Detection of soy, pea and wheat proteins in milk powder by near infrared spectroscopy

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Introduction

The economic value of milk and milk products is, to a large extent, determined by the fat and protein content. Consequently, the risk of fraud, by replacing them with products of lower value, can be identified. Some of these aspects—such as dilution of milk and milk products, milk fat replacement, milk fat fractionation, milk protein replacement[1,2] and manipulation of the protein composition—have been dealt with in several papers and studies. In this context, the development of a method for the detection of non-milk protein in dairy products is an important step in EU efforts to fight against fraud. Replacement of milk protein with cheaper proteins is usually realised by the addition of vegetable protein isolates such as soy, pea and wheat isolates, on the basis of their cost and their functional properties (yield increase, foaming formation, etc…). There may be a risk that this fraud is carried out in products such as milk powder, yoghurt and processed cheese. Difficulties and limitations encountered by using the methods and techniques developed[3–10] have resulted in the lack of an official method able to detect this kind of fraud. Recently, a research project, financed by the Measurement and Testing Programme of the European Commission,[11] has worked towards a proposed method, based on electrophoretic and/or ELISA principles. However, some questions regarding analytical costs, analysis time and the availability of specific antibodies have directed the research towards faster and cheaper procedures able to provide suitable results.

Near infrared (NIR) spectroscopy, when applied to the dairy field, also proved a fast and feasible technique to determine the chemical composition of different types of dairy products and to detect anomalies in milk and cheese defects.[12–16]

A preliminary approach showed the possibility to detect soy, pea and wheat isolates in milk powder by NIR with satisfactory accuracy. This work aimed to improve the NIR prediction power carrying out separate calibration curves, each designed to detect a single vegetable protein used in milk powder adulteration.

Materials and methods

Samples

Two hundred and twenty-four samples of genuine and adulterated skimmed milk powder containing 0–5% of selected vegetable isolates were used. Genuine (44 samples) and adulterated (1, 2 and 5%) samples were prepared by NIZO (Ede, Wageningen, The Netherlands) in April, May and October 1999 for the purposes mentioned within the EU Project[11] and were strictly controlled for homogeneity,

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Table 1. Detection of soy and other proteins by NIR.

Code	Description	% TN	% Protein (TN × 6.25)
A	Soya protein isolate [Supro 500 E]	13.59	85.0
K	Soya protein isolate [Europrod.595]	13.45	84.1
C	Pea protein isolate [Pisane HD]	13.44	84.0
L	Wheat gluten [SWP 100]	12.88	80.5

stability and solubility. Samples were prepared using liquid skimmed milk, applying two different heat treatments: pasteurisation (107 samples) and UHT process (107 samples) using the same spray-drying process. Four commercial vegetable isolates were used: two isolates from soy (A and K; 99 samples), one from pea (C; 62 samples) and one from wheat (L; 63 samples). Table 1 gives a description of vegetable protein isolates. Mixtures at 3 and 4% were made by mixing calculated amount of genuine and adulterated (5%) samples, taking care to accurately blend the two components.

The protein content was measured by the Dumas method.[17] This method was used to determine the protein content of each vegetable isolate, the milk protein content and the protein content of each calibrated mixture before and after the spray-drying process. The percentage of vegetable protein was expressed as [vegetable protein (g) / total protein (g)] * 100].

NIR measurements

NIR spectra were recorded with a holographic grating spectrometer (Bran+Luebbe InfraAlyzer 500, Bran+Luebbe GmbH, Germany) at 1100 to 2500 nm at 4 nm intervals (351 data points).

Light absorption was expressed as log (R^{-1}) values. Measurements were carried out in reflectance mode. The instrument was equipped with a sample cell for solids (code no. 189-0564F, Bran+Luebbe). Samples were analysed in duplicate. Each spectrum was the mean of two spectra, collected rotating the cell by 90°. NIR spectra were collected at room temperature and data were processed by using Sesame Software (Bran+Luebbe). Different data pre-treatments were applied: i.e. raw absorbance data, data normalisation (obtained scaling all measured values between 0 and 1) and first derivative of absorbance values. Pre-treated data were processed by using partial least squares regression (PLSR), using the full spectrum.

Separated calibrations were performed using, respectively, 80, 50 and 50 out of the 99, 62 and 63 samples for soy, pea and wheat adulterated samples. Cross-validation protocol of Sesame software was applied to calculate the optimal number of PLS factors for each calibration and the standard error of prediction (SEP_{CV}). Remaining samples (19 soy, 12 pea, 13 wheat) were used for prediction. The sets were prepared by random selection of samples maintaining roughly constant representation of the addition percentages. Regression coefficients (R^2), *SEE*

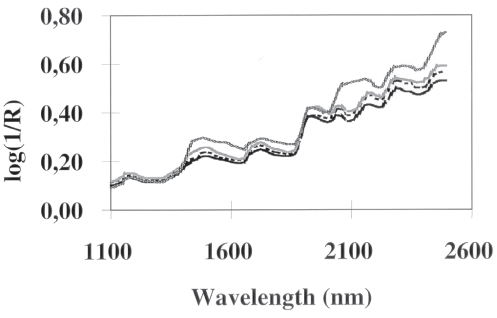

Figure 1. Examples of NIR spectra for genuine skim milk powder (pasteurised .....), soy isolate (A—), pea isolate (C - - -) and wheat isolate (L—).

Table 2. Detection of soy and other proteins by NIR.

Added vegetable protein (*n*)	PLSR factors	*CV* segments	R^2	*SEE*	$RMSEP_{all}$	$RMSEP_{cal}$	$RMSEP_{pred}$	$RMSEP_{cv}$
soy (99)	9	80	0.994	0.193	0.191	0.181	0.229	0.301
pea (62)	9	50	0.997	0.148	0.176	0.133	0.295	0.294
wheat (63)	10	50	0.997	0.152	0.150	0.134	0.198	0.339

(standard error of estimation), *RMSEP* (standard error of prediction) for calibration and prediction sets and $RMSEP_{all}$ (standard error of prediction on whole sets of samples) values were calculated.

Capillary electrophoresis and ELISA procedures

Determination of the content of soy, pea and wheat isolates in milk powder samples was already carried out in the development of the mentioned EU Project,[11] where several collaborative studies were realised among eight European participant laboratories in order to evaluate accuracy and detection limits of both methods. In this study, results obtained by CE and ELISA techniques are compared with NIR predicted values.

Results and discussion

An example of NIR spectra for genuine skimmed milk powder, soy, pea and wheat isolates, collected from 1100 nm to 2500 nm, is shown in Figure 1 as absorbance values *vs* wavelengths.

NIR predictions

The information, collected in a preliminary study,[18] proved the suitability of NIR for detecting vegetable proteins in milk powder and suggested that this method may be used as an accurate screening procedure in routine analyses for quality control.

Soy, pea and wheat protein isolates in milk powder

PLS calibration for soy, pea and wheat protein adulteration was performed using nine factors (F) and 80 cross-validation segments (CVS), 10 F and 50 CVS, 10 F and 50 CVS on first derivative normalised spectra, respectively. NIR prediction results are presented in Table 2. The corresponding correlation coefficients, expressed as R^2, between predicted and actual values (% vegetable protein/total proteins) and number of samples (*n*) are also presented. Figures 2, 3 and 4 show the relationship between the actual percentage of added proteins and NIR predicted values.

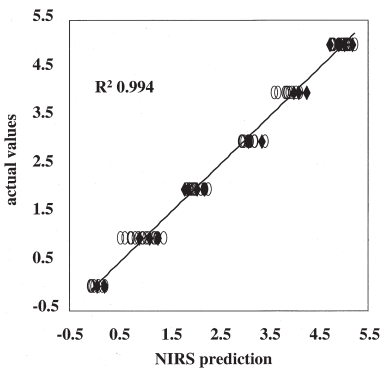

Figure 2. Relationship between the actual percentage of added soy proteins and NIR predicted values by using PLSR [(0) calibration set, (◆) validation set].

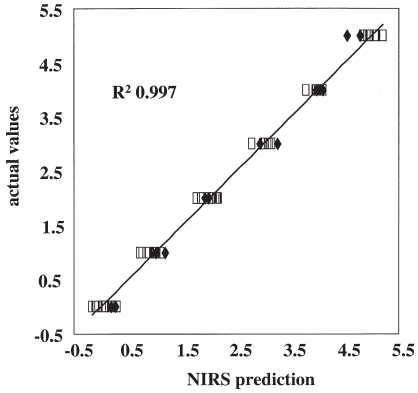

Figure 3. Relationship between the actual percentage of added pea proteins and NIR predicted values by using PLSR [(□) calibration set, (◆) validation set].

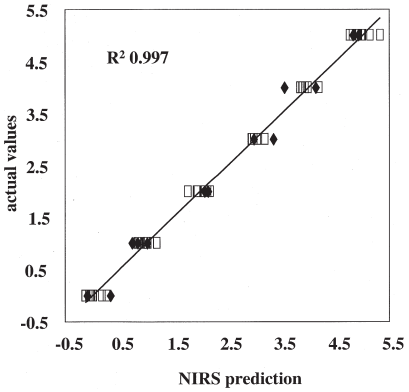

Figure 4. Relationship between the actual percentage of added wheat proteins and NIR predicted values by using PLSR (□) calibration set, (◆) validation set].

NIR predictive power against capillary electrophoresis results

Figures 5 and 6 show the comparison between capillary electrophoresis (CE) results and NIR prediction values. Lines indicating the confidence interval (P = 95%) are also shown. This plot just refers to the sets of pasteurised samples, adulterated with increasing amounts of soy (Figure 5) and pea (Figure 6) isolates. We can note that the variability of data associated with the application of the CE procedure was higher than the NIR variations. In this case, the actual adulteration percentage was known, so NIR could be calibrated against true values, resulting in a precise calibration with satisfactory results in the prediction and cross-validation steps (see Table 2). Conversely, if we needed to calibrate against CE results, NIR prediction would be affected by the standard error associated with the applied reference method, resulting in a higher variability and a worse prediction. Quantitative data

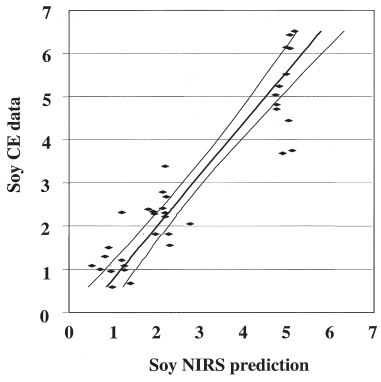

Figure 5. Comparison between capillary electrophoresis (CE) results and NIR prediction values (soy).

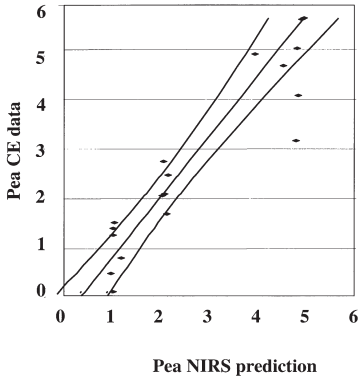

Figure 6. Comparison between capillary electrophoresis (CE) results and NIR prediction values (pea).

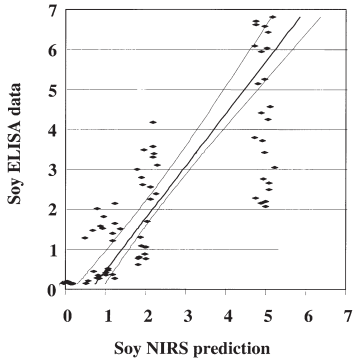

Figure 7. Comparison between ELISA results and NIR prediction values (soy).

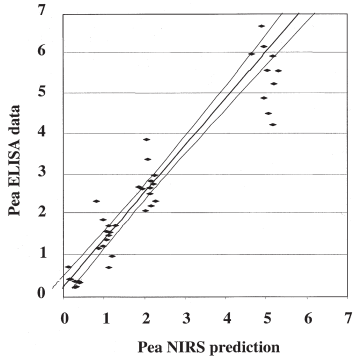

Figure 8. Comparison between ELISA results and NIR prediction values (pea).

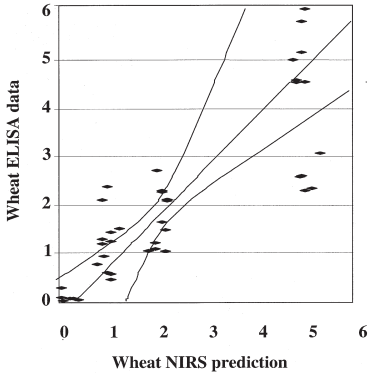

Figure 9. Comparison between ELISA results and NIR prediction values (wheat).

also show the possibility of correctly determining the amount of adulterant proteins in milk powder by using the CE procedure and to discriminate between 1, 2 and 5% additions, but NIR prediction values were characterised by higher accuracy and precision, easier sample preparation and were less time consuming.

NIR predictive power against competitive ELISA data

The comparison between ELISA and NIR results, obtained analysing all sets of samples, is reported in Figures 7–9, which show separated plots for soy, pea and wheat adulterated samples, respectively. Lines indicating the confidence interval (P = 95%) are also shown. A large variability of response and a partial overlapping of quantitative values were found when the ELISA procedure was applied and, also, if genuine samples were, in all cases, correctly classified. Considerations made before about accuracy and precision can be confirmed. We would just point out that the CE and ELISA methods showed satisfactory results, on the basis of data obtained by eight different laboratories, and represented an index of method reproducibility, but NIR prediction seems to be more precise in estimating the actual content of vegetable added proteins, with low *SEE* values.

Conclusions

NIR may have some advantages, such as rapidity, ease of use, no sample preparation and low operator skill required, over existing methods, based on electrophoretic and immunochemical principles.

NIR was able to determine more accurately than the other two techniques the percentage of adulteration in the analysed samples. Furthermore, dedicated calibrations could also help in identifying what type of vegetable protein isolate was added.

A more complete validation of the described NIR procedure needs further investigations, such as: (i) the identification of this kind of adulteration in different skimmed milk powders, (ii) the possibility of identifying the presence of vegetable hydrolysed isolates in milk powder, (iii) the detection of non-milk proteins in yoghurt and processed cheese, (iv) the detection of other vegetable proteins in milk and dairy products; (v) the data collection in order to calculate reproducibility index.

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