Analysis of Cow Milk by Near-infrared Spectroscopy

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Abstract

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In this work, the major components (total solids, fat, protein, casein, urea nitrogen, lactose, and somatic cells) were determined in cow milk by near-infrared spectroscopy. Fifty calibration samples of milk were analysed by reference methods and by FT NIR spectroscopy in reflectance mode at wavelengths ranging from 4000 to 10 000 cm^{-1} with 100 scan. Each sample was analysed three times and the average spectrum was used for calibration. Partial least squares (PLS) regression was used to develop calibration models for the milk components examined. Determined were the highest correlation coefficients for total solids (0.928), fat (0.961), protein (0.985), casein (0.932), urea nitrogen (0.906), lactose (0.931), and somatic cells (0.872). The constructed calibration models were validated by full cross validation. The results of this study indicated that NIR spectroscopy is applicable for a rapid analysis of milk composition.

Keywords: near-infrared spectroscopy; milk; fat; protein

The traditional methods for determining the quality of milk and its major components are slow and expensive. Near-infrared spectroscopy of foodstuffs is a relatively recent technique. The advantages of NIR spectroscopy compared to the present methods include a higher rapidity and a simultaneous, non-destructive measurement of a number of milk constituents as well as a great potential for on-line analysis. This method was used to measure the contents of various constituents in homogenised milk (Sato *et al.* 1987; Rodriguez-Otero *et al.* 1997; Ru & Glatz 2000). Tsenkova *et al.* (1999, 2000) reported on the analysis of non-homogenised milk samples; these authors determined the highest positive coefficients for fat, lactose, and total protein. Kukačková *et al.* (2000) used a fibre optic probe to analyse raw milk.

Our work deals with the determination of the major components (total solids, fat, protein, casein, urea nitrogen, lactose and somatic cells) in non-homogenised cow milk in reflectance mode.

MATERIAL AND METHODS

About 50 samples of cow milk were analysed for calibration and validation of the calibration performed. A wavelength scanning instrument, FT NIR was used with a scanning range from 4000 to 10 000 cm^{-1} and with 100 scan in reflectance mode. Samples of milk were warmed to 40°C, agitated, cooled to 20°C and transferred to Petri bowls. The measured area was spaced by a metallic mirror. Each sample was analysed three times and the average spectrum was used for calibration. The whole spectrum area has been tested. The same samples were employed for full cross validation by software FT NIR Reference Analysis. Total solids were determined by drying a known mass of milk at 102 ± 1°C and subsequent weighing to determine the mass loss (Cvak *et al.* 1992). Fat content was determined by Gerber method (Marshall 1992). Total protein and casein were determined spectrophotometrically

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using the apparatus PRO-MILK from Foss Electric, Denmark. Urea nitrogen was determined spectrophotometrically at the wavelength of 420 nm after the reaction with p-dimethylaminobenzaldehyde (Gajdůšek *et al.* 1996). Lactose was determined polarimetrically (Cvak *et al.* 1992). Somatic cell count was determined on Fossomatic apparatus from Foss Electric.

RESULTS AND DISCUSSION

The results are recapitulated in Table 1. All results were evaluated using the variation statistics (ANOVA) of the statistic package Unistat (Table 2).

The calibration model was created by PLS algorithm (Partial least squares). PLS factors include spectral interference and information on concentration. PRESS dependence on factors used on calibration with PLS method is an important diagnostic implement. It can calculate the optimal number of factors.

In an optimal course, PRESS shows a sharp fall and the next fall is gradual. The optimal number of PLS factors will be found if the value of PRESS is minimal. A high number of PLS factors indicates a decreasing ability of prediction because PRESS includes spectral interference. The numbers of PLS factors for urea nitrogen, fat, total solids, and somatic cells were low which demonstrated the robustness of these models (Table 1). Tsenkova *et al.* (1999, 2000) reported PLS factors for fat, total protein, and lactose to be from 8 to 10 which corresponds to our results on PLS factors for protein (14), casein (10) and lactose (11).

Dependable calibration takes place in the case of the value of the calibration coefficient of variation CCV being lower than 5%, and the value of the prediction coefficient of variation being lower than 10%. For all components (with the exception

Table 1. The calibration and validation of the results obtained in the determination of the components in raw cow milk

Calibration component	*n*	Average	Number PLS factors	Transformation
Solids (%)	50	12.64	6	none
Fat (%)	56	4.16	4	none
Protein (%)	60	3.30	14	none
Casein (%)	57	2.62	10	none
Urea nitrogen (mg/100 ml)	52	11.19	2	none
Lactose (%)	56	4.91	11	none
Somatic cells (1000/ml)	45	244.8	2	none

Calibration component	Calibration			Cross validation		
	R	SEC	CCV (%)	*R*	SEP	PCV (%)
Solids (%)	0.9277	0.46	3.64	0.8958	0.55	4.35
Fat (%)	0.9612	0.30	7.21	0.9534	0.33	7.93
Protein (%)	0.9848	0.05	1.37	0.9504	0.08	2.42
Casein (%)	0.9319	0.09	3.44	0.8508	0.13	4.96
Urea nitrogen (mg/100 ml)	0.9058	1.63	14.57	0.8251	2.18	19.48
Lactose (%)	0.9307	0.08	1.63	0.7498	0.15	3.05
Somatic cells (1000/ml)	0.8720	65.21	26.64	0.8538	69.51	28.39

R – correlation coefficient; SEC – standard error of calibration; SEP – standard error of prediction; CCV – calibration coefficient of variation; PCV – prediction coefficient of variation; *n* – number of samples

Table 2. Results of ANOVA (P = 0.05)

Calibration component	n	xNIR	xREF	d	s_d	P	F_{crit}
Protein (%)	60	3.3015	3.3017	–0.0002	0.0452	0.9972	3.9215
Fat (%)	56	4.1643	4.1643	0.0000	0.3007	1.0000	3.9274
Solids (%)	50	12.6362	12.6362	0.0000	0.4591	1.0254	3.9381
Casein (%)	57	2.6219	2.6215	0.0004	0.0892	0.9938	3.9258
Lactose (%)	56	4.9104	4.9109	–0.0005	0.0794	0.9892	3.9274
Urea nitrogen (mg/100 ml)	52	11.1904	11.1898	0.0006	1.6309	0.9994	3.9342
Somatic cells (1000/ml)	45	244.8222	244.8000	0.0222	65.2419	0.9993	3.9493

n – number of samples; xNIR – average of NIR values; xREF – average of reference values; d – difference between average NIR and reference values

s_d – standard error of difference; P – statistics values ; F_{crit} – significance factor

of urea nitrogen and somatic cells) the values of the coefficients of variation were smaller than 5%. With urea nitrogen and somatic cells, the value of the prediction coefficient of variation was higher than 10%.

The determined correlation coefficients for calibration were for total solids 0.928, fat 0.961, protein 0.985, casein 0.932, urea nitrogen 0.906, lactose 0.931 and somatic cells 0.872, and the correlation coefficients for validation found were for total solids

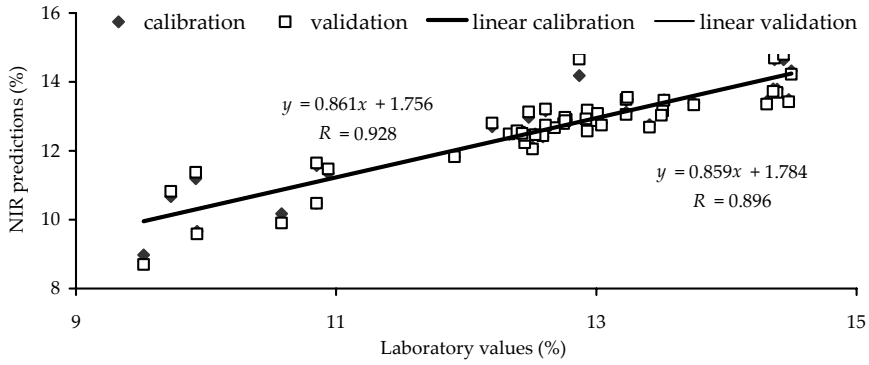

Figure 1. Calibration and validation results of solids

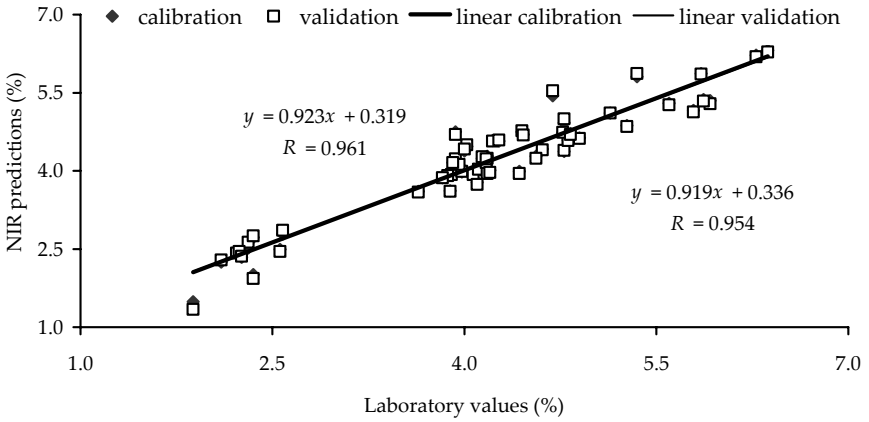

Figure 2. Calibration and validation results of fat

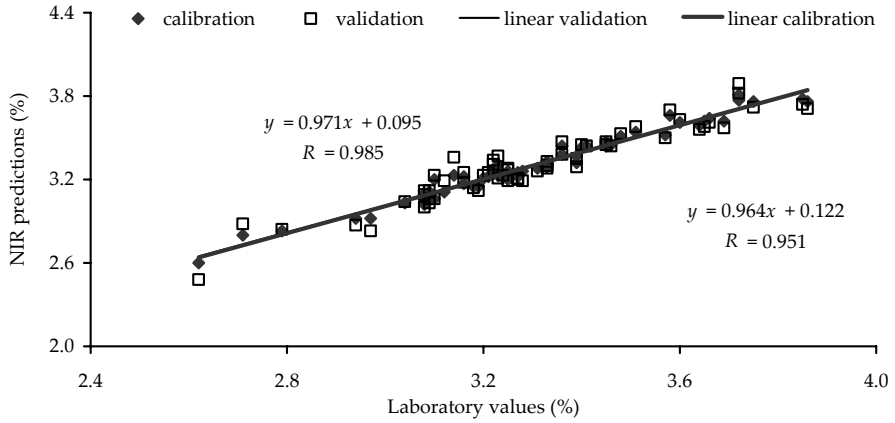

Figure 3. Calibration and validation results of protein

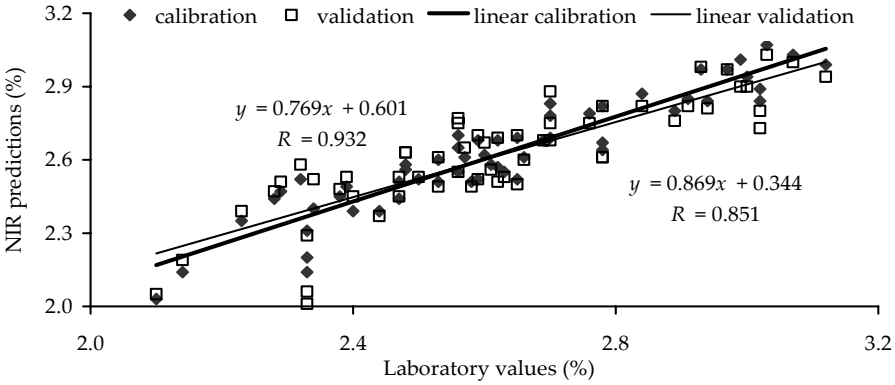

Figure 4. Calibration and validation results of casein

0.896, fat 0.953, protein 0.950, casein 0.851, urea nitrogen 0.825, lactose 0.750, and somatic cells 0.854 (Table 1, Figures 1–7). Tsenkova *et al.* (1999, 2000) and Ru and Glatz (2000) obtained similar results for non-homogenised milk. Kukačková *et al.* (2000) found the best calibration results for the prediction of total solids 0.975, fat 0.967, and protein 0.965.

The results of the reference values of samples and of the calculated values of NIR were statistically analysed by ANOVA test in UNISTAT. Statistically significant differences were not found between the

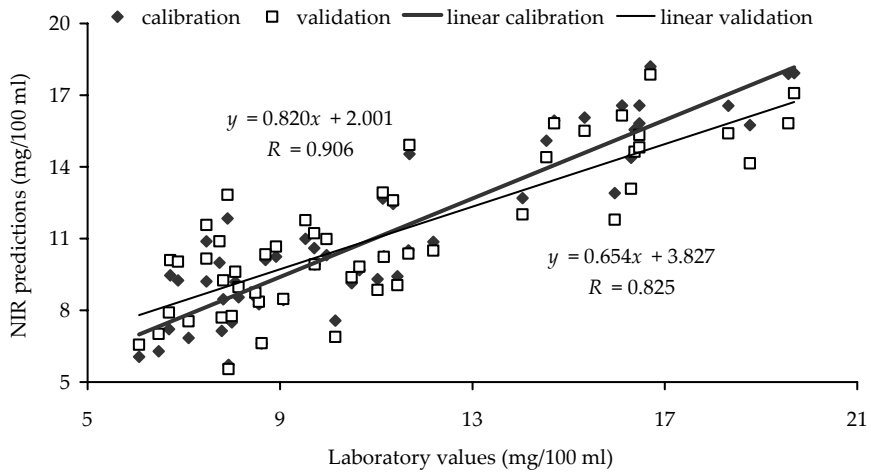

Figure 5. Calibration and validation results of urea nitrogen

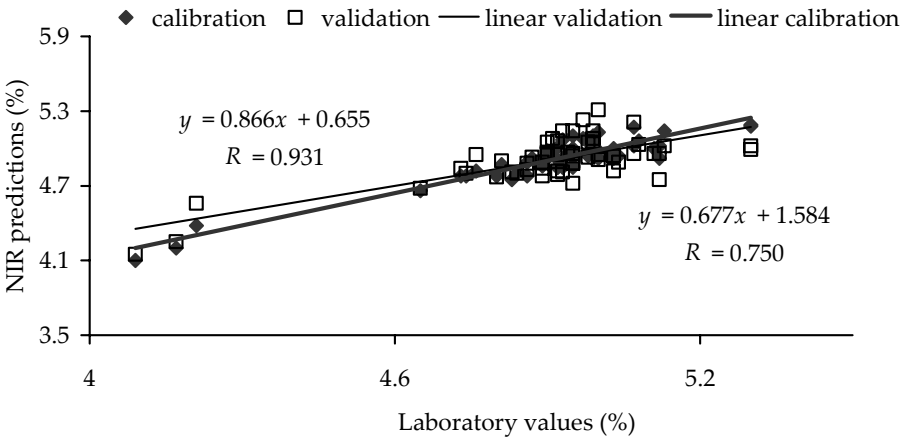

Figure 6. Calibration and validation results of lactose

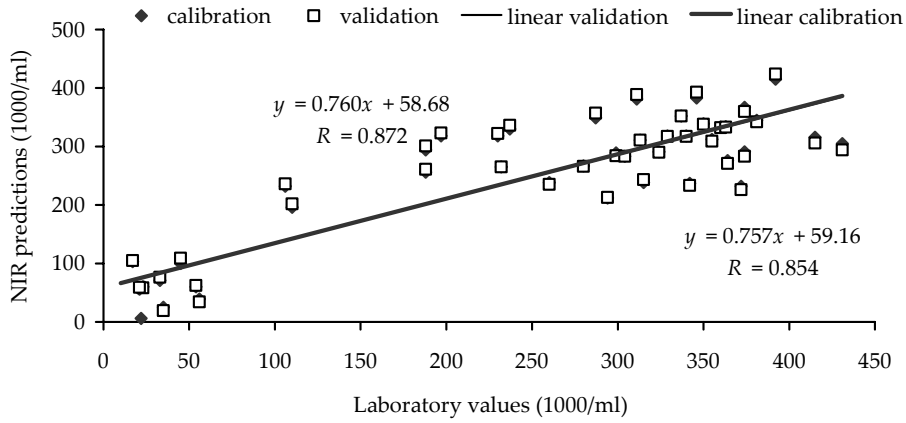

Figure 7. Calibration and validation results of somatic cells

reference values and the calculated values of NIR (Table 2).

Conclusions

Correlation coefficients obtained for the components of calibration were higher than 0.90 (with the exception of somatic cells). The correlation coefficients were determined as follows: for total solids 0.928, fat 0.961, protein 0.985, casein 0.932, urea nitrogen 0.906, lactose 0.931, and somatic cells 0.872. The coefficient of correlation for lactose is low, together with a high number of PLS factors it shows a small degree of robust method. The whole calibration is influenced by three samples with a very low concentration of lactose. It is necessary to make this calibration for lactose more accurate.

Cross validation indicates the possibility of using NIR spectrometry to determine the basic ingredients of milk. The correlation coefficients of validation were for total solids 0.896, fat 0.953, protein 0.950, casein 0.851, urea nitrogen 0.825, lactose 0.750, and somatic cells 0.854.

Statistically significant differences between the reference values and the calculated values of NIR were not found.

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Souhrn

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Pomocí NIR spektroskopie jsme stanovovali obsah jednotlivých složek nehomogenizovaného kravského mléka, především obsahu sušiny, tuku, celkových bílkovin, kaseinu, laktosy, močovinového dusíku a somatických buněk. Měření bylo prováděno u 50 vzorků mléka na přístroji FT NIR Nicolet Antaris v rozsahu vlnových délek 4 000–10 000 cm^{-1} se 100 scany a rozlišením 8. Vzorky mléka byly zahřáty na 40 °C, protřepány a ochlazeny na 20 °C. IR spektra vzorků byla měřena na integrační sféře v režimu reflektance (technika měřící absorpci záření po odrazu paprsku od povrchu vzorku, který byl umístěn v Petriho misce, a měřící prostor vymezen zrcátkem). Kalibrace byla vyhotovena pomocí PLS metody. Každý vzorek byl proměřen třikrát a pro kalibraci bylo použito průměrné spektrum. Vytvořené kalibrační modely pro jednotlivé složky mléka byly ověřeny křížovou validací. Pro vytvoření validačního modelu byla použita stejná sada vzorků jako pro kalibraci. Zhodnocení výsledků bylo provedeno na základě korelace mezi referenčními hodnotami a hodnotami vypočtenými z kalibračních rovnic a na základě směrodatných odchylek kalibrace a validace (SEC a SEP). Zjištěné korelační koeficienty pro kalibrace: sušina 0,928, tuk 0,961, čisté bílkoviny 0,985, kasein 0,932, močovinový dusík 0,906, laktosa 0,931 a somatické buňky 0,872. Korelační koeficienty pro validaci byly zjištěny pro sušinu 0,896, tuk 0,953, bílkoviny 0,950, kasein 0,851, močovinový dusík 0,825, laktosu 0,750 a somatické buňky 0,854. Hodnoty sledovaných ukazatelů složení mléka naměřené NIR spektrofotometrií byly metodou ANOVA statisticky porovnány s hodnotami naměřenými referenčními metodami. Mezi oběma metodami stanovení nebyly zjištěny statisticky průkazné rozdíly.

Klíčová slova: NIR spektroskopie; mléko; bílkoviny, tuk

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