ANALYSIS OF SHEEP COLOSTRUM BY NEAR-INFRARED SPECTROSCOPY

ANALÝZY OVČÍHO MLEZIVA NIR SPEKTROSKOPIÍ

Jankovská R., Šustová K., Kráčmar S.

Ústav technologie potravin, Agronomická fakulta, Mendelova zemědělská a lesnická univerzita v Brně, Zemědělská 1, 613 00 Brno, Česká republika.

E-mail: rjankov@mendelu.cz

ABSTRACT

Our work deals with of changes in basic composition of sheep colostrum (during first 72 hours after parturition) and the possibility of determination the major components and total essential, total nonessential and total amino acids in sheep colostrum by near-infrared spectroscopy. Changes in colostral levels of dry matter, protein, fat, lactose, pH, total essential, total nonessential and total amino acids were followed in 10 cross-bred (F_{111}) ewes of East-Friesian breed x Improved Valachian sheep (EF87IV) on the 2nd and subsequent lactations within the time interval of 2 to 72 hours after parturition. About 90 samples of sheep colostrum were analysed by reference methods and by FT NIR spectrophotometer was used with a scanning range from 4 000 to 10 000 cm^{-1} and with 100 scan in reflectance mode. Samples of sheep colostrum were warmed to 40 °C, agitated, cooled to 20 °C, and transferred to Petri dishes. 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. Partial least squares PLS regression was used to develop calibration models for examined sheep colostrum. They were determined the highest correlation coefficient for crude protein 0.985, true protein 0.983, dry matter 0.994, fat 0.965, lactose 0.866 pH 0.832, ΣEAA 0.940, ΣNEAA 0.958 and ΣAA 0.977. The same samples were employed for full cross validation by software FT NIR Reference Analysis. Statistically significant differences between the reference values and the calculated values of NIR were not found (p=0.05).

Keywords: Near-infrared spectroscopy; Sheep colostrum; Protein; Fat; Lactose; pH; Amino acids

ABSTRAKT

Práce řeší změny základních složek ovčího mleziva (sušiny, dusíkatých látek, čistých bílkovin, tuku, laktózy, pH a aminokyselinového složení) v průběhu 2 – 72 hodin po porodu a možnost použití blízké infračervené spektroskopie pro stanovení těchto složek. Měření bylo prováděno u 90 vzorků mleziva na přístroji FT NIR Antaris v rozsahu vlnových délek od 4 000 do 10 000 cm^{-1}. Kalibrace byla vyhotovena pomocí metody PLS (metoda nejmenších částečných čtverců). 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, SEP). Bylo dosaženo poměrně vysokých korelačních koeficientů kalibrace pro dusíkaté látky 0.985, čisté bílkoviny 0.983, sušinu 0.994, tuk 0.965, laktózu 0.866 a pH 0.832. Hodnoty sledovaných ukazatelů byly statisticky metodou ANOVA porovnány s hodnotami naměřenými referenčními metodami. Nebyly zjištěny statisticky průkazné rozdíly mezi oběma metodami stanovení (p=0.05).

Klíčová slova: blízká infračervená spektroskopie; ovčí mlezivo; sušina; dusíkaté látky; tuk; laktóza; pH; aminokyseliny

INTRODUCTION

Near infrared spectroscopy (NIR) covers the range of the electromagnetic spectrum between 780 and 3000 nm. In this range it is possible to study low energy electronic transitions, overtones, and combinations of hydrogen stretching and bending vibrations (C-H, N-H, O-H) that have high frequencies and are well suited to quantitative analysis applications. A NIR spectrum can provide useful information about hydrogen bearing functional groups in a molecule even though it does not necessarily characterise a complete structure.

Traditional methods of evaluation of the quality of milk and its major components are relatively slow and rather expensive. Near-infrared spectroscopy of foodstuffs is a new analytical method. The advantages of NIR involve above all higher rapidity, simultaneous, non-destructive measurement of a number of milk constituents and a great potential for on-line analysis. For each particular component, NIR spectroscopy requires calibration of the instrument to a recognised laboratory method know as a reference method. Calibration samples should cover the whole concentration range and all reasonable variations.

This method was used to estimate the content of various constituents in both homogenised non-homogenised milk samples (Sato et al., 1987; Rodriguez-Otero et al., 1997; Ru and Glatz, 2000). Tsenkova et al. (1999, 2000) found in raw, non-homogenised milk the highest correlation coefficients for fat, lactose and total protein. Kukačková et al. (2000) evaluated the total solids, fat and protein in raw milk using a fibre optic probe. Tarkošová and Čopíková (2000) used the FT-NIR spectroscopy to establish calibration equations with the aim of determining sucrose, lactose, fat and moisture in chocolate. Jankovská and Šustová (2003) determined the major components (total solids, fat, protein, casein, urea nitrogen, lactose, and somatic cells) in non-homogenised cow milk.

The aim of our work was to evaluate the major components (dry matter, protein, fat, lactose, pH) and essential, nonessential and total amino acids in non-homogenenised sheep colostrum without the need of previous preparation of the sample by NIR spectroscopy.

MATERIALS AND METHODS

Colostrum samples were taken 2, 12, 24, 36, 48 and 72 hours after parturition. After sampling, colostrum was homogenised in batches of ca 100 – 300 ml and the homogenised samples were frozen. Following traits were determined: dry matter (gravimetrically, drying a known mass of milk at 102±1 °C and weighing it afterwards to determine the loss mass), total nitrogen according to Kjeldahl using Kjeltec Auto 1031 Analyzer (manufacturer Tecator), crude protein was calculated as N x factor of 6.37, true protein (spectrophotometrically; the apparatus Pro-Milk II, Foss Electric, Denmark), fat (Gerber method), lactose (polarimetrically as monohydrate), values of pH using the apparatus Inola (Germany). Contents of amino acids in colostrum samples were simultaneously estimated using the classical chromatographic analysis. The used reference method were analysed by Official Journal L 206, 29/07/1978 P. 0043 – 0055.

About 90 samples of sheep colostrums were analysed for calibration and validation of the calibration performed. A wavelength scanning instrument FT NIR, was used with a scanning range from 4 000 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 dishes. 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 calibration model was created by PLS algorithm (partial least squares). The same samples were employed for full cross validation by software FT NIR Reference Analysis.

All results were evaluated using the variation statistic analysis (ANOVA). Correlation matrices and regression function were calculated according to Snedecor and Cohran (1967) when using the statistical package Microsoft® Excel 2000 and Unistat 5.1.

RESULTS AND DISCUSSION

The purpose of this study was see the possibility of determining the crude protein (CP), the true protein (TP), the dry matter (DM), the fat (F), the lactose (L) the pH, the total essential (ΣEAA), the total nonessential (ΣNEAA) and the total amino acids (ΣAA) by NIR spectroscopy.

Table 1 shows the average of reference values and the number of PLS factors. Figure 2 explains how a predicted error sum of squares (PRESS) plot was used to determine the optimal number of PLS factors for the dry matter. The PLS factor is a set of principal components that contain spectral and concentration information and so describe the variations in a PLS method. The optimal number of factors can be chosen as a first local minimum in the PRESS plot. The numbers of PLS factors for CP, DM, F, L, pH, ΣEAA, ΣNEAA, ΣAA were low which demonstrated the robustness of these models. The high number of PLS factors for true protein shows a small degree of robust method. Figure 1 shows a good correlation between predicted values and known reference values for dry matter.

Table 2 and 3 show calibration and validation results provided by the PLS algorithm for determination of crude and true protein, dry matter, fat, lactose, pH, total essential, total nonessential and total amino acids. The following statistical values (correlation coefficient r and standard error of calibration SEC, Table 2) were obtained: 0.985 and 0.69% for CP; 0.983 and 0.34% for TP; 0.994 and 0.47% for DM; 0.965 and 0.47% for F; 0.866 and 0.67% for L; 0.832 and 0.09% for pH; 0.940 and 0.64% for ΣEAA; 0.958 and 0.59% for ΣNEAA; 0.977 and 0.85% for ΣAA. Cross validation was used to prove the performance of the calibration model and indicates the possibility of using NIR spectrometry to determine the basic ingredients of sheep colostrum. The same samples were employed for full cross validation (correlation coefficient r and standard error of prediction SEP, Table 3). In table 2 and 3 are described the statistically significant differences for correlation coefficient of calibration and validation. Tsenkova et al. (1991, 2000) and Ru and Glatz (2000) determined analogous results of non-homogenised cow 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 for the cow milk. Tarkošová and Čopíková (2000) investigated the use of NIR spectroscopy for determination of sucrose 0.980, lactose 0.920, fat 0.970 and moisture 0.970 in the chocolate. The results obtained indicated that NIR spectroscopy could be related to the composition analysis of chocolate. Jankovská and Šustová (2003) also concluded that NIRS is a suitable method for a rapid analysis of milk composition. They found the following values of correlation coefficients of calibration: 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 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 reference values and the calculated values of NIR (Table 4).

CONCLUSIONS

Near infrared spectroscopy combined with PLS regression is a simple and rapid technique for compositional analysis of raw milk. The major components, namely crude protein, dry matter and fat, can be determined with sufficient accuracy. The lactose and pH cannot be determined with acceptable precision. Statistically significant differences between the reference values and the calculated values of NIR were not found. NIR spectroscopy is a suitable technique for rapid analysis of sheep colostrums without any sample pre-treatment and is acceptable for industrial practice.

Supported by the Ministry of Education, Youth and Sports of the Czech Republic (Project No. 4321 00001).

REFERENCES

Jankovská R., Šustová K. (2003): Analysis of cow milk by near-infrared spectroscopy. Czech J. Food Sci., *21*, 4, 123 - 128.

Kukačková O., Čurda L., Jindřich J. (2000): Multivariate calibration of raw cow milk using NIR spectroscopy. Czech J. Food Sci., *18*, 1, 1 - 4.

Tarkošová J., Čopíková J. (2000): Fourier transform near infrared spectroscopy applied to analysis of chocolate. Near Infrared Spectrosc., *8*, 251-257.

Tsenkova, R., Atanassova, S., Toyoda, K., Ozaki, Y., Itoh, K., Fearn, T. (1999): Near-Infrared spectroscopy for dairy management: measurement of unhomogenized milk composition. J. Dairy Sci., *82*, 2344-2351.

Official Journal L 206, 29/07/1978 P. 0043 – 0055: Eighth Commission Directive 78/633/EEC of 15 June 1978 establishing Community methods of analysis for the official control of feedingstuffs.

Tsenkova, R., Atanassova, S., Itoh, K., Ozaki, Y., Toyoda, K. (2000): Near infrared spectroscopy for bio monitoring: Cow milk composition measurement in a spectral region from 1,100 to 2,400 nanometers. American Sci. of Animal., *78*, 515-522.

Rodriguez-Otero, J.L., Hermida, M., Centeno, J. (1997):Analysis of Dairy Products by Near-Infrared Spectroscopy: A Rewiew. J. of Agric. Food Chem., *45*, 8, 2815–2818.

Ru, Y.J., Glatz, P.C. (2000): Application of Near Infrared Spectroscopy (NIR) for Monitoring the Quality of Milk, Cheese, Meat and Fish. Asian-Aus. J. Anim. Sci., *13*, 7, 1017–1025.

Sato, T., Yoshino, M., Farukawa, S., Someya, Y., Yano, N., Uozumi, J., Iwamoto, M. (1987): Analysis of milk constituents by the near infrared spectrophotometer method. Jpn. J. Zootech. Sci., *58*, 698-706.

Tab. 1: Calibration components

Calibration components	n	Average	Number PLS factors
Crude protein (%)	90	9,67	5
True protein (%)	87	8,19	10
Dry matter (%)	84	22,66	5
Fat (%)	87	7,92	5
Lactose (%)	90	4,07	3
pH	87	6,59	3
ΣEAA	90	4.47	5
ΣNEAA	90	4.67	7
ΣAA	90	4.47	5

Tab. 2: Parameters of the regression function $y'_i = a + bx_i$ for the calibration model

Components	a ± bx$_i$			SEC	CCV(%)	r	
Crude protein (%)	0.2858	+	0.9704	0.69	7.14	0.985	
True protein (%)	0.2826	+	0.9827	0.34	4.15	0.983	**
Dry matter (%)	0.2770	+	0.9877	0.47	2.07	0.994	*
Fat (%)	0.5488	+	0.9309	0.47	5.93	0.965	*
Lactose (%)	1.0160	+	0.7505	0.67	16.46	0.866	
pH	2.0732	+	0.6855	0.09	1.37	0.832	***
ΣEAA	0.5147	+	0.8849	0.64	0.10	0.940	
ΣNEAA	0.3822	+	0.9182	0.59	1.30	0.958	
ΣAA	0.4214	+	0.9548	0.85	1.90	0.977	

Tab. 3: Parameters of the regression function $y'_i = a + bx_i$ for the validation model

Components	a ± bx$_i$			SEP	PCV(%)	r	
Crude protein (%)	0.4208	+	0.9544	0.94	9.72	0.972	
True protein (%)	1.3127	+	0.8424	0.92	11.23	0.871	
Dry matter (%)	0.5188	+	0.9770	0.68	3.00	0.988	
Fat (%)	0.8437	+	0.8917	0.62	7.83	0.943	
Lactose (%)	1.2395	+	0.6920	0.81	19.90	0.797	
pH	2.3454	+	0.6441	0.11	1.67	0.768	***
ΣEAA	0.6030	+	0.8662	0.82	1.80	0.901	
ΣNEAA	0.4350	+	0.8993	0.79	1.70	0.923	
ΣAA	0.2740	+	0.9782	1.38	3.10	0.943	

* = P<0.05; ** = P<0.01; *** = P<0.001

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

Tab. 4: Parameters of basic components in sheep colostrum as estimated by NIR reference values and their mutual comparison by paired T-test

Components	n	xNIR	xREF	d	S.D.
Crude protein (%)	90	9.670	9.670	0.000	0.69
True protein (%)	87	8.187	8.187	0.001	0.34
Dry matter (%)	84	22.662	22.661	0.000	0.47
Fat (%)	87	7.926	7.939	-0.013	0.47
Lactose (%)	90	4.073	4.073	0.000	0.67
pH	87	6.594	6.593	0.000	0.09
∑EAA	90	4470.20	4470.20	0.000	0.63
∑NEAA	90	4670.97	4670.93	0.040	0.58
∑AA	90	9322.50	9322.86	-0.360	0.84

xNIR = mean of the NIR values; xREF = mean of the reference values; d = difference of mean of NIR and reference values

Figure 1. Calibration and validation results of dry matter

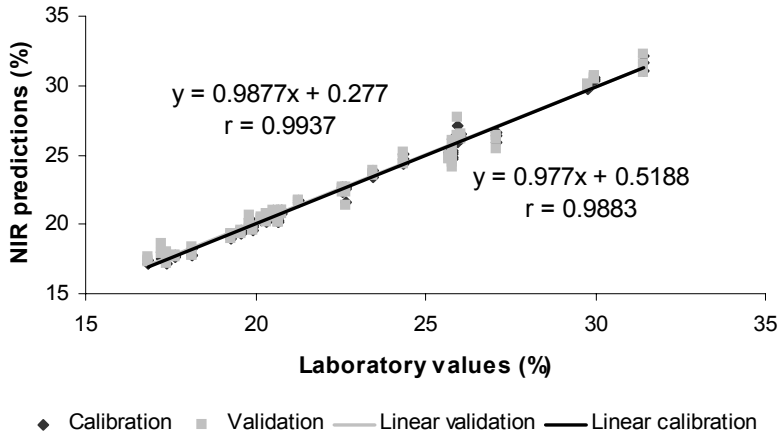

Figure 2. Predicted residual error sum of squares (PRESS) plot for dry matter