Determination of Selected Parameters of Quality of the Dairy Products by NIR Spectroscopy

Jana RŮŽIČKOVÁ and Květoslava ŠUSTOVÁ

Department of Food Technology, Mendel University of Agriculture and Forestry Brno, Brno, Czech Republic

Abstract

Růžičková J., Šustová K. (2006): **Determination of selected parameters of quality of the dairy products by NIR spectroscopy**. Czech J. Food Sci., **24**: 255–260.

The possibility of the application of near-infrared spectroscopy to the analysis of the selected parameters of quality of the dairy products was followed. The contents of solids and fat, as well as pH in yoghurts (also the titrable acidity), milk semolina, and milk rice were determined. The samples were analysed by reference methods and by FT NIR spectroscope at integrating sphere within reflectance mode in the wavelength range of 10 000–4 000 cm^{-1} with 100 scans. To develop the calibration model for the components examined, the partial least squares (PLS) was used and this model was validated by full cross validation. The highest correlation coefficients were found with yoghurt: 0.998 (solids), 0.989 (fat), 0.875 (pH) and 0.989 (titrable acidity), with milk semolina: 0.967 (solids), 0.983 (fat) and 0.992 (pH), and with milk rice: 0.987 (solids), 0.990 (fat) and 0.852 (pH). The results of this study showed the availability of NIR spectroscopy for a quick and non-destructive analysis of the dairy products.

Keywords: NIR spectroscopy; dairy products; dry matter; fat; pH; titrable acidity of yoghurt

Spectroscopy of the NIR region (near infrared region) offers a wide range of applications in the control of the quality indicators of raw materials and intermediary products, as well as final products, in the field of food production. This method is used especially for the determination of the main constituents (dry matter, proteins, fat, and saccharides), for example in the case of native milk and colostrum. However, the use of NIR spectroscopy is much wider and includes the determination of sensoric and physico-chemical parameters (density, freezing point, pH, size of particles) (Rodríquez-Otero *et al*. 1997). NIR spectroscopy is used not only in the pharmaceutical industry and the oil and lubricant industry, but also in the food industry (Garcia-Alvarez *et al*. 2000; Ru & Glatz 2000; Prevolnik *et al*. 2005). NIR application is widely used in the milk industry for the analysis of the composition of milk (Sasic & Ozaki 2001; Čurda *et al*. 2002), cheeses (Blazquez *et al*. 2004), or butter (Hermida *et al*. 2001). However, only a few works deal with the use of NIR spectroscopy for the determination of the basic indicators in the case of dairy products. One of them is the work by Paradkar and Irudayaraj (2002), who concentrated on the determination of cholesterol in the dairy products.

The aim of our work was to evaluate the use of NIR spectroscopy for the quantitative analysis of the dairy products. The analysis determined the contents of dry matter and fat, and also pH in the case of milk semolina and milk rice, as well as titratable acidity in the case of yoghurt.

MATERIAL AND METHODS

Material. The following dairy products were used for the quantitative analysis: white yoghurts

with the declared fat contents of 0.1%, 3.5%, and 11%, fruit yoghurts (fat contents of 0.1%, 2.9%, and 9%), and, furthermore, samples of chocolate flavoured milk rice and milk semolina with the fat contents of 2.5% and 6%, respectively. For the creation of the calibration models of the spectrophotometer, we needed samples of various pH value ranges, titratable acidity, contents of fat and of dry matter. For this reason, the samples were diluted with water or cream after complete homogenisation. For the purpose of diluting, cream with the declared fat content of 31% was used. In order to widen the calibration of titratable acidity, samples of yoghurts were used which were produced in the laboratory of the Mendel University of Agriculture and Forestry Brno from half-fat milk by means of a yoghurt culture (type Rx, Lactoflora, Collection strain No. 22), using a cultivation temperature of 42°C. The samples for the determination of titratable acidity were collected during the fermentation process.

Methods. The monitored constituents were referentially determined in the laboratory of the Department of Food Technology at the Mendel University of Agriculture and Forestry Brno. In the case of milk rice and semolina, the reference gravimetric method was used to determine the dry matter content. This method consists of drying the weighed sample to a constant weight at 102 ± 2°C. The dry matter content in yoghurt was also determined using the reference method, however, with the addition of ZnO into the sample (Czech State Standard ČSN 57 1450 – Ostatní mléčné výrobky, jogurt, kasein apod.).

For the determination of the fat content, the method of acidic destruction of proteins by means of Gerber acid with the addition of amyl alcohol was used, modified according to the methods used by the dairy plant. The content of fat was read on the scale of a butyrometer after centrifugation (ČSN 57 1450).

The pH (active acidity) values were obtained using a WTW pH 95 pH-meter with a SenTix 97 probe, with a built-in temperature sensor. Prior to the individual measurements, the apparatus was calibrated at pH 4 and pH 7.

Titratable acidity depends on the content of organic acids, mainly lactic acid, and, simultaneously, on the content and composition of mineral substances and proteins. It was determined only in the case of white yoghurt, as the method can only be used with colourless samples. For this reason, titratable acidity was not determined in the case of milk semolina and rice. The acidity was determined by titrating 50 g of yoghurt with a 0.25M solution of NaOH using the phenolphthalein indicator until the development of a rose colour according to Soxhlet-Henkel (Czech State Standard No. 57 1450).

NIR method. All samples were simultaneously measured using an FT NIR Antaris apparatus supplied by the ThermoNicolet company, in the spectral range of 10 000–4 000 cm^{-1} with 100 scans at resolution 8 and scanning time ca 1 minute. The spectra were measured on an integration sphere in the reflectance mode (a technique measuring the absorption of radiation after reflection from the layer of the sample) in a 25 ml beaker using aluminium foil. Each sample was scanned three times and the average spectrum was used for calibration. The measurements and subsequent adjustments of the spectra were carried out using the Omnic program.

Statistic methods. Calibration models were created using a PLS algorithm (least squares method) (Haaland & Thomas 1988a, b) of the TQ Analyst program. The PLS factors used in the calibration models include spectral and, simultaneously, concentration information. A very important diagnostic tool is the relation of the presupposed residual error of the sum of the squares (PRESS) to the number of factors used to calibrate the individual quality indicators, which enables the estimation of the optimum number of factors. A high PLS factor value means accurate prediction, as PRESS also includes spectral noise. Approximately 50 samples of each product were used, in the case of titratable acidity 80 samples of white yoghurt.

The results were evaluated on the basis of correlation between the reference values and the values calculated from the calibration equations obtained, and on the basis of the size of standard errors of calibration (SEC), and of validation (SEP). The suitability of the resulting model is also estimated according to correlation coefficient (R). The closer is the R value to 1, the more suitable can the model be considered. A further indicator of the reliability of the model is the value of the calibration coefficient of the CCV variation, which should not exceed 5%, and the value of the PCV prediction coefficient 10%.

The evaluation of the possible difference between the reference and the predicted (NIR) values was carried out by means of ANOVA statistical analysis

in the Microsoft Excel program, using a parametric Z-test. The values were compared on the levels of significance of $P < 0.05$ and $P < 0.01$.

RESULTS AND DISCUSSION

The calibration and validation results for yoghurt are presented in Table 1, from which it follows that the calibration model for the determination of the content of dry matter meets the conditions of a reliable model. In the fat model, the variation coefficient limit values were exceeded (CVV 11.73% and PCV 16.41%). This was probably caused by an error in the Gerber method for the determination of the fat content. The value of the correlation coefficient *R* for the calibration of the fat content is 0.989. The correlation coefficient *R* of pH are lower (calibration 0.875; validation 0.788); however, they do not show a negative influence on the robustness of the model. The titratable acidity calibration model meets the conditions for a reliable model. We attained the correlation coefficient values of 0.989 for calibration, and 0.979 for validation, and, simultaneously, the calibration variation coefficient (CVV 4.60%), as well as the prediction coefficient (PCV 6.43%), from which it follows that the coefficients met the condition of reliability. The model can be considered robust and applicable in practice.

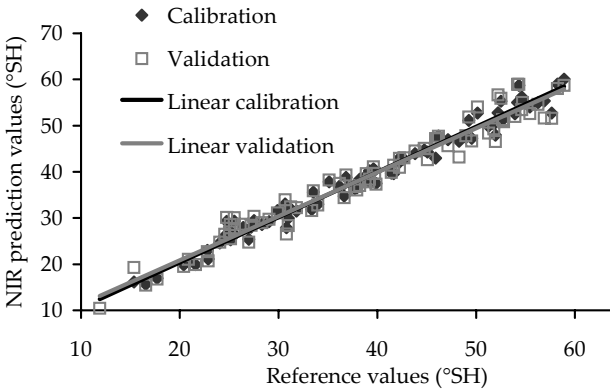

Figure 1. Results of calibration and validation of titrable acidity of yoghurt

Table 1. Calibration and validation results of yoghurt

Calibration component	*n*	$\bar{x}_p$	$S_{\bar{x}}$	min	max	PLS
Solids (%)	50	15.09	3.22	10.32	22.48	8
Fat (%)	50	5.90	4.72	0.12	14.69	6
pH	50	4.11	0.06	4.00	4.24	5
Titrable acidity (°SH)	80	38.40	12.19	11.88	58.91	7
	Calibration					
	$a \pm bx$	*R*	SEC	CCV(%)		
Solids (%)	0.067 ± 0.996*x*	0.998	0.218	1.44		
Fat (%)	0.126 ± 0.979*x*	0.989	0.692	11.73		
pH	0.930 ± 0.774*x*	0.875	0.028	0.68		
Titrable acidity (°SH)	0.812 ± 0.979*x*	0.989	1.77	4.60		
	Validation					
	$a \pm bx$	*R*	SEP	PCV(%)		
Solids (%)	0.234 ± 0.984*x*	0.989	0.460	3.05		
Fat (%)	0.194 ± 0.967*x*	0.978	0.968	16.41		
pH	1.296 ± 0.685*x*	0.788	0.038	0.91		
Titrable acidity (°SH)	1.833 ± 0.952*x*	0.979	2.47	6.43		

Explanation for Table 1 to 3

n – number of samples; $\bar{x}_p$ – average values; $S_{\bar{x}}$ – standard deviation; min – minimum values; max – maximum values; PLS – number of factors; $a \pm bx$ – linear regression line; *R* – correlation coefficient; SEC – standard error of calibration; CCV – calibration coefficient of variation; SEP – standard error of prediction; PCV – prediction coefficient of variation

Table 2. Calibration and validation results of milk semolina

Calibration component	n	$\bar{x}_p$	$S_{\bar{x}}$	min	max	PLS
Solids (%)	47	30.04	2.17	25.73	33.30	10
Fat (%)	49	5.89	2.18	2.89	10.26	6
pH	49	6.45	0.33	5.84	6.77	15
	Calibration					
	$a \pm bx$	R	SEC	CCV(%)		
Solids (%)	1.968 ± 0.935x	0.967	0.554	1.84		
Fat (%)	0.196 ± 0.967x	0.983	0.396	6.72		
pH	0.104 ± 0.984x	0.992	0.041	0.64		
	Validation					
	$a \pm bx$	R	SEP	PCV(%)		
Solids (%)	3.197 ± 0.893x	0.916	0.881	2.93		
Fat (%)	0.262 ± 0.955x	0.974	0.491	8.34		
pH	0.146 ± 0.977x	0.959	0.096	1.49		

Figure 1 gives a graphic illustration of the dependence curve of the titratable acidity of yoghurts. It is obvious that a strong link exists between the reference and predicted values, as there was an almost perfect overlap of the regression lines of both calibration and validation.

The results obtained with milk semolina and rice are given in Tables 2 and 3. It follows from the values that the model of the dry matter content does not exceed either the calibration or the validation limits of the reliability coefficients, and is robust. Minor errors are obvious in the fat determination models in the case of the calibration coefficients of the CCV variation (semolina 6.72%, rice 5.84%), as well as in the case of the PCV prediction coefficients (semolina 8.34%, rice 9.16%). This is in accordance with the model concerning yoghurt, and can be caused by an error when measuring the fat using the Gerber method.

In the case of the pH model, there is a noticeable difference between milk semolina and rice. In the case of semolina, the correlation coefficient values

Table 3. Calibration and validation results of milk rice

Calibration component	n	$\bar{x}_p$	$S_{\bar{x}}$	min	max	PLS
Solids (%)	50	31.00	2.31	25.87	34.50	10
Fat (%)	46	5.14	2.08	1.77	9.41	11
pH	48	6.57	0.06	6.37	6.65	10
	Calibration					
	$a \pm bx$	R	SEC	CCV(%)		
Solids (%)	0.819 ± 0.974x	0.987	0.375	1.21		
Fat (%)	0.105 ± 0.980x	0.990	0.300	5.84		
pH	1.782 ± 0.729x	0.852	0.034	0.52		
	Validation					
	$a \pm bx$	R	SEP	PCV(%)		
Solids (%)	1.078 ± 0.965x	0.970	0.562	1.81		
Fat (%)	0.230 ± 0.955x	0.974	0.471	9.16		
pH	3.099 ± 0.528x	0.617	0.064	0.97		

Table 4. Statistical evaluation of reference and NIR values by Z-test

	n	xNIR	xREF	d	SD	*P*
Yoghurt						
Solids (%)	50	15.09	15.09	0.001	0.22	
Fat (%)	50	5.90	5.90	0.0004	0.69	
pH	50	4.11	4.11	0.0002	0.03	
Titrable acidity (°SH)	80	38.40	38.40	–0.002	1.77	
Milk semolina						
Solids (%)	47	30.06	30.06	–0.0002	0.75	
Fat (%)	49	5.89	5.89	0.0008	0.40	
pH	49	6.45	6.45	0	0.04	
Milk rice						
Solids (%)	50	31.00	31.00	0.0002	0.38	
Fat (%)	46	5.14	5.14	0	0.30	
pH	48	6.57	6.57	–0.0006	0.05	

n – number of samples; xREF – avarage of reference values; xNIR – average of NIR values; d – difference between average NIR and reference values; SD – standard error of difference; *P* – statistics values: $^{*}P < 0.05$; $^{**}P < 0.01$

of 0.992 for calibration and 0.959 for validation were attained; however, in the case of rice, these values are much lower (0.852 and 0.617, respectively). However, the calibration variation coefficient in the case of milk rice did not exceed the limit confirming the reliability of the model (CCV 0.52%). The same is true for the PCV prediction coefficient of 0.97%. Therefore, it can be concluded that the present model is functional for milk rice, although low correlation coefficients are attained.

All the results of the reference values and NIR values were statistically verified using a parametric Z-test. No statistically conclusive difference ($z–z_t$) was found between the evaluated values in any of the indicators determined.

CONCLUSIONS

The results proved the possibility of determining the selected qualitative indicators (dry matter, fat, pH, titratable acidity) by means of NIR spectroscopy in the case of dairy products. Relatively high correlation coefficients were attained in almost all the models evaluated. The only exception was the lower values of R (0.852 calibration and 0.617 validation) in the case of the pH of milk rice. However, all the other indicators verifying the reliability of the calibration model (CCV and PCV) were right. Statistical verification by means of the Z-test did not show any statistically conclusive differences between the reference and the predicted NIR values in any of the cases. Because of this, the method of NIR spectroscopy can be recommended for practical application. The use of NIR spectroscopy to determine the contents of the selected components of products is highly applicable in practice, in spite of higher initial costs connected with the purchase of the apparatus. The unquestionable advantage of this method is its speed, the possibility of analysing a high number of samples, non-destructiveness, and minimal consumption of chemicals. The models can be still extended in the future, due to which they may become more precise.

References

Blazquez C., Downey G., O'Donnell C., O'Callaghan D., Howard V. (2004): Prediction of moisture, fat and inorganic salts in processed cheese by near infrared reflectance spectroscopy and multivariate data analysis. Journal of Near Infrared Spectroscopy, **12**: 149–157.

Čurda L., Kukačková O., Novotná M. (2002): Near-infrared spectroscopy and its application to milk and dairy products analysis. Chemické Listy, **96**: 305–310.

Garcia-Alvarez M., Huidobro J.F., Hermida M., Rodriguez-Otero J.L. (2000): Major components of honey analysis by near-infrared transflectance spectroscopy. Journal of Agricultural and Food Chemistry, **48**: 5154–5158.

Haaland D.M., Thomas E.V. (1988a): Partial least-squares methods for spectral analyses. 1. Relation to other quantitative calibration methods and the extraction of qualitative information. Analytical Chemistry, **60**: 1193–1202.

Haaland D.M., Thomas E.V. (1988b): Partial least-squares methods for spectral analyses. 2. Application to simulated and glass spectral data. Analytical Chemistry, **60**: 1202–1208.

Hermida M., Gonzalez J.M., Sanchez M., Rodriguez-Otero J.L. (2001): Moisture, solids-non-fat and fat analysis in butter by near infrared spectroscopy. International Dairy Journal, **11**: 93–98.

Paradkar M.M., Irudayaraj J. (2002): Determination of cholesterol in dairy products by infrared techniques: 2. FT-NIR method. International Journal of Dairy Technology, **55**: 133–138.

Prevolnik M., Candek-Potorkar M., Skorjanc D., Velikonja-Bolta S., Skrlep M., Znidarsic T., Babnik D. (2005): Predicting intramuscular fat content in pork and beef by near infrared spectroscopy. Journal of Near Infrared Spectroscopy, **13**: 77–85.

Rodríguez-Otero J.L., Hermida M., Centero J. (1997): Analysis of dairy products by near-infrared spectroscopy. Journal of Agricultural and Food Chemistry, **45**: 2815–2819.

Ru Y.J., Glatz P.C. (2000): Application of near infrared spectroscopy (NIR) for monitoring the quality of milk, cheese, meat and fish. Asian-Australian Journal of Animal Science, **13**: 1017–1025.

Sasic S., Ozaki Y. (2001): Short-wave near-infrared spectroscopy of biological fluids. 1. Quantitative analysis of fat, protein, and lactose in raw milk by partial least squares regression and band assignment. Analytical Chemistry, **73**: 64–71.

Received for publication November 30, 2005
Accepted for corrections August 11, 2006

Corresponding author:

Ing. Jana Růžičková, Mendelova zemědělská a lesnická univerzita v Brně, Agronomická fakulta,
Ústav technologie potravin, Zemědělská 1, 613 00 Brno, Česká republika
tel.: + 420 545 133 262, fax: + 420 545 133 190, e-mail: xruzic10@node.mendelu.cz