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ANALYSIS OF EWE'S MILK BY FT NEAR INFRARED SPECTROSCOPY: MEASUREMENT OF SAMPLES ON PETRI DISHES IN REFLECTANCE MODE

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Abstract

ŠUSTOVÁ, K., KUCHTÍK, J., KRÁČMAR, S.: *Analysis of ewe's milk by FT Near Infrared spectroscopy: measurement of samples on Petri dishes in reflectance mode*. Acta univ. agric. et silvic. Mendel. Brun., 2006, LIV, No. 2, pp. 131–138

Our work deals with a possibility of determination of basic composition (dry matter, fat, protein, casein, lactose and urea nitrogen) of ewe's milk and colostrum by FT NIR spectroscopy. Samples of milk were warmed to 40 °C, agitated, cooled to 20 °C, transferred into Petri dishes and analysed by reference methods and by FT NIR in reflectance mode. The measured area was spaced by a metallic mirror. Statistically significant differences between the reference values and the calculated values of NIR were not found (p=0.05). Results of calibration for ewe's milk determined the highest correlation coefficients: dry matter 0.983, fat 0.989, true protein 0.997, casein 0.977, lactose 0.980 and urea nitrogen 0.973. The study showed that NIRS method, when samples of milk are measured on Petri dishes, is a useful technique for the prediction of dry matter, fat, protein and casein in ewe's milk.

near-infrared spectroscopy, ewe's milk, dry matter, fat, protein, lactose, chemical composition

There are many factors which shape a quality of ewe's milk. In general, the composition of milk changes significantly due to the action of many factors in the course of whole lactation, (JELÍNEK et al., 1991; ŽIŽLAVSKÝ et al., 1989; KRÁČMAR et al., 1998; ANTUNOVIČ et al., 2001; GAJDŮŠEK et al., 2003). Traditional methods of the estimation of quality of milk and its major components (Kjeldahl method, Gerber method) are relatively slow and rather expensive. The Milko-Scan is used as an instrumental method for cow´s milk analysis (O'SULLIVAN et al., 1999). NIR spectroscopy of foodstuffs is one of the new analytical methods. It has numerous advantages, e.g. rapidity, non-destructive sample analysis and, especially, a great potential for on-line analysis (RU & GLATZ, 2000; ALBANELL et al., 2003). FT NIR method has been used to measure the content of various constituents in homogenised cow milk (SATO et al., 1987; RODRIGUEZ-OTERO, HERMIDA & CENTENO, 1997; RU & GLATZ, 2000; ŠAŠIČ & OZAKI, 2001). A few authors have reported analysis of non-homogenised milk samples (CHEN, IYO & KAWANO, 1999; LAPORTE & PAQUIN, 1999). TSENKOVA et al., 1999; TSENKOVA et al., 2000 and TSENKOVA, ATANASSOVA, & TOYODA, 2001) determined the highest positive coefficients for fat, lactose and crude protein in non-homogenised milk. KUKAČKOVÁ, ČURDA & JINDŘICH, 2000 and TURZA et al., 2002 evaluated the total solids, fat and protein in raw milk using a fibre optic probe. JANKOVSKÁ & ŠUSTOVÁ, 2003 determined the major components (total solids, fat, protein, casein, urea nitrogen, lactose, and somatic cells) in non-homogenised cow milk by reflectance mode on the Petri dishes. Few applications of NIRs techniques for analysis of goat's milk (ALBANELL et al., 2003) and ewe's milk have been reported.

The objective of our experiments was to determine contents of basic components of ewe's milk using the FT NIR method, when samples of milk are measured on Petri dishes. Our attention was paid mainly to determination of dry matter, fat, protein, casein, urea nitrogen and lactose.

MATERIALS AND METHODS

Materials and reference values

Ewe's milk samples were taken during the lactation. Experimental animals did not show any health problems during the whole study period.

Following data were obtained by a reference method: dry matter (DM) gravimetrically method, by oven drying at 102 ± 2 °C to constant weight (ČSN ISO 6731). Total nitrogen (TN) was assessed according to Kjeldahl method using Kjeltec Auto 1031 Analyzer, Foss Tecator, Sweden (MARSHALL, 1992) and crude protein (CP) was calculated as N x factor of 6.38. True protein (TP) were determined spectrophotometrically - the apparatus Pro-Milk II, Foss Electric, Denmark. Fat (F) was determined by Gerber method (ČSN ISO 2446). Lactose (L) was determined polarimetrically as monohydrate (CVAK, PETERKOVÁ & ČERNÁ; 1992). Urea nitrogen (UN) was analysed by method of GAJDŮŠEK, JELÍNEK & HAMPL; 1996. All measurements were made in duplicate.

FT NIR analysis

A wavelength scanning instrument FT NIR Antaris (ThermoNicolet, USA) was used with a scanning range from 4 000 to 10 000 cm^{-1} in reflectance mode. Samples of ewe's 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. An average of 100 spectral scans was taken for each sample. Diffuse reflectance was recorded as log 1/R. 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 partial least squares (PLS) algorithm (HAALAND & THOMAS, 1988). The same samples were employed for full cross validation by software FT NIR Reference Analysis. 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 (correlation coefficient – r and SEP) were used to determinate the final calibration equation.

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

RESULTS AND DISCUSSION

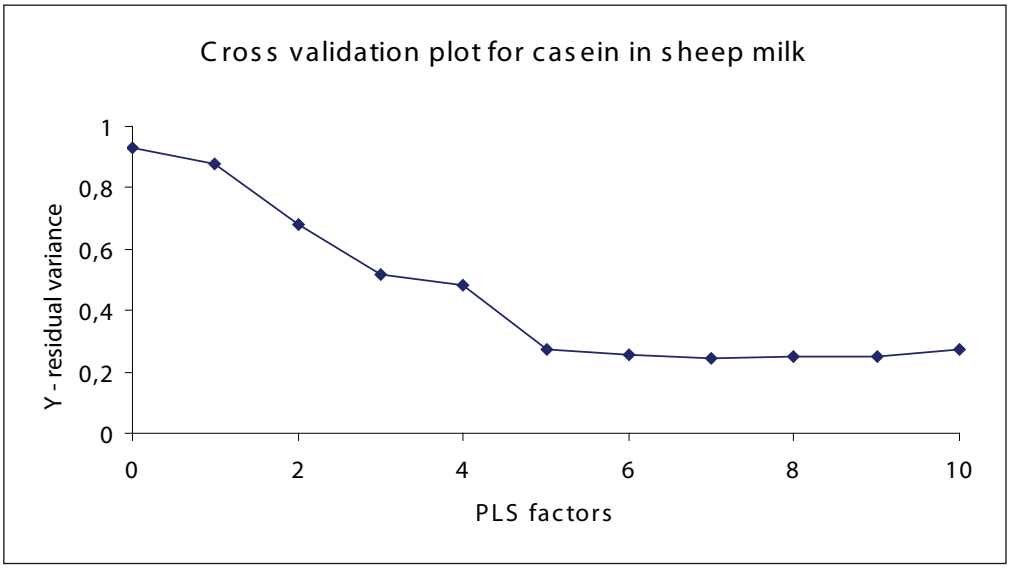
The calibration model for the ewe's milk was created using 73 samples for estimation of DM, 79 samples for F, 76 samples for TP, 46 for C, 59 for UN and 79 for L. The calibration model was constructed using the PLS algorithm. PLS factors used in our calibration model involved spectral information and, at the same time, also concentration data. The dependence of predicted residual error sum of squares (PRESS) on the number of factors used for calibration of components of the PLS method is an important diagnostic tool and enables to estimate the optimal number of factors. A high value of PLS factors indicates the accuracy of prediction because PRESS involves also the spectral noise.

Means of reference values, their standard deviations and PLS factors are presented in Table I. Numbers of PLS factors for DM, F, C and UN are relatively low and the PRESS function shows a decreasing tendency. For P and L, however, the number of factors is higher but the PRESS function again decreases. It can be concluded that due to a high number of samples all models are robust. Dependence of PRESS function on the number of PLS factors used for calibration of estimation of C in milk is presented in Figure 1.

I: *Mean, range and standard deviation (SD) for each of the data sets used as a calibration or test set*

Component	N	Mean	Minimum	Maximum	SD	Number of PLS factors
Ewe's milk						
Dry matter (%)	73	16.82	14.67	19.72	0.95	9
Fat (%)	79	7.24	4.37	10.77	1.52	8
True protein (%)	76	6.08	4.71	9.30	0.78	14
Casein (%)	46	4.55	2.97	6.67	0.90	7
Lactose (%)	79	4.71	3.59	5.33	0.34	13
Urea Nitrogen (mg $100mL^{-1}$)	59	67.41	33.69	102.11	15.90	7

N – number of samples



1: *Dependence of the PRESS function on the number of PLS factors used for calibration of estimation of casein in ewe's milk*

Calibration and validation results of estimations of individual milk components are presented in Tables II and III. Unfortunately, these results cannot be compared with any other studies dealing with the determination of basic components of ewe's milk. Until now only results of studies on cow's milk and dairy products were published. TSENKOVA et al. (1999); TSENKOVA et al. (2000) and TSENKOVA, ATANASSOVA, & TOYODA (2001) determined the highest positive coefficients for fat, protein, lactose and total solids. RU & GLATZ (2000) published similar results with non-homogenised cow's milk. KUKAČKOVÁ, ČURDA & JINDŘICH (2000) reported the best calibration results for prediction of DM, F and P (in cow's milk was 0.975, 0.967 and 0.965, respectively). JANKOVSKÁ & ŠUSTOVÁ (2003) determined, in samples of unhomogenized raw cow's milk, the following values of correlation coefficients: DM 0.928; F 0.961; P 0.985; C 0.932; UN 0.906 and L 0.931. ŠAŠIČ & OZAKI (2001) mentioned smaller correlation for protein (0.924 up 0.960), very low correlation for lactose (0.696) and very high correlation for fat (0.996) in homogenized cow's milk. ALBANELL et al. (2003) analysed homogenized goat's milk (fat 0.98, protein 0.96, casein 0.91, total solid 0.94) and unhomogenized goat's milk (fat 0.97, protein 0.95, casein 0.92, total solid 0.95) with lower results of correlation.

II: *Parameters of the regression function* $y'_i = a + bx_i$ *for the calibration model*

Component	$a \pm bx_i$	SEC	CCV(%)	R
Ewe's milk				
Dry matter (%)	0.571 ± 0.966	0.18	1.07	0.983
Fat (%)	0.158 ± 0.978	0.23	3.18	0.989
True protein (%)	0.039 ± 0.994	0.06	0.99	0.997
Casein (%)	0.208 ± 0.954	0.19	4.18	0.977
Lactose (%)	0.177 ± 0.962	0.07	1.49	0.980
Urea Nitrogen (mg $100mL^{-1}$)	3.571 ± 0.947	3.66	5.43	0.973

SEC – standard error of calibration; CCV – calibration coefficient of variation; R – correlation coefficient

III: *Parameters of the regression function $y'_i = a + bx_i$ for the validation model*

Component	a ± bx_i	SEP	PCV(%)	R
Ewe's milk				
Dry matter (%)	1.600 ± 0.905	0.33	1.96	0.940
Fat (%)	0.209 ± 0.971	0.27	3.73	0.984
True protein (%)	0.053 ± 0.991	0.09	1.48	0.994
Casein (%)	0.268 ± 0.941	0.24	5.27	0.963
Lactose (%)	0.315 ± 0.933	0.09	1.91	0.962
Urea Nitrogen (mg 100mL^{-1})	23.295 ± 0.658	9.07	13.41	0.822

R – correlation coefficient; SEP – standard error of prediction; PCV – prediction coefficient of variation

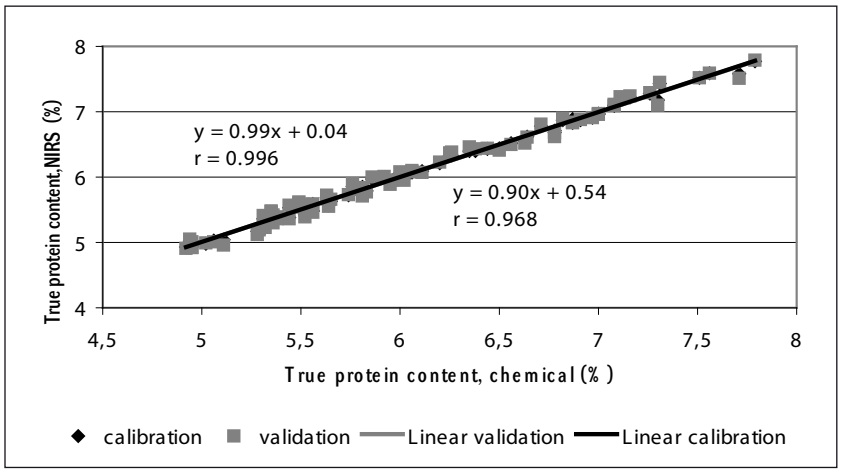
IV: *Parameters of basic components in ewe's milk as estimated by NIR reference values and their mutual comparison by paired Z-test*

Component	N	NIR	REF	d	SD
Ewe's milk					
Dry matter (%)	73	16.822	16.823	–0.001	0.94
Fat (%)	79	7.243	7.243	0.000	1.51
True protein (%)	76	6.078	6.078	0.000	0.77
Casein (%)	46	4.549	4.549	0.000	0.88
Lactose (%)	79	4.709	4.709	0.000	0.33
Urea Nitrogen (mg 100mL^{-1})	59	67.408	67.408	0.000	15.47

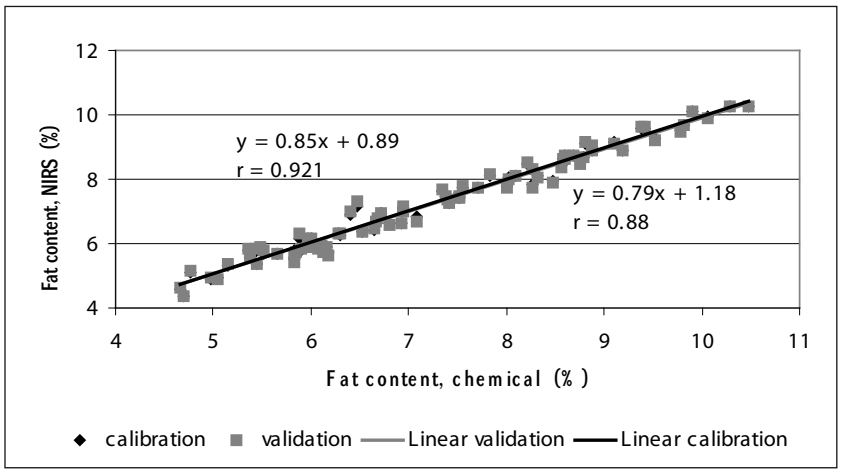
* $P<0.05$; N – number of samples; NIR – mean of the NIR values; REF – mean of the reference values; d – difference of mean of NIR and reference values; SD – standard deviation of mean

The validation model was constructed on the base of cross validation using the same set of samples (i. e. those, which were used for calibration). This validation checks reliability of the calibration model and is characterised by the standard error of prediction (SEP). Expected values of calibration coefficient of variation (CCV) and of prediction coefficient of variation (PCV) lower than 5% and 10%, respectively, are considered to be very reliable. In our experiments, values of CCV were lower than 5% for all components excepting urea nitrogen in milk. In this case also the PCV value was higher than 10%. It seems that this was caused by a narrower variation of measured values so that the reliability of the constructed model could be negatively influenced. ALBANELL et al. (2003) determined results of SEC and SEP identical to our results. LAPORTE & PAQUIN (1999) achieved lower results of SEC (0.12 for fat, 0.06 for crude protein, 0.04 for true protein and 0.05 for casein) and SEP (0.07 for fat, 0.06 for crude protein and casein, 0.05 for true protein. All these authors concluded that the NIRS method could be used for a quick analysis of cow's milk components.

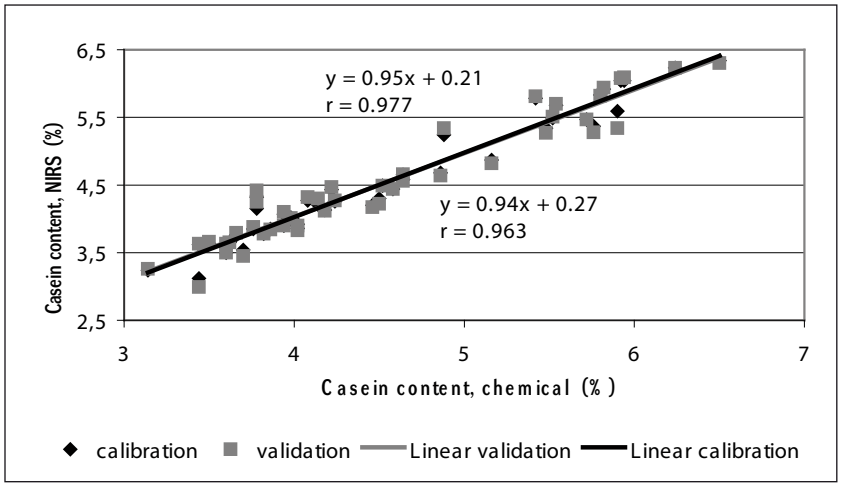
The linear dependence of the reference results versus results predicted by the PLS algorithm for determination of true protein, fat, casein, lactose and urea nitrogen in ewe's milk is illustrated in Figures 2–6. Obviously, there is a good correlation between predicted values and known chemical reference values.



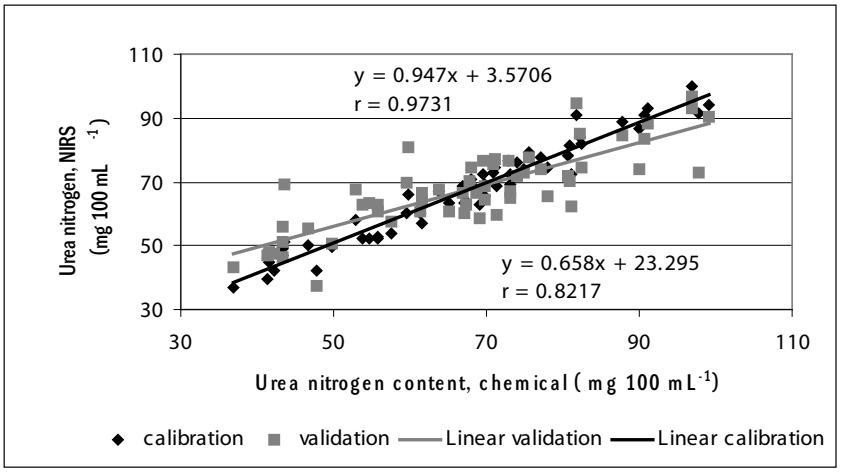
2: *Relationship of calibration and validation results of true protein in ewe's milk*



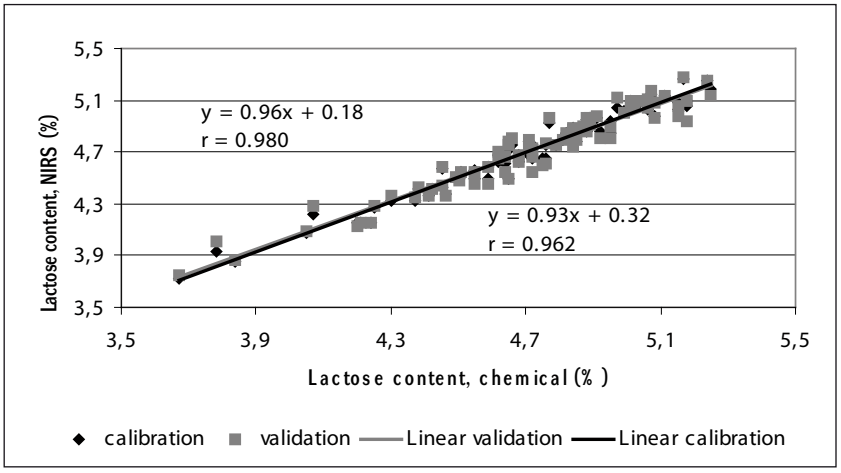
3: *Relationship of calibration and validation results of fat in ewe's milk*



4: *Relationship of calibration and validation results of casein in ewe's milk*



5: *Relationship of calibration and validation results of urea nitrogen in ewe's milk*



6: *Relationship of calibration and validation results of lactose in ewe's milk*

Values predicted by means of NIRS method were tested using a paired Z-test and are presented in Table 4 together with reference values. Statistically significant differences between the reference values and the calculated values of NIR were not found.

CONCLUSIONS

Statistical results showed sufficient accuracy on predictions of major components of ewe's milk using reflectance mode on Petri dishes. NIRS method is suitable for the estimation of individual components of ewe's milk on the base of exact reference methods. The major components, namely crude protein, dry matter and fat, can be determined with sufficient accuracy. The lactose was not determined with acceptable precision. Near infrared spectroscopy combined with PLS regression is a simple and rapid technique for compositional analysis of ewe's milk. Use of Fourier transform near-infrared reflectance spectroscopy measurement of samples on Petri dishes is a suitable technique for rapid analysis of ewe's milk without any sample pre-treatment and is acceptable for industrial practice.

SOUHRN

Analýza ovčího mléka FT NIR spektrometrem; měření vzorků na Petriho misce reflektanční kyvetou

Práce se zabývá možností stanovení základních složek ovčího mléka (sušiny, tuku, čistých bílkovin, kaseinu, laktózy a močoviny) FT NIR spektrometrem. Vzorky mléka byly před vlastním měřením zahřáty na teplotu 40 °C, protřepány a ochlazeny na 20 °C. Poté byly umístěny na Petriho misku a proměřeny kovovým zrcátkem na integrační sféře. Výsledky kalibračních korelačních koeficientů: sušina 0,983; tuk 0,989; čisté bílkoviny 0,997; kasein 0,977; laktóza 0,980 a močovina 0,973. Hotové kalibrační modely byly ověřeny křížovou validací. Z-testem nebyly zjištěny rozdíly mezi referenčními hodnotami a hodnotami naměřenými NIR spektrometrem (p=0,05). Na základě uvedených výsledků vyplývá, že NIR spektrometr je možno použít k rychlé analýze základních složek ovčího mléka.

blízká infračervená spektroskopie, ovčí mléko, sušina, tuk, bílkoviny, kasein, laktóza, chemické složení

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