectral data and was used to examine the possible grouping of samples (*17*, *18*). Discrimination models were developed using the dummy regression technique described elsewhere (*21*−*24*). 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).

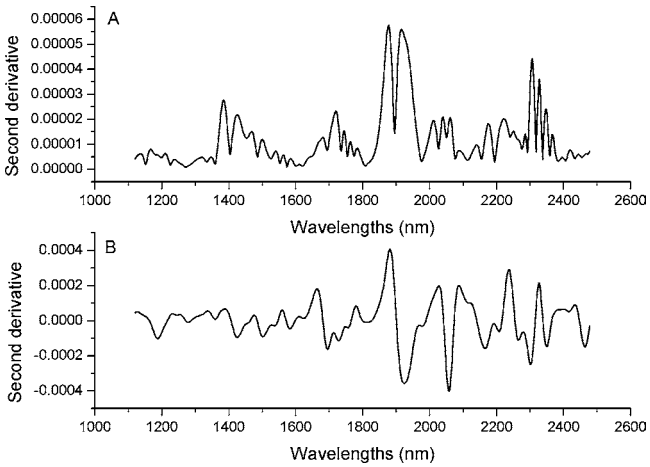

Figure 1. Second derivative of the near-infrared mean spectrum of fishmeal samples: (**A**) standard deviation; (**B**) mean spectrum (all samples).

The DPLS models were developed using a nonmetric dummy variable (set to 1 = blue whiting, 2 = others, and 3 = salmon). The classification of the fishmeal samples according to fish species was on the basis of the 0.5 cutoff value. Linear discriminant analysis (LDA) like DPLS is a supervised classification technique in which the number of categories and the sample that belong to each category are previously defined (*25*, *26*). The method produces a number of orthogonal linear discriminant functions, equal to the number of categories minus one, that allow the samples to be classified in one or another category (*25*). LDA was carried out using JMP software (version 5.01, SAS Institute Inc., Cary, NC) on the PCA sample scores on components 1−3, which gave the highest level of separation in the PCA models developed. Cross-validation (CV) was used when LDA models were developed (*25*). Both PCA and DPLS models were developed using full internal CV. Full CV estimates the prediction error by splitting the calibration samples into groups (four in this case). One group was reserved for validation, and the remaining groups were used for calibration (leave-one-out) (*17*, *18*, *26*). The process was repeated until all groups had been used for validation once. Statistics calculated included the standard error of calibration (SEC), the coefficient of determination in calibration (R^2_{cal}), and the standard error in cross-validation (SECV).

RESULTS AND DISCUSSION

Figure 1A shows the standard deviation of the second derivative of the NIR mean spectrum. The second derivative of the mean spectrum (**Figure 1B**) allows identification of smaller absorption bands. The second derivative of fishmeal samples presents absorption bands in the NIR region at 1200, 1460, and 1936 nm related to O−H stretch first overtone and O−H stretch second overtone, related with water content (*6*, *20*, *27*, *28*). Absorption bands around 1500 and 1800 nm are related with N−H stretch first overtone, respectively (*27*, *28*). The absorption band at 1726 nm is related with C−H stretch first overtone bands associated with oil content. This band acts as a reference point; its wavelength does not change across the spectra of a set of similar foods (*27*, *28*). Bands at 2058 and 2174 nm are related to the peptide absorption of the amide group and had high correlation either with protein or with TVN content in the fishmeal samples (*20*−*27*). The SD (**Figure 1A**) showed that water (variable moisture content), oil, compounds containing nitrogen, and proteins are the most important chemical param-

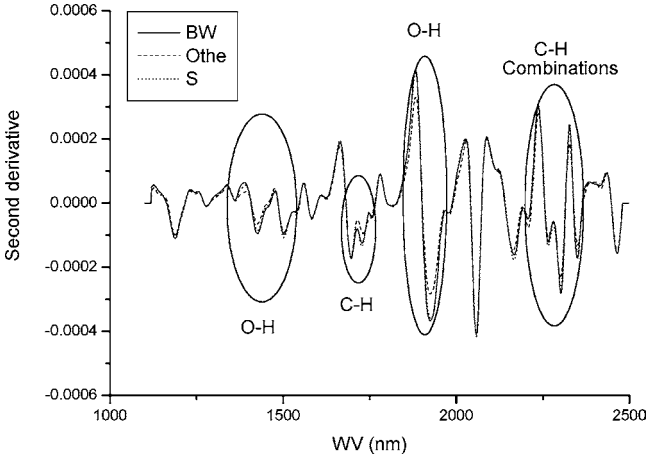

Figure 2. Second derivative of the near-infrared mean spectrum of fishmeal samples made with blue whiting (BW), salmon (S), and other fish species.

eters explaining the variation in the fishmeal samples. The variation in oil content is mainly due to the different fish species and seasonality. **Figure 2** shows the second derivative of the mean spectrum of fishmeal made with blue whiting, salmon, and other fish species. As described before, water, oil, and protein are the chemical parameters that explain the differences among the fishmeal samples analyzed.

A PCA was performed on the spectra (raw and second derivative, 1100 to 2500 nm) to examine qualitative differences between fishmeal samples related with the different sources of fish. **Figure 3** shows the scores for the first two principal components (PC) derived from the raw NIR spectra of fishmeal samples. A separation between the samples related with the different fish species was observed. A clear separation among fishmeal samples made with salmon and the other two fish species (blue whiting and others) was observed along PC1. Both blue whiting and other fish species were different from salmon, mainly with regard to protein and oil content. Samples labeled as others also included some batches of salmon as well as blue whiting, mackerel, and herring fish species. Therefore, this result suggests that discrimination between fishmeal samples is possible and that different spectral attributes of the fishmeal batches are associated with characteristics of the fish used. To investigate the basis for the observed separation between the fishmeal batches, the PCA eigenvectors were analyzed. **Figure 4** shows the eigenvectors for the first three PCs used to separate fishmeal samples according to fish species The first three PCs account for 99% of the spectral variation in the fishmeal samples analyzed. PC1 explains 92% of the total variance in the samples, and the highest eigenvectors were found around 1440 nm (O−H first overtone) and at 1926 nm (O−H stretch first overtone) associated with water content. PC2 explains 6% of the variation, and the highest eigenvectors were found around 1450 and 1920 nm, both related to O−H overtones associated with water content (*20*, *27*), around 1726 nm (stretch C−H first overtone), associated with oil content, and around 2306 and 2346 nm, associated with either unsaturated lipids or compounds containing aromatic groups such as amino acids (e.g., tyrosine and tryptophan) (*6*, *20*, *27*, *28*). Fishmeal samples made with salmon are clustered along PC2. Those samples showed great variability in both moisture and oil contents. PC3 explains 1% of the variation and seems to be the mirror image of the spectra of the fishmeal samples. PC3 probably would account for variations in particle size among the fishmeal samples analyzed. Classification based on spectral data is not a trivial task; a single data set can reveal several distinct groups or geometrical exploration based on scores plots (*29*). However, the clusters related with the different fishmeal samples indicated that the NIR spectra contain information related with the species of fish used to elaborate the product.

Table 1 shows the descriptive statistics for the chemical composition in the fishmeal samples analyzed. Differences were observed in protein and oil content among the fishmeal samples analyzed due to the different species. Similar results were reported by other authors (*30*). **Table 2** shows calibration statistics from DPLS regression models developed on either the raw spectra or after second derivative. The results indicate that DPLS models developed accounted for 88−94% of the variability in the fishmeal samples made with different fish species. Loadings of the DPLS calibration models for the discrimination between fishmeal samples were similar to the eigenvectors described for the PCA analysis (not shown). **Table 3** shows the LDA classification models for the fishmeal samples. Similar to DPLS, >80% of the samples were classified correctly. It is not surprising that the fishmeal made with other fish species

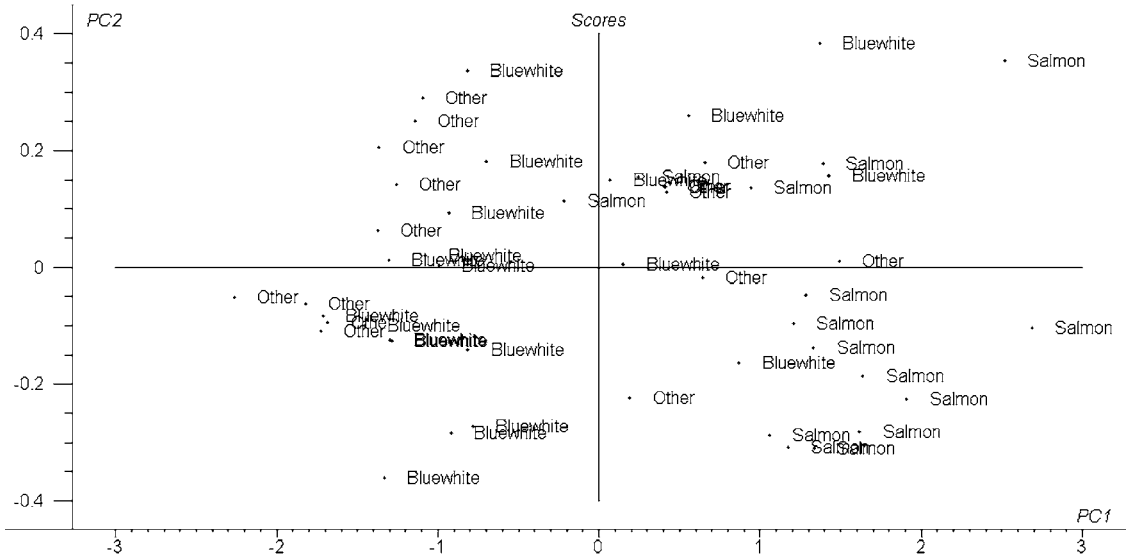

Figure 3. Score plots of the first two principal components indicating the separation among fishmeal samples (PC1, 92%; PC2, 6%) (raw spectra, 1100–2500 nm).

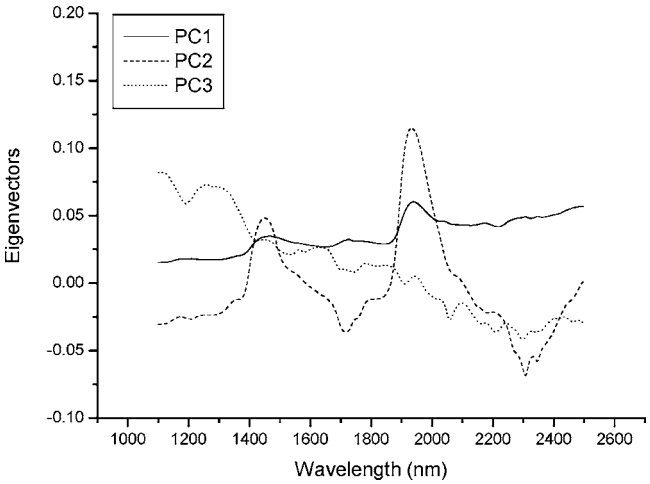

Figure 4. Eigenvectors for the first three-principal components of fishmeal samples (raw spectra, 1100–2500 nm).

Table 1. Descriptive Statistics of the Chemical Composition in the Fishmeal Samples[a]

	blue whiting ($n = 20$)	other ($n = 20$)	salmon ($n = 20$)
crude protein, g kg^{-1}	68.5a	67.9a	65.9b
oil, g kg^{-1}	65.0a	69.1a	83.4b
moisture, g kg^{-1}	7.5a	7.45a	8.3a
total volatile N, mg g^{-1}	143.5a	143.9a	143.8a

[a] Different letters in a row indicate statistical differences ($p < 0.05$).

Table 2. Dummy PLS Regression Statistics Using Raw and Second-Derivative Spectra (1100–2500 nm)[a]

spectra	R^2_{cal}	SECV	T	% correctly classified
raw	0.88	0.39	10	80
second derivative	0.94	0.28	8	82

[a] R^2_{cal}, coefficient of determination in calibration; SECV, standard error in cross-validation; *T*, number of PLS terms used to develop the calibration models.

Table 3. LDA Classification of Fishmeal Samples According to Fish Species (Actual Value in Raw, Predicted in Column)

	blue whiting	other	salmon	% classification
blue whiting	16	4	0	80
other	4	14	2	70
salmon	0	2	18	90
total	20	20	20	

did not achieve good classification. Taking into account these results, we may consider the classification models to be sufficiently feasible and robust for the purposes of classifying fishmeal batches according to their raw material. The ability of the NIR model to classify the fishmeal 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 the higher the variability between fish species in those chemical entities (e.g., water, protein, or oil content), which respond in these regions of the spectrum, the better the accuracy of the model. The results also suggested that other characteristics (e.g., amino acids, amines, or other nitrogen compounds) in either the raw fish or its byproduct would explain the discrimination among samples. The NIR technique has a traditional and unfair reputation as a black box method (*31*); however, this study demonstrates that a rapid screening of fishmeal was achieved using the composite chemical and physical optical information recorded in the NIR spectrum of the fishmeal samples. As suggested by other authors, NIR spectroscopy might be used as a first line of defense against accidental contamination (e.g., cross-contamination) or fraudulent practices or in screening of the raw material (*13*, *16*). Nevertheless, NIR spectroscopic methods might provide initial screening in the food chain and enable more costly methods to be used more productively on suspect specimens and will be easily implemented in feed mills. Raw fish is filleted, skinned, and trimmed to produce fillets, and these byproducts are used to make the fishmeal by the industry. To be able to control and optimize the processing of the fishmeal, it is important to measure and analyze the chemical composition of the raw material. It would be valuable to determine key parameters such as the chemical composition (moisture, oil, and protein) and other parameters such as TVN or salt content related with both storage and conservation of the material before entry into the fishmeal factory (*18*). Additionally, qualitative analysis might be possible using NIR spectroscopy and will be incorporated in routine NIR analysis in the feed industry.

The identification of the fish species used to make the fishmeal was possible using NIR spectroscopy and chemometrics. More than 80% of the samples were classified correctly into the three groups assigned according to the fish species. These results also suggested that NIR spectroscopy might be used by the feed mills for the identification and authentication of the product produced. The work reported here constitutes a preliminary study and requires further development. Further studies are needed to improve the calibration specificity, accuracy, and robustness and to extend the discrimination to other fish species.

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