Use of Fourier Transform Near-Infrared Reflectance Spectroscopy for Rapid Quantification of Castor Bean Meal in a Selection of Flour-Based Products

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Methodology was developed and evaluated for the rapid detection of castor bean meal (CBM) containing the toxic protein ricin by using Fourier transform near-infrared (FT-NIR) spectroscopy and multivariate techniques. The method is intended to be a prototype to develop a more general approach to detect food tampering. Measurements were made on an FT-NIR system using a diffuse reflection-integrating sphere. Flours spiked with caffeine, crystalline sugar, and corn meal, 1−20% w/w, were used as test articles to evaluate the methodologies. Food matrices (bleached flour, wheat flour, and blueberry pancake mix) spiked with CBM (0.5−8% w/w) were analyzed. Multiplicative scatter correction transformed partial least-squares regression models, using a specific NIR spectral region, predicted CBM contamination in foods with a standard error of cross-validation of <0.6% and a coefficient of determination (R^2) of >94%. Models discriminated between flour samples contaminated with CBM and other protein sources (egg white, soybean meal, tofu, and infant formula). CBM had loading spectra with bands characteristic of amide groups (4880 and 4555 cm^{-1}) and lipids (5800, 5685, 4340, and 4261 cm^{-1}).

Keywords: *FT-NIR spectroscopy; food contaminants; castor bean meal*

INTRODUCTION

Diffuse reflectance near-infrared (NIR) spectroscopy has been studied for the analysis of plant and animal products (Williams and Norris, 1987). Applications include the determination of common constituents of foodstuffs such as moisture, protein, lipid, sugar, starch, dietary fiber, and organic acids (Osborne and Fearn, 1986); physical characteristics of grains (Delwiche et al., 1996); structural changes resulting from processing and storage (Millar et al., 1996); and byproducts of microbial fermentation (Hall et al., 1996). The use of NIR spectroscopy to assess food quality has been investigated by monitoring juice degrees Brix in pineapple and flesh dry matter in mango (Guthrie and Walsh, 1997), ethanol in wines (Buchanan et al., 1988), and hydroperoxides in fats and oils (Dong et al., 1997). Other potential applications of NIR spectroscopy and multivariate analysis include food authentication (Downey et al., 1997; Downey and Boussion, 1996; Sirieix and Downey, 1993; Osborne et al., 1993) and food adulteration (Twomey et al., 1995; Evans et al., 1993). Due to the use of NIR techniques in quality and process control applications, the potential exists to extend the technology to monitor for food tampering.

NIR absorption spectroscopy is based on the relatively weak and broad overtone and combination bands of fundamental vibrational transitions associated mainly with C−H, N−H, and O−H functional groups (Shenk et al., 1992). The NIR technique provides fast and accurate measurements of chemical components, can be applied to small amounts of sample, is nondestructive (Williams and Norris, 1987), and gives information about structural and physical properties of materials (Millar et al., 1996; Liu et al., 1994a). In addition, the NIR bands are 10−100 times less intense than the corresponding mid-infrared fundamental bands. This enables direct analysis of samples that are highly absorbing and strongly light scattering without dilution or extensive sample preparation (Shenk et al., 1992; Hall et al., 1996). Furthermore, the use of the absorption information obtained with an interferometer by Fourier transform near-infrared (FT-NIR) spectroscopy improves spectral reproducibility and wavenumber precision in comparison to results from dispersion instruments (McClure et al., 1996).

The main limitations of NIR spectroscopy are the strong dependence of reflectance on the scattering properties of the sample and the existence of extensively overlapping absorption bands, which may confound any peak of interest (Osborne and Fearn, 1986). Multivariate calibration methods provide analysts with a means of overcoming these problems by developing empirical models that relate the multiple spectral intensities from many calibration samples to the known analyte concentrations in these samples (Thomas and Haaland, 1990). Principal component regression (PCR) and partial least-squares regression (PLSR) are factor analysis methods that reduce the calibration spectral intensity data at many frequencies to a relatively small number of intensities in a transformed full-spectrum coordinate system (Haaland and Thomas, 1988). PCR and PLSR have the potential to estimate the component concen-

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tration and chemical and physical properties (loading vectors, vector of final calibration regression coefficients, and spectral residuals) from the NIR spectra (Haaland and Thomas, 1988). PLSR has been particularly successful in developing multivariate calibration models for NIR spectroscopy because it uses the concentration information (*Y* variable) actively in determining how the regression factors are computed from the spectral data matrix (*X*), reducing the impact of irrelevant *X* variations in the calibration model (Bjorsvik and Martens, 1992; Martens and Naes, 1989). This capability provides a more information-rich data set of reduced dimensionality and eliminates data noise, which results in more accurate and reproducible calibration models (Martens and Naes, 1987). Advances in FT-NIR spectroscopic instrumentation and multivariate data analysis techniques have significant potential for the determination of changes in food composition that may be indicative of the addition of extraneous material, including agents such as ricin that could be potential threats.

The seeds of the castor plant (*Ricinus comunis*) contain the extremely potent cytotoxic protein ricin. Ricin specifically and irreversibly inactivates eukaryotic ribosomes, promoting cell death by inhibiting protein synthesis. It is composed of a ribosome-inactivating enzyme (A-subunit) linked to a galactose/*N*-acetylgalactosamine-binding lectin (B-subunit) by a single disulfide bond. It enters the cells by endocytosis, after binding via its B-subunit to surface components containing terminal galactose residues. The B-subunit may also facilitate delivery of the A-subunit into the cell cytosol, where its ribosomal substrate is located, thus allowing the A-subunit to enzymatically inactivate rRNA (Wellner et al., 1995; Lord et al., 1994).

Castor oil, which has several industrial applications (e.g., lubricant, coatings, textile processing, and soaps), is derived from castor bean seeds. After expression of the oil, a residue (castor bean meal, CBM) remains in which ricin is contained (Lord et al., 1994; Core, 1981). The water-soluble ricin does not partition into the extracted oil, provided that no cross-contamination occurs during its production (Vuoso, 1999). CBM has been used as an ingredient in some animal feeds after the ricin is denatured to reduce its toxicity. Ricin is denatured by heating the CBM for 20 min at 140 °C using steam or an autoclave (Vuoso, 1999; Rao, 1970), although some studies have shown that heat treatment is not effective in reducing all toxicity (Vuoso, 1999).

Several cases of accidental or intentional (suicidal or homicidal) intoxication from ingestion of castor bean seeds have been reported (Ellenhorn and Barceloux, 1988). Severe hemorrhagic gastritis and dehydration resulted in a 21-year-old male after ingestion of 30 castor bean seeds estimated to contain 10.5 mg of ricin (Kopferschmitt et al., 1983). Acute intoxication in adults has occurred after ingestion of 2−15 seeds, and death has been estimated to occur at a ratio of 1 mg/kg of body weight. Moreover, 1−3 seeds could be lethal in children who are more susceptible to the toxic effects of ricin (severe dehydration) (Ellenhorn and Barceloux, 1988). Intoxication from consumption of castor bean seeds depends on whether the seed remains intact. Consumption of whole intact seeds does not lead to intoxication (Ellenhorn and Barceloux, 1988). Contamination of samples with milled CBM could augment the severity of the intoxication due to accessibility of the ricin for intestinal absorption. CBM is readily available and could be used to deliberately contaminate the food supply, thus making it a potential threat (Wellner et al., 1995).

The objective of this research was to develop methodology for the rapid detection of CBM, containing the highly cytotoxic protein ricin, in flour-containing products by using FT-NIR spectroscopy and multivariate methods.

MATERIALS AND METHODS

Samples and Sample Preparation. Enriched bleached and wheat flour, blueberry pancake mix, granulated cane sugar, stone ground yellow corn meal, pasteurized dried egg white, and tofu were purchased from local supermarkets and, caffeine was purchased from Sigma Chemical Co. (St. Louis, MO). The tofu was vacuum-dried at 40 °C to remove most of the water. Defatted CBM was provided by Dr. Mark Poli (Toxinology Division, U.S. Army Medical Research Institute of Infectious Diseases, Frederick, MD). Defatted soybean meal was provided by Dr. Scott Taylor (USDA−ARS, Peoria, IL). All test articles were ground (Thomas Wiley cutting mill) through a sieve (20 mesh) before addition to the flour.

Bleached flour was spiked with sugar and corn meal at levels ranging from 2 to 20% w/w and with caffeine at levels ranging from 0.1 to 8% w/w to test the methodologies. Bleached flour, wheat flour, and blueberry pancake mix (BPM) were contaminated with CBM at levels ranging from 0.5 to 8% w/w. The wheat flour and BPM matrices were also contaminated with other protein sources (egg white, defatted soybean, or infant formula) at levels ranging from 1 to 4% w/w.

Extraction of Ricin from CBM. CBM (2.5 g) was extracted with 25 mL of 0.02 M HCl (Wannemacher et al., 1992), homogenized, and centrifuged at 8000*g* for 30 min. The resulting supernatant was saved, and the residue was re-extracted with 15 mL of 0.02 M HCl and centrifuged at 8000*g* for 20 min. The supernates from both extractions were combined and assayed for protein and ricin content.

Protein Analysis of CBM Extract. The colorimetric detection and quantification of total protein on the CBM extract was done by using the bicinchoninic acid (BCA; Pierce, Rockford, IL) protein assay (Smith et al., 1985; Wiechelman et al., 1988). Bovine serum albumin (BSA) was used as the standard protein against which to measure the concentration of a sample protein.

Detection of Ricin by Colorimetric Enzyme-Linked Immunosorbent Assay (ELISA). The affinity-purified goat anti-ricin antibody (IgG) was provided by Dr. Mark Poli (Toxinology Division, U.S. Army Medical Research Institute of Infectious Diseases). Biotinylated anti-ricin was prepared with *N*-hydroxysuccinimidyl-6-(biotinamido) hexanoate (EZ-Link NHS-LC-Biotin; Pierce) according to the manufacturer's directions and extensively dialyzed against 10 mM ammonium acetate overnight at 4 °C. A BCA protein analysis (Pierce) was done on the dialyzed product to determine the amount of biotinylated anti-ricin IgG. An aliquot (0.5 mg) of biotinylated anti-ricin IgG was dissolved in 0.5 mL of a solution containing 6 mg/mL RIA grade BSA (Pierce). The product was lyophilized, reconstituted in 0.5 mL of SuperFreeze conjugate stabilizer (Pierce), and stored at −20 °C until used.

The ELISA assay for the detection and quantification of ricin was done following the procedure described by Poli et al. (1994). The assay utilizes an affinity-purified goat polyclonal antibody to adsorb ricin from solution. A standard curve, at levels of 0, 2.5, 5, 7.5, and 10 ng of ricin/mL, was prepared by diluting a 5 mg/mL ricin stock solution (Vector Laboratories, Burlingame, CA) in phosphate-buffered saline (PBS) assay buffer (60 mM PBS with 0.05% Tween 20 at pH 7.4; Sigma Chemical Co.). The CBM extracts were diluted 1/5000 in PBS assay buffer. The biotinylated anti-ricin antibody was used to form a sandwich with the ricin present in the samples. NeutrAvidin D-conjugated alkaline phosphatase (1:3000 dilution with PBS assay buffer; Pierce) and the substrate *p*-nitrophenyl phosphate (pNPP-104 at 1 mg/mL in 1 M TrisBase

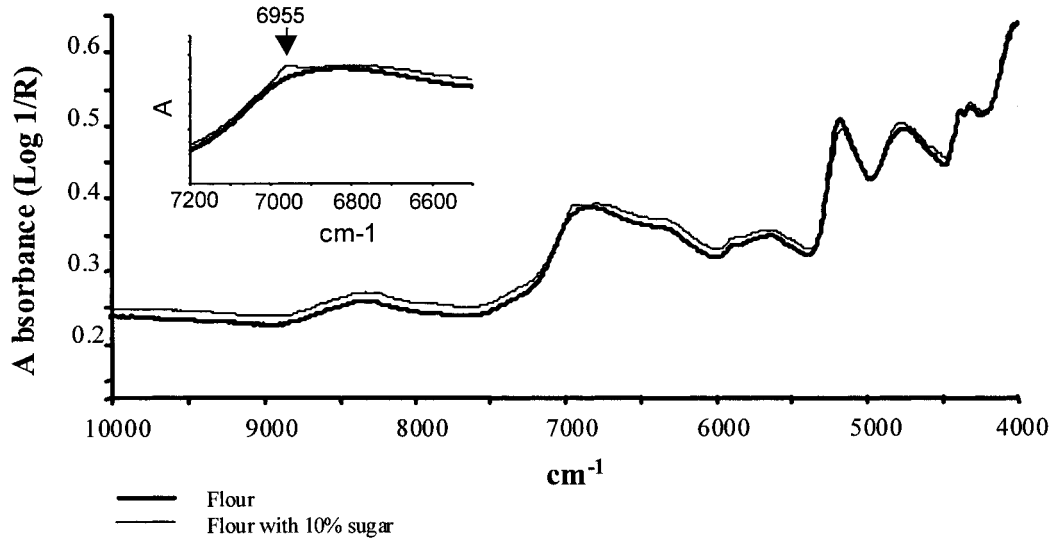

Figure 1. FT-NIR spectra of bleached flour contaminated with crystalline cane sugar.

at pH 9.8, containing 0.03% $MgCl_2$; Sigma Chemical Co.) were used for color development. The reaction was allowed to proceed for 30 min at room temperature, and the optical density was measured at 405 nm on a SpectraMax 340 microtiter plate reader (Molecular Devices, Sunnyvale, CA).

FT-NIR Measurements. All FT-NIR spectra were recorded using a Perkin-Elmer Spectrum Identicheck system operating at 8 cm^{-1} resolution. The mirror velocity was 0.30 cm/s. Measurements were made by using the internal solid analysis by diffuse reflection integrating sphere, equipped with a PbS detector. The absorbance spectrum was obtained by ratioing the single beam spectrum against that of the background, Spectralon (a Teflon-based material manufactured by Lab-Sphere, North Sutton, NH). The FT-NIR spectra were recorded from 10000 to 4000 cm^{-1} at intervals of 4 cm^{-1}. Fifty interferograms were co-added followed by strong Beer-Norton apodization. The total number of data points was 1501 for each spectrum. Samples were measured in capped transparent glass vials (Kimble Glass, Inc., Vineland, NJ) containing 5 g of material. Measurements were also made on a 40 mm diameter Petri dish base (Perkin-Elmer, Norwalk, CT) that were filled with material. Prior to calibration, the FT-NIR reflectance data was mean centered and baseline corrected using offset. The FT-NIR data was further transformed by multiplicative scatter correction (MSC) pretreatment to correct for scatter effects (Isaksson and Naes, 1988).

Multivariate Analyses. PLSR was applied to generate calibration models using a Quant Plus (Perkin-Elmer, CT) software system. The optimum number of latent variables (LVs) used for prediction was determined by full cross-validation (leave-one-out approach). The model producing the first local minimum standard error of prediction (SEP) was selected as the best model for the spectral data set. The resulting models were evaluated in terms of loading vectors, standard error of estimate (SEE), standard error of cross-validation (SECV), coefficient of determination (R^2), and F value for the calibration models. The SEE gives an indication of the quality of fit of the regression and is calculated as the square root of the residual variance divided by the number of degrees of freedom. The SECV is an estimate of the SEP (magnitude of error expected when independent samples are predicted using the model). The coefficient of determination gives the proportion of variability of the property that is described by the model. The F value can be viewed as a measure of the signal-to-noise ratio in the model as it determines whether the property variance is significantly better than the residual property variance. If the value of F is low, the calibration is not robust and the performance on future unknown samples is likely to be substantially worse than on calibration samples. If F is large, then the calibration should have approximately the same accuracy on future unknown samples as on the calibration samples.

The X residuals and leverage were used for the evaluation of outliers. Calibration models were evaluated graphically by looking for a random distribution of residuals. An observation with large residuals or an unusual residual pattern normally indicates an outlier. The leverage of a calibration sample was used to determine its potential contribution to the estimated calibration model. Any observation with abnormal residual or leverage was reanalyzed and eliminated if necessary, after which the calibration model was repeated.

Principal component analysis (PCA) was carried out by Pirouette pattern recognition software (InfoMetrix, Inc. version 2.51 for Windows NT, Woodinville, WA). PCA was applied to the spectral data of the different protein sources.

RESULTS AND DISCUSSION

Flour Samples Spiked with Different Food Ingredients—Test Articles. Bleached flour samples were spiked with different test articles (caffeine, cane sugar, and corn meal) to optimize conditions for the development of calibration models that can predict the level of contamination of the sample from FT-NIR measurements and evaluate the loading spectra for important chemical information. FT-NIR spectra (Figure 1) of flour were comparable to those reported by Kays et al. (1996) and Murray and Williams (1987). Addition of cane sugar to flour, at levels >5%, resulted in a distinct FT-NIR absorbance peak at 6955 cm^{-1} (Figure 1) related to the first overtone of the O−H structural vibration of sucrose (Osborne and Fearn, 1986). Most of the changes in the shape of NIR peaks, however, are barely noticeable, and mathematical models are needed to extract the relevant information for qualitative and quantitative analysis (Hruschka, 1987).

PLSR can extract pertinent information from complex spectra of several chemicals by describing the main types of NIR variations and relating the chemical data to the NIR data (Bjorsvik and Martens, 1992) and was used to generate the calibration models. Also, PLSR has been successfully applied to quantitative analysis of NIR data (Bjorsvik and Martens, 1992; Haaland and Thomas, 1988; Martens and Naes, 1987).

Multivariate analyses demonstrated that sample homogeneity was an important variable for the calibration model. The coefficient of determination (R^2) in-

Table 1. Multivariate Analysis by PLSR of Contaminated Bleached Flour Samples

contaminant	no. of standards	no. of LVs[a]	SEE	SECV	multiple correlation	% variance (R^2)	F value	bias[b]
caffeine using capped vials	30	3	0.36	0.44	0.99	98.0	396	−0.0019
cane sugar using capped vials	30	3	0.94	1.04	0.98	95.1	154	−0.0128
cane sugar using glass dishes	30	3	0.48	0.56	0.99	98.9	699	−0.0082
corn meal using capped vials	50	2	2.15	2.23	0.92	85.2	132	−0.0118
corn meal using glass dishes	50	3	1.85	2.10	0.94	89.0	121	0.0032
cane sugar and corn meal matrix								
A. sugar	22	1	1.22	1.34	0.98	95.8	461	0.0728
B. corn meal	22	3	1.59	1.88	0.97	93.7	88	0.016
CBM using glass dishes	16	1	0.36	0.39	0.99	97.8	535	−0.0117
CBM using capped vials	16	2	0.56	0.68	0.98	96.6	173	0.0531

[a] In the case of the corn meal model, increasing the number of latent variables (LVs) slightly increased the percent explained variance (R^2); however, the significance test indicated that more LVs do not contribute to the regression model (p value > 0.05) and simply account for variation in the spectra without improving the description of the property variation. Increasing the number of LVs did not reveal any additional structural information. [b] The overall bias of the models was negligible.

creased from 42 to 98% when cane sugar was milled before its incorporation into the flour samples. Olinger and Griffiths (1993) reported that particle size and morphology must be considered to avoid the effects of specular reflection. Transformed spectra (second derivative) did not improve the calibration models.

The FT-NIR calibration results for spiked bleached flour are presented in Table 1. The cross-validated calibration models used the first few latent variables with SECV ranging from 0.4 to 2.2% and explained 85−98% of the variability, depending on the test article. Performance of models for flour samples spiked with corn meal showed the highest SECV (2.2%) and lowest R^2 (85%) and F value (120). Corn meal and flour have very similar spectral features that can account for the reduced accuracy of this calibration model.

The orientation and density or compactness of material in the sample cell influence the NIR reflectance, and fairly large errors can occur if the samples are not sufficiently well packed (Williams, 1987). The vials were overfilled and the base was tamped to eliminate the variability due to the differences in compactness of the material (Williams, 1987), and the material placed on Petri dishes was compressed by using a stainless steel weight placed on top of the cell surface. The type of sample cell (vial versus Petri dish) used for the FT-NIR reflectance measurements had an effect on the performance parameters of the PLSR calibration models (Table 1). Overall, models developed from samples measured on Petri dishes gave better statistics (lower SECV and higher F values and R^2) than samples measured in vials. Petri dishes that allow reproducible reflectance measurements were selected by the manufacturer for their spectral and geometrical properties, whereas the glass vials have a curved base and variable thickness that affect the optics of light reflected to the detector and thus increase the noise.

Qualitative information regarding areas of the spectrum associated with the highest variation in a calibration set were indicated by the loading weights. Frequencies of high variation reflect combinations of different chemical and physical phenomena (Bjorsvik and Martens, 1992; Kays et al., 1998). Examination of the loadings for flour samples spiked with caffeine showed that the absorption peaks at 8840, 7390, 5987, 5850, 4446, and 4135 cm^{-1} largely influenced the spectral variation (Figure 2A). The spectral features exhibited by LV1 were similar to the features of the reflectance spectrum of a caffeine standard and the features of the discriminant factor 1 profile for dried coffee extract reported by Downey and Boussion (1996). In the case

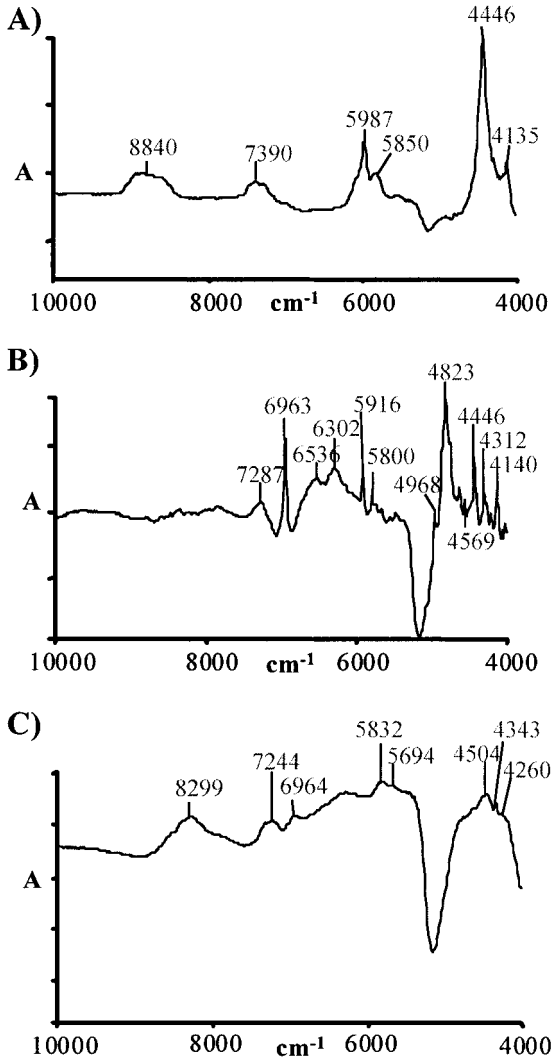

Figure 2. PLSR loading spectra (latent variable 1) for bleached flour samples spiked with different materials: (A) caffeine; (B) sugar cane; (C) corn meal.

of flour spiked with cane sugar, the spectral features exhibited by LV1 (Figure 2B) were associated the characteristic absorbances of carbohydrate O−H and C−H groups. The FT-NIR spectrum of crystalline sugar was very similar to that reported by McClure et al. (1996). Sharp bands at 6963 and 4823 cm^{-1} dominated the first loading, which are indicative of the importance of O−H groups in crystalline sucrose, as previously reported by Kays et al. (1998). The bands near 6960,

6595, and 6325 cm^{-1} have been assigned to the first overtones of stretching modes of free OH, intramolecular hydrogen-bonded OH, and intermolecular hydrogen-bonded OH groups, respectively (McClure et al., 1996). Bands in the 5950−5700 cm^{-1} region and near 7270 cm^{-1} have been associated with first overtones of CH stretching modes and combinations of CH vibrations, respectively. In addition, bands in the region from 4400 to 4033 cm^{-1} have been related to C−H groups of carbohydrates (Kays et al., 1998). Evaluation of loading weights of flour samples spiked with corn meal (Figure 2C) appeared to contain spectral features associated with C−H structural overtones of aliphatic groups. The main areas of absorption were 8297, 7244, 6292, 5833, 5693, 4504, 4334, and 4260 cm^{-1}. Tkachuk (1987) reported characteristic NIR absorption signals for oil at 7209, 5807, 5681, 4336, and 4269 cm^{-1}, which are close to some of the absorption bands in the corn meal loadings. Also, absorption at 8424 cm^{-1} has been reported to be characteristic of proteins (Tkachuck, 1987), and bands near 6645 cm^{-1} arise from the first overtones of NH stretching modes (McClure et al., 1996).

The application of FT-NIR spectroscopy and PLSR multivariate techniques allowed the detection and quantification of different test agents in the matrix (bleached flour). Empirically, it was observed that contaminants, that is, corn meal, which are similar to the matrix (flour) were quantitatively modeled with less precision than those contaminants (i.e., caffeine and sugar cane) which showed distinctive spectral features. The spectral information obtained with LV1 was associated with the FT-NIR spectra of the pure standard (caffeine or cane sugar), which indicates that the models are explaining most of the chemical contributions of the contaminants with the first factor. Corn meal LV1 showed the contributions of C−H overtones of aliphatic groups regardless of increased baseline noise. The use of Petri dishes for FT-NIR reflectance measurements instead of glass vials improved the performance of the calibration models, mainly by decreasing the SECV. Nevertheless, the models generated for flour that was contaminated with CBM using capped glass vials gave acceptable performance with SECV of 0.7% and R^2 of 97% (Table 1).

Contamination of Flour-Containing Products with CBM. The defatted CBM used to contaminate the flour products had an average protein content of 7.2 ± 0.5 g/100 g of CBM, based on BSA standard, and an estimated ricin level of 497 ± 60 mg/100 g of CBM, which represents 6.1% of the total protein. These ricin levels (~450 mg/100 g of CBM) agree with those reported by Wannemacher et al. (1992) using ELISA and the findings of Lord et al. (1994), which suggest that ricin accounts for >5% of the total protein present in mature *Ricinus comunis* seeds (~7−12 mg of ricin/g of seed).

FT-NIR reflectance measurements were made by using capped vials as sample cells to ensure safe handling of the toxic material. The FT-NIR spectra of pure CBM and other protein-containing products were evaluated (Figure 3). Although similar gross spectral features were observed among samples, PCA was able to group the samples into separate clusters (Figure 4).

Cross-validated (leave-one-out) PLSR models were developed to evaluate the level of CBM contamination in different matrices (bleached flour, wheat flour, and

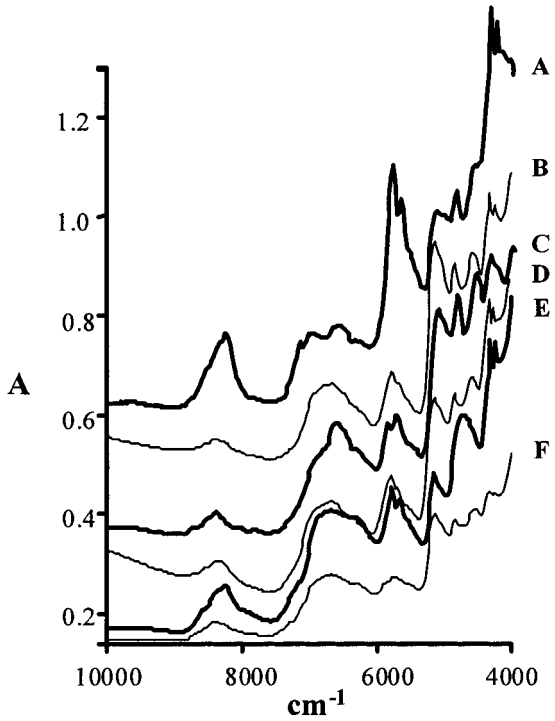

Figure 3. FT-NIR spectra of protein-containing products: (A) egg yolk; (B) tofu; (C) egg white; (D) CBM; (E) powdered milk; (F) soybean meal.

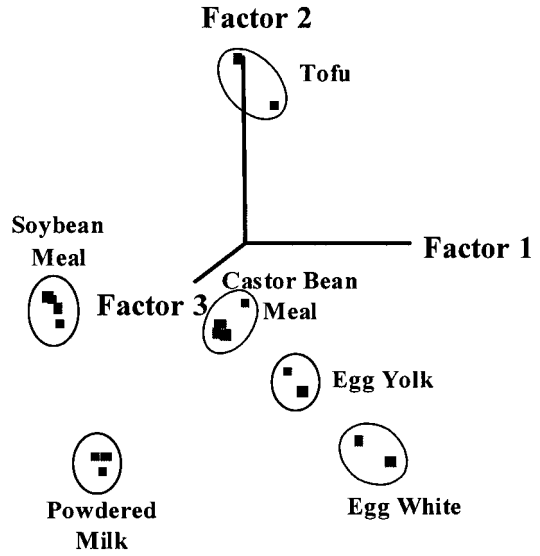

Figure 4. PCA scores plot for FT-NIR spectra of protein-containing products, considered in the region 10000−4000 cm^{-1}.

BPM) and to infer relevant chemical information from the mixture spectra by using the loading vectors (Haaland and Thomas, 1988). Most of the variance was explained by the first three latent variables (>92%). The prediction error (SECV) estimated from the test objects in the calibration set (internal validation) ranged from 0.35 to 0.57%. This compares well with the prediction errors reported for similar food matrices which showed values for moisture (0.16−0.19%), protein (0.40−0.63%), and wet gluten (1.37−2.20) in milled wheat flours (Sorvaniemi et al., 1993); for total dietary fiber (1.5%), sugar (1.9%), and fat (1.8%) in cereal products (Kays et al., 1996, 1998); and for crude protein (0.13%) and protein fractions glutenin (0.44%), gliadins (0.49%), and albumin and globulins (0.27%) in hard red winter wheat

Table 2. Multivariate Analysis by PLSR of Flour Samples Contaminated with CBM

product	spectral range[a]	no. of standards	no. of LVs	SEE	SECV	multiple correlation	% variance (R^2)	F value	bias
bleached flour	A	70	4	0.50	0.55	0.98	96.2	400	−0.003
	B	70	4	0.48	0.53	0.98	96.3	405	−0.003
wheat flour	A	94	3	0.56	0.57	0.97	94.0	472	0.0009
	B	94	3	0.55	0.56	0.97	94.5	543	−0.0005
BPM	A	80	3	0.36	0.38	0.98	95.6	543	0.0013
	B	80	2	0.34	0.35	0.98	95.8	855	0.0006

[a] Spectral ranges used for data acquisition: (A) 10000−4000 cm^{-1}; (B) 10000−7600, 6100−5600, and 4950−4000 cm^{-1}.

flour (Delwiche et al., 1998). Multivariate analysis gave coefficient of determination (R^2) of >94%, F values (model robustness) >400, and negligible bias effect for flour products contaminated with CBM. The models generated using the complete spectrum (10000−4000 cm^{-1}) or selected regions (10000−7500, 6100−5600, and 4950−4000 cm^{-1}) showed very similar performances (Table 2).

The predictive ability of the models was evaluated on independent samples of wheat flour and BPM contaminated with CBM, egg white, soybean meal, or infant powder formula. Models that used the complete spectrum were able to correctly predict the levels of CBM contamination but gave false positive values (>SECV) for wheat samples containing other contaminants (Table 3). Changes in the residual moisture content of samples can interfere with the predictive ability of the NIR calibration model (Kays et al., 1997). The FT-NIR spectral regions from 7600 to 6100 cm^{-1} and from 5600 to 4950 cm^{-1} were primarily affected by the changes in moisture. Removal of the bands that were influenced by the water signal from the PLSR model improved the accuracy of prediction of the contaminated wheat samples (Table 3) because the apparent positive values were lower than the SECV of the models. The samples were prepared at different times over a one month period from wheat flour that was stored in a hood. Changes in the moisture content due to storage conditions could have affected the prediction ability. In the case of BPM samples, both PLSR models (complete spectrum and selected regions) gave similar predictive ability for CBM levels and other contaminants. The samples used for the calibration model and independent predictions for the BPM were prepared at different time periods but from sealed containers. Storage in sealed containers may have avoided any changes in moisture content of the product, and therefore the calibration was not affected by the elimination of the water spectral regions. The improved performance of the optimized model (selected spectral ranges) was indicated by the prediction of samples that contained only the matrix (control), with estimated levels close to zero and highest values of 0.1% w/w. These results showed that the PLS model could detect differences from the control at levels >0.1% due to the presence of extraneous compounds in the matrix and positively predict CBM contamination at levels above their SECV, based on the limits established by the calibration process.

Contamination levels of ricin in the samples ranged from 2.5 to 40 mg/100 g of flour. We were able to quantify CBM contamination at levels >0.6% (3 mg of ricin/100 g) and >0.3% (1.5 mg of ricin /100 g) for wheat flour and BPM, respectively. This detection level is similar to the low levels of ricin calculated to be present in two to three seeds that were ingested by a man (age 44) and a child (age 12), resulting in nausea and vomiting but without major clinical complications such as violent abdominal pains, copious diarrhea, severe hemorrhagic gastritis, dehydration, or death (Malizia et al., 1977).

Table 3. Prediction of the Level of CBM Contamination in Wheat Flour and BPM Samples

level of contamination (%)				prediction (%) PLS model	
CBM[a]	egg white[b]	defatted soybean meal[b]	powdered milk[b]	MSC 10000−4000 cm^{-1}	MSC 10000−7500, 6100−5600, 4950−4000 cm^{-1}
A. Wheat Flour					
control[a]				ND; 0.6[c]	ND; 0.1
1.0				1.3	1.1
1.5				1.6	1.5
2.0				2.0	2.0
2.5				2.8	2.7
	1.0			0.5	ND; 0.1
	1.5			1.0	0.1
	2.0			0.9	ND
		1.0		ND	ND
		2.0		0.8	0.2
		4.0		0.9	ND; 0.6
			1.0	0.1	ND
			2.0	0.6	ND
			4.0	0.6	ND
B. Blueberry Pancake Mix					
control[d]				ND; 0.2	ND; 0.1
1.0				0.9	1.0
1.5				1.6	1.6
2.5				2.3	2.3
	1.5			ND	ND; 0.2
	2.3			0.3	ND; 0.1
	3.9			0.2	0.2
		1.0		0.2	0.2
		2.1		ND; 0.4	ND
		3.0		0.3	ND
		4.1		0.4	ND
			1.3	ND	ND
			2.1	ND	ND
			2.9	ND	0.3
			3.5	0.1	0.2

[a] Values for CBM and control represent averages from six replicates. [b] Values for egg white, defatted soybean meal, and powdered milk represent averages from two replicates. [c] ND corresponds to prediced negative values by the calibration methods. Values given with ND represent the highest level predicted. [d] Values for CBM and control represent averages from three replicates.

The PLSR loadings (Figure 5) displayed important absorption features of CBM. Examination of the loading spectra suggests that the effects of LV1 were related to the CBM because of similar spectral pattern and absorption bands at 8370, 5800, 5685, 4860, 4555, 4340, and 4261 cm^{-1} for the different matrices evaluated. The use of a castor oil standard (Figure 6) confirmed that bands at 5800, 5685, 4340, and 4261 cm^{-1} for LV1 were correlated to residual oil in the CBM. Bands in the 5000−4550 cm^{-1} region have been related to amides modes (Liu et al., 1994a) with bands near 4850 and 4600 cm^{-1} assigned to combinations of amide A/I and amide B/II, respectively (Wang et al., 1994). Robert et al. (1999)

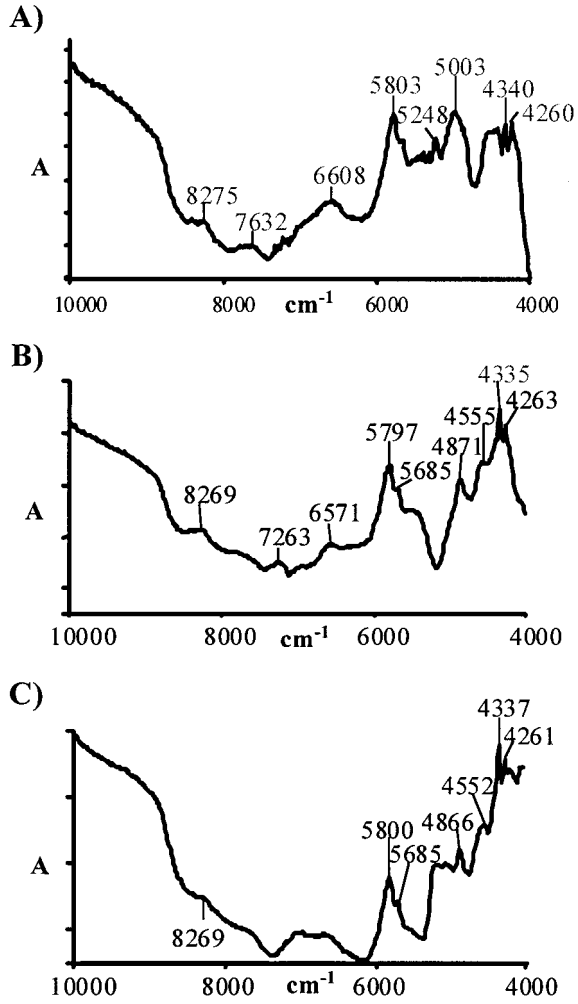

Figure 5. PLSR loading spectra (latent variable 1) of samples contaminated with CBM: (A) bleached flour; (B) wheat flour; (C) BPM.

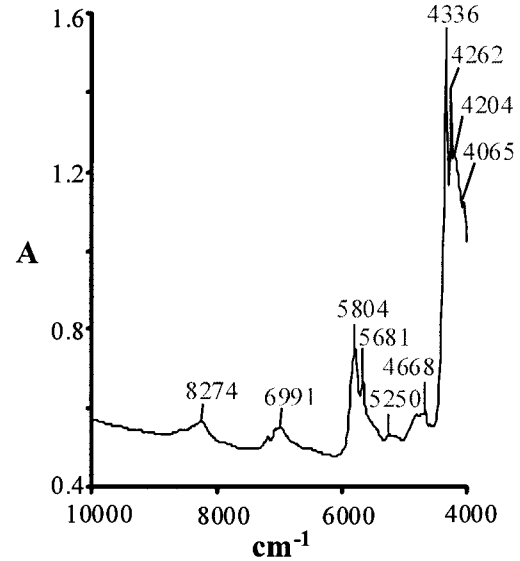

Figure 6. FT-NIR spectra of castor oil standard.

reported that proteins (myoglobin, β-lactoglobulin, and β-casein) exhibited common peaks at 4866, 4608, and 4255 cm^{-1} attributed to combination bands of amide A/I, amide I/II, and CH stretch/CH deformation.

Figure 7 shows the spectral contributions of the first three latent variables for the BPM matrix. A complex

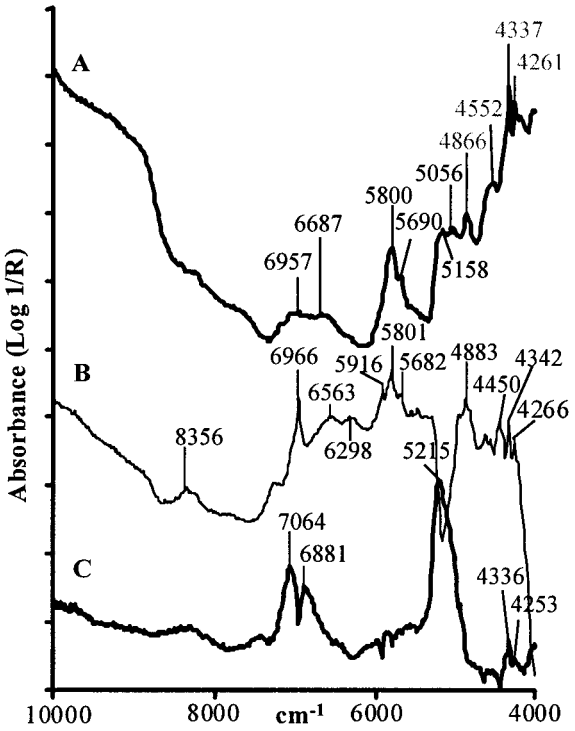

Figure 7. PLSR loading spectra of BPM contaminated with CBM: (A) LV1, explained 62.3% of the spectral variability; (B) LV2, explained 19.7% of the spectral variability; (C) LV3, explained 9.7% of the spectral variability.

matrix, BPM contained several ingredients, including enriched flour, corn meal, sugar, soybean and cottonseed oils, and cellulose gum. As mentioned before, spectral features of LV1 were related to the castor bean FT-NIR signal, whereas spectral features of LV2 and LV3 were characteristic of the components of the matrix. LV2 showed bands associated with stretching and bending OH modes of sugars (6966, 6563, 6298, 5916, and 4825 cm^{-1}), N−H combination of amide II (4883 cm^{-1}), C−H overtones and combination regions of lipids (8356, 5801, 5682, 4342, and 4266 cm^{-1}), and C−H groups of the carbohydrates (4450−4340 cm^{-1}) (McClure et al., 1996; Kays et al., 1996; Li et al., 1999). LV3 showed FT-NIR signals associated with the broad OH absorption combination bands of water at 5200 cm^{-1} (Espinoza et al., 1999) and the OH stretching and HOH deformation modes of polysaccharides (5190 cm^{-1}). The band at 6881 cm^{-1} can be related to the first overtones of NH stretching modes in the 6900−6200 cm^{-1} region (Liu et al., 1994b), and the spectral band at 7064 cm^{-1} can be associated with combination modes of CH_2 (Shenk et al., 1992; Liu et al., 1993).

Conclusions. FT-NIR and multivariate techniques (PLSR) allowed for rapid detection of CBM in selected flour-containing products. The PLSR models were able to differentiate between CBM contamination and the addition of other rich-protein products to the matrices. On the basis of the PLSR models developed, quantification of CBM contamination could be determined at levels >0.3% (1.5 mg of ricin) and 0.6% (3 mg of ricin) w/w in selected wheat flour and BPM, respectively. On the basis of human intoxication reports, ingestion of ~1 mg of ricin will produce mild intoxication in adults (nausea, vomiting), whereas higher levels will result in more complicated clinical symptoms associated with ricin toxicity.

ABBREVIATIONS USED

BPM, blueberry pancake mix; BSA, bovine serum albumin; CBM, castor bean meal; ELISA, enzyme-linked immunosorbent assay; FT-NIR, Fourier transform near-infrared; IgG, anti-ricin antibody; LV, latent variable; MSC, multiplicative scatter correction; NIR, near-infrared; PbS detector, lead sulfide; PCA, principal component analysis; PCR, principal components regression; PLSR, partial least-squares regression; R^2, coefficient of determination; SECV, standard error of cross-validation; SEE, standard error of estimate; SEP, standard error of prediction.

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