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Application News

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IRTracer-100, LabSolutions IR, Chemometrics

Quantitative Determination of Protein, Total Fat, and Carbohydrate Contents in Milk by FT-NIR Spectroscopy Method

❑ Introduction

The traditional methods of analyzing main constituents in milk, for example the Kjeldahl method for protein, Röse-Gottlieb or Mojonnier methods for fat, and polarimetry method for lactose, are time consuming and expensive. The near infrared spectroscopy (NIR) method provides a simultaneous quantitation of a number of milk constituents like proteins, fats and carbohydrates. It is a non-destructive and rapid measurement. Sample dilution is not necessary and even comparatively thick samples can be measured. The NIR spectrum consists of combination bands and overtone vibrations of the fundamental middle infrared bands, and mainly –CH, –OH, and –NH bonding vibrations are observed in the NIR region. However, unlike fundamental middle-IR bands, NIR bands are generally weak intensity, broad and overlapping. Because of the high similarity in NIR spectra, chemometrics data analysis such as principle component regression (PCR), multi-linear regression (MLR) or partial least squares (PLS) regression is required to correlate spectral data with the reference values of measuring components. Here, we introduce a method for quantitative determination of protein, total fat and carbohydrate contents in milk using NIR spectrometry and PLS quantitative calibration method.

❑ Experimental

Twelve commercially available cow milks were measured over the range of 3850 cm^{-1} to 10000 cm^{-1} by FT-NIR transmission method with a pathlength of 1 mm. The measurement conditions used are shown in Table 1. Each sample was measured three times and out of the twelve milk samples, ten were used as references to establish a PLS calibration curve using LabSolutions IR workstation with Chemometrics function. While, Milk 07 and Milk 08 were used as samples for quantitative determination of protein, total fat and carbohydrate.

Table 1. Instrument and Analytical Conditions

Instruments	: IRTracer-100, Near-Infrared Kit
Resolution	: 8 cm^{-1}
Accumulation	: 100
Apodization	: Happ-Genzel
Detector	: InGaAs

Table 2: Labelled nutritional contents of 12 milk samples

Sample	g / 100 mL		
	Protein	Total Fat	Carbohydrate
Milk 01	3.2	3.8	4.8
Milk 02	3.7	1.3	5.7
Milk 03	3.8	0.1	4.9
Milk 04	4	3.7	3.9
Milk 05	4	4	5
Milk 06	3.7	1.5	5
Milk 07	3.5	3.6	5
Milk 08	5	1	5.3
Milk 09	5	1	5.5
Milk 10	3.3	4.1	5
Milk 11	3.2	1.2	7
Milk 12	3.2	3.4	4.9

❑ Results and Discussion

Figure 1 shows the overlapped NIR spectra of Milk 01 to Milk 12 from 5300 cm^{-1} to 10000 cm^{-1}.

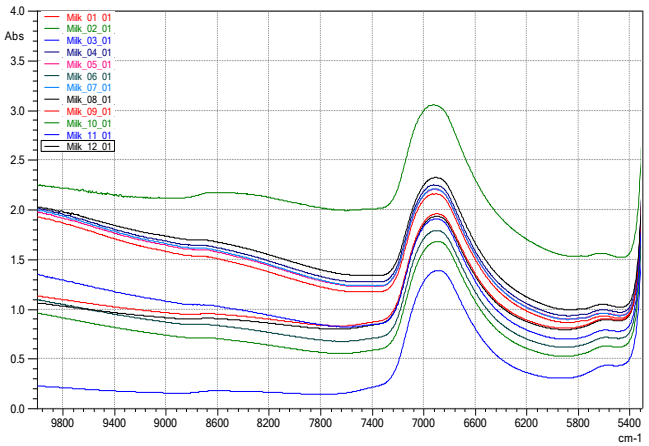

Figure 1: Overlapped NIR spectra of twelve milk samples, Milk 01 to Milk 12.

Second derivative spectra were actually used in the PLS data analysis for better resolution of overlapping and shoulder peaks, as well as removal of baseline fluctuation. Good correlation coefficients of greater than 0.95 were obtained for the PLS calibration modeling with low Mean Squared Error of Prediction (MSEP) and Standard Error of Prediction (SEP) as shown in Table 3.

Table 4 shows the quantitation results of Milk 07 and Milk 08 by the PLS method. From the repeated measurements, the percentage variation from mean is less than 10%. The measured values were very closed to the labelled values. In general, for greater accuracy more calibration samples will be required for establishment of PLS calibration.

Table 3. PLS calibration parameters of protein, total fat and carbohydrate in milk using ten reference samples

Calibration Table			
Algorithm	PLS II		
Number of components	3		
Number of references	30 (three measurements per sample)		
Range [cm^{-1}]	5300 – 10000		
Pre-process	MSC (5500 - 9000) Derivative, Order=2, Points=23		
Scale	Autoscale		
Component	Protein	Total Fat	Carbohy-drate
Number of factors	5	5	5
Correlation coefficient	0.9556	0.9968	0.9743
MSEP	0.0839	0.0062	0.0491
SEP	0.2897	0.0786	0.2216

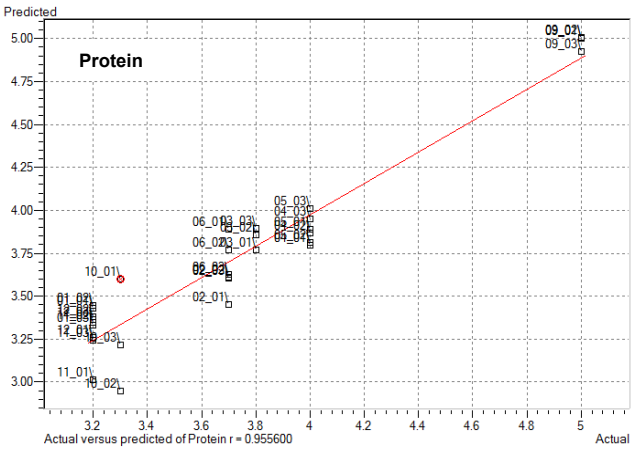

Figure 2. PLS calibration for protein predicted versus actual values (labelled).

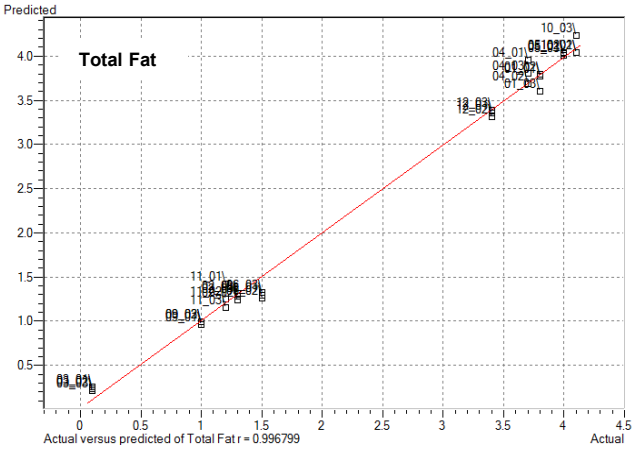

Figure 3. PLS calibration for total fat predicted versus actual values (labelled)

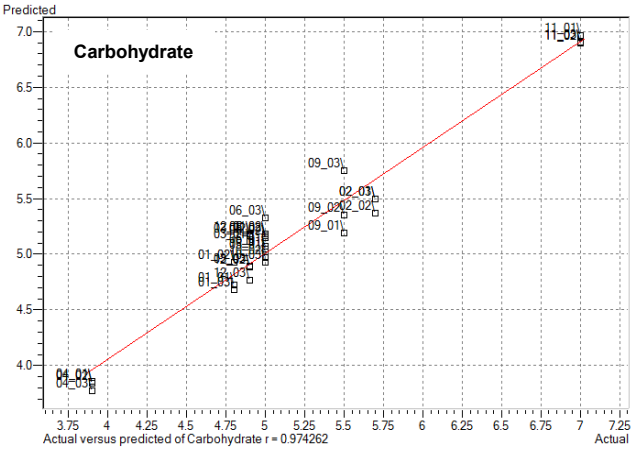

Figure 4. PLS calibration for carbohydrate predicted versus actual values (labelled).

Table 4. Quantitation results of protein, total fat and carbohydrate in samples Milk 07 and Milk 08

Sample	Predicted (g/100 mL)				Labelled (g/100 mL)
	Milk 07-1	**Milk 07-2**	**Milk 07-3**	**Mean**	
Protein	3.49	3.65	3.25	3.46	3.5
Total Fat	3.76	3.74	3.97	3.82	3.6
Carbohy-drate	5.51	4.81	4.81	5.04	5
Sample	**Milk 08-1**	**Milk 08-2**	**Milk 08-3**	**Mean**	**Labelled**
Protein	4.82	4.84	4.82	4.83	5
Total Fat	0.88	0.86	0.91	0.88	1
Carbohy-drate	5.46	5.21	5.32	5.33	5.3

❑ Conclusions

The FT-NIR with PLS data analysis offers an alternative quantitation method for protein, total fat and carbohydrate contents in milk without the need for sample pre-treatment. Furthermore, the method is cost effective and faster than the traditional methods.

❑ References

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2. Mariana C. M. P. BRANDÃO; Alexandre P. CARMO; Maria José V. BELL; Virgílio C. ANJOS: Characterization of Milk by Infrared Spectroscopy. Rev. Inst. Latic. “Cândido Tostes”, Mar/Abr, nº 373, 65: 30-33, 2010
3. Shimadzu Application News No.A475 Quantitative Analysis of Fat in Milk by UV-Vis-NIR Reflectance Spectroscopy and Multivariate Analysis

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