Multivariate Calibration of Raw Cow Milk using NIR Spectroscopy

OLGA KUKAČKOVÁ, LADISLAV ČURDA and JOSEF JINDŘICH

Institute of Chemical Technology – Department of Dairy and Fat Technology, Prague, Czech Republic

Abstract

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At present, mid-IR spectroscopy is the preferred rapid method for milk analysis. NIR spectrometers have some advantages in comparison with mid-IR. The purpose of this study was to see a possibility of determining the total solids, fat and protein in milk. The total number of preserved raw cow milk samples was 65. NIR spectra were recorded in duplicate on the NIRS 6500 instrument (NIRSystems, Inc.) using a fiber optic probe. The reference method for milk samples used for calibration model was determined by oven drying to measure the total solids, by Gerber method to measure fat content, and by Kjeldahl method to measure protein content. A commercial spectral analysis NIRS 3 software was used to process and develop chemometric models. The calibration was calculated using the modified partial least squares (MPLS) regression. The following statistical values (correlation coefficient *R* and standard error of prediction SEP) were obtained: $R = 0.975$ and SEP = 0.160% for milk total solids, $R = 0.967$ and SEP = 0.179% for milk fat content, $R = 0.965$ and SEP = 0.049 % for protein content. Using the NIR instrument the multivariate models suitable for rapid fundamental analysis of milk were developed.

Keywords: near infrared spectroscopy; raw cow milk; total solids; fat, protein

The methods monitoring fundamental milk composition (content of total solids, fat and protein) are necessary in dairy industry. The fundamental milk analysis is a basic step to ensure milk quality and safety. It is also important for payments to farmers. Compositional analysis of milk using spectroscopy and especially the use of mid-infrared spectroscopy is well established within the dairy industry. But this technique cannot be used for the other dairy products except liquid milk. This problem is solved by the NIR spectroscopy.

Unlike the conventional analytical methods, which demand time-consuming preparation and processing of samples, the NIR spectroscopy is a non-destructive multicomponent method and in addition it is a rapid and accurate technique for a wide range of analytical applications. It has emerged as an interesting alternative to common chemistry in analysis and quality control of agricultural and food products. NIR spectroscopy requires almost no sample preparation, does not require any chemicals and does not produce any chemical waste (OSBORNE 1981).

NIR has been applied to major components of milk (HALL & CHAN 1993), whey powder (OSBORNE & FEARN 1986; RODRIGUEZ-OTERO *et al.* 1998), to classify milk powder according to thermal treatment (DOWNEY *et al.* 1990), to analyze cheese (FRANKHUIZEN & VAN DER VEEN 1985; FRANK & BIRTH 1982; WEHLING & PIERCE 1988; PIERCE & WEHLING 1994; RODRIGUEZ-OTERO *et al.* 1995; WITTRUP & NOERGAARD 1998), fermented milk products (RODRIGUEZ-OTERO *et al.* 1997), and butter (FRANKHUIZEN 1992).

For each particular component, NIR spectroscopy requires calibration of the instrument by a generally accepted laboratory method known as a reference method. Calibration samples should cover the whole concentration range and all reasonable variations. The aim of our work was to evaluate the major components (total solids, fat, protein) of raw cow milk without the need of previous preparation of the sample by NIR spectroscopy.

MATERIAL AND METHODS

Samples: Sixty-five raw cow milk samples were used as calibration standards. The analytical ranges were from 11.89 to 14.72% for the total solids, from 3.50 to 6.15% for fat, and from 3.15 to 3.73% for protein. All samples were preserved by addition of 0.06% (w/w) $K_2Cr_2O_4$. Samples in polyethylene bottles (250ml) were before measurement first heated to 40°C in water bath and then cooled to 20°C.

Reference Analysis: The total solids were determined by oven drying at 102°C to constant weight (CVAK *et al.* 1992) and the fat by Gerber method (MARSHALL 1992). Crude protein in milk samples was calculated from total nitrogen content using conversion factor 6.38. Total nitrogen was assessed by Kjeldahl method using Kjeltec Auto 1030 Analyzer (MARSHALL 1992). All determinations were made in duplicate.

Apparatus: Spectra were obtained by the wavelength scanning instrument NIRS 6500 (NIRSystems, Inc.) using of a fiber optic probe. A scanning range from 400 to 2498 nm (25 000–4 003 cm^{-1}) and wavelength increments of 2 nm were used. An average of 25 spectral scans was taken for each sample. Diffuse reflectance was recorded as log 1/R. Each sample was scanned twice and the average spectra were used for calibration.

Stastistics: NIRS 3, version 3.10 (ISI) software for MS DOS was used to evaluate the data and to develop chemometric models (ANONYM 1995). Scatter correction was performed by Standard Normal Variate Transformation (SNV) and detrend method and then transformed with the first derivative, which was calculated by using a subtraction gap and smoothing segment of 4 data points (treatment 1,4,4). Calibration was used by Modified Partial Least Square (MPLS) regression and the correlation coefficient (R) was determined. During development of MPLS calibration model some samples with higher standard error of calibration were found. These samples impair calibration model and therefore they were rejected as outliers. Six outlying samples of total solids and protein, and five outlying samples of fat were removed because of their higher deviations. The calibration of the compositional analysis of milk was verified by segmented cross validation (8 segments chosen from the set). The selection of optimum number of PLS terms for the calibration was based on the standard error of prediction (SEP), which should be minimised. The statistical parameters (R and SEP) were used to determinate the final calibration equation.

RESULTS AND DISCUSSION

The NIR absorbance spectrum of raw cow milk samples with different fat content is presented in Fig. 1. The obvious absorption bands located around 1450 nm and 1940 nm are due to the content of water in the sample. Small peaks at 1720 nm and 1760 nm correspond to a CH$_2$ group, which is typical of fat (DÍAZ-CARRILLO *et al.* 1993).

The correlation coefficient R and standard error of prediction SEP for all constituents obtained by MPLS (using a full spectrum) are shown in Table 1. In the case of the total solids and fat content, the best values were obtained when treatment 1,4,4 was used, and when scatter correction was made by SNV and detrend, both with 4 terms in

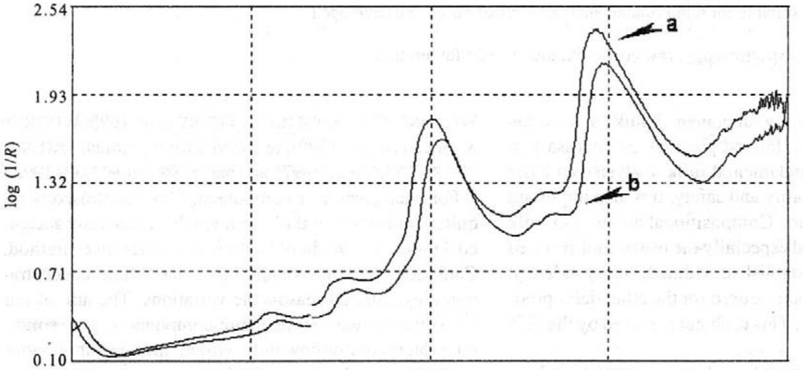

Fig. 1. Examples of NIR spectra of raw cow milk (a – low fat milk; b – high fat milk)

Table 1. The best calibration results for prediction of major components in raw cow milk

Component	Amount of samples	No. of PLS terms	Transformation	R	SEP [%]
Total solids	59	4	1st derivative SNV + detrend	0.975	0.160
Fat	60	4	1st derivative SNV + detrend	0.967	0.179
Protein	59	6	1st derivative None	0.965	0.049

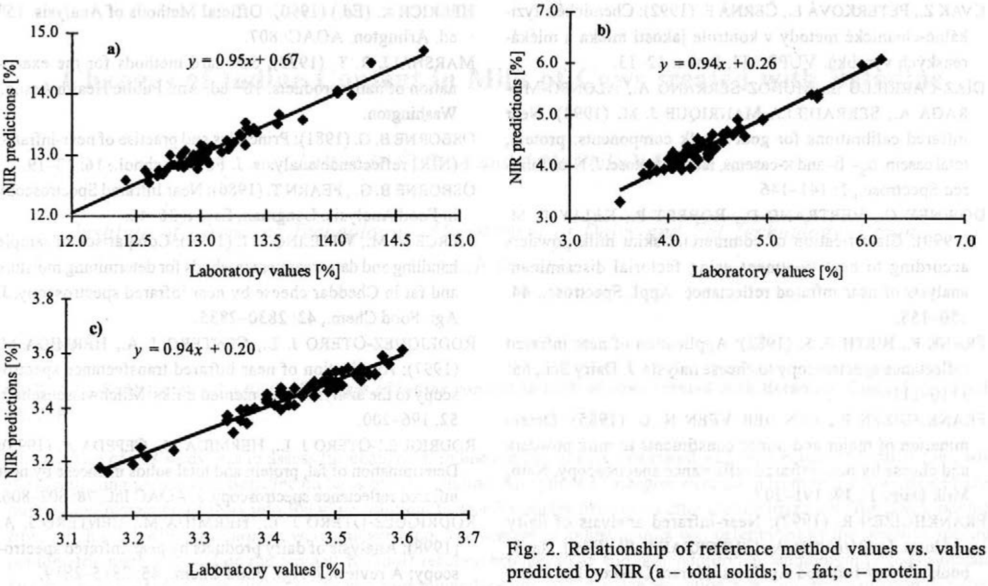

Fig. 2. Relationship of reference method values vs. values predicted by NIR (a – total solids; b – fat; c – protein]

the calibration. For the calibration of protein content the best treatment also turned out to be 1,4,4 without scatter correction, with 6 terms. Very similar values were obtained with the use of different mathematical treatments. The absolute value of correlation coefficient *R* should approach 1 and the standard error of prediction (SEP) should be in the same range as the reference method error.

For protein analysis, the narrow analyte span reduces the size of the correlation coefficient. Improvements could be expected if the protein calibration range would be extended. Calibration results of proteins are also influenced by variability of non-protein nitrogen content. The protein values are in agreement with published literature (HALL & CHAN 1993); the total solids of raw milk are not mentioned in literature. HALL & CHAN (1993) published a higher correlation coefficient ($R = 0.999$) for fat in comparison to the result obtained in our experiment. It could be explained by the different range of fat content (0.05–5.47%) in the quoted literature, which does not correspond with raw cow milk. The range of fat content of raw cow milk samples used in this work is summarized in Table 1. The narrow analyte reduces the magnitude of the correlation coefficient and in these instances, the SEP better indicates analytical performance (HALL & THOMAS 1993), but in the mentioned article (HALL & CHAN 1993) only standard error of calibration (SEC) is given. These authors have SEC for fat 0.04%, we obtained SEC = 0.12% probably because different reference methods were used in the compared studies (Mojonnier, resp. Gerber method). The accuracy of NIR method depends on the used concentration range and on accuracy of reference method.

The linear dependence of the reference results versus results predicted by the MPLS algorithm for determination of total solids, fat and protein in raw cow milk is shown in Fig. 2. Obviously, there is a good correlation between predicted values and known chemical reference values. The slope of the linear regression line for all constituents was close to the value 1; the intercept of the linear regression line for all constituents was close to the value 0. It proves good the agreement between the results obtained from the NIR measurement and the reference method. In our study, we obtained the spectra by the use of the fiber optic probe, whereas the spectra in published literature (HALL & CHAN 1993) were collected by the use of cuvette in diffuse transmittance. Fiber optic probe has advantages of faster application and easier way of cleaning and maintenance than the use of cuvettes. Moreover fiber optic probe enables connection of NIR instrument with a processing line, which provides real time information, saves raw material and enhances the quality of final product.

Conclusions

NIR spectroscopy is a suitable technique for rapid analysis of raw cow milk without any sample pre-treatment and is acceptable for industrial practice.

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Souhrn

KUKAČKOVÁ O., ČURDA L., JINDŘICH J. (2000): **Využití multivariační analýzy v NIR spektroskopii syrového mléka.** Czech J. Food Sci., **18**: 1–4.

V současné době se pro rychlé stanovení složení mléka používá spektroskopie ve střední infračervené oblasti, přičemž měření v blízké infračervené oblasti má ve srovnání s MIR některé výhody. Cílem této studie bylo ověřit možnosti stanovení sušiny, tuku a bílkovin. Bylo změřeno 65 vzorků syrového nakonzervovaného mléka na přístroji NIRS 6500 (NIRSystems, Inc.) s použitím vláknové optiky. Pro kalibraci byly provedeny srovnávací analýzy – sušina byla stanovena vážkově, tuk Gerberovou metodou a bílkoviny podle Kjeldahla. Kalibrace byla vyhodnocena pomocí spektrálního analytického programu NIRS 3 užitím modifikované metody nejmenších čtverců (MPLS). Pro sušinu byla zjištěna hodnota korelačního koeficientu $R = 0,975$ a standardní odchylky predikce SEP = 0,160 %, pro tuk $R = 0,967$ a SEP = 0,179 % a pro bílkoviny $R = 0,965$ a SEP = 0,049 %. Lze konstatovat, že metodu NIR je možné použít pro rychlé stanovení základních složek mléka.

Klíčová slova: NIR spektroskopie; syrové kravské mléko; sušina; tuk; bílkoviny

Corresponding author:

Ing. OLGA KUKAČKOVÁ, Vysoká škola chemicko-technologická, Ústav technologie mléka a tuků, Technická 5, 166 28 Praha 6, Česká republika, tel.: + 420 2 24 35 32 65, fax: + 420 2 31 19 990, e-mail: olga.kukackova@vscht.cz