TECHNICAL COMMUNICATIONS

Determination of Fat, Protein, and Total Solids in Ovine Milk by Near-Infrared Spectroscopy

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Analysis by near-infrared spectroscopy (NIRS) was investigated as a means of predicting quality parameters of ovine milk. Calibration equations were developed with samples of ovine milk obtained from a flock of Manchega and Lacaune dairy ewes at different stages of lactation for a wide variation in milk composition. Prediction equations for milk protein, fat, and total solids content were developed by use of reflection or transflection methods to measure absorbance values. Accuracies of measurements were compared. R^2 (squared multiple correlation coefficient) values were satisfactory in most cases. The highest R^2 value for milk protein content (0.92) was obtained in transflectance mode with unhomogenized milk. The highest R^2 values for fat (0.99) and total solids (0.98–0.96) content were obtained in both a transflectance mode without sample conditioning and in a transflectance mode with milk homogenized at 40°C. To validate the calibration, an independent set of 40 milk samples was used. The best r^2 (simple correlation coefficient) values for protein, fat, and total solids were 0.92, 0.97, and 0.92, respectively. The study showed that NIRS is a potentially useful technique for evaluating the composition of unhomogenized ovine milk.

Regular monitoring of the fat and protein contents of bovine milk is a basic and important operation in the dairy industry, because the value of raw milk depends on these 2 components.

In Europe and the Mediterranean region, large numbers of ewes are milked to produce milk for the manufacture of cheese and other dairy products. The fat and protein contents of ovine milk are of interest because composition affects the quality of dairy products.

The development of automated infrared instruments for rapid determination of milk components has made it possible to analyze routinely large numbers of milk samples for milk control and selection programs. Physical measures for simultaneous determination of several constituents (mid-infrared or near-infrared spectroscopy [NIRS]) have replaced chemical methods for routine analysis of milk and milk products. Mid-infrared region (2.5 to 25 μm) corresponds to the fundamental absorbing bands, and the near-infrared region (0.8 to 2.5 μm) corresponds to overtones and combinations.

Milk analyzers that use mid-infrared transmittance are the most common type of electronic milk testing equipment used by the dairy industry today for measurement of milk fat and protein contents. All infrared milk analyzers are equipped with homogenizers that reduce the diameters of milk fat globules to a small, uniform size. A homogenizer is an essential component of a mid-infrared transmittance milk analyzer (1, 2).

NIRS is widely used, especially by the food industry, because it is rapid and nondestructive. Calibrations and checking systems have been described for bovine milk and dairy products (3–9), but few applications of NIRS techniques to analysis of sheep milk are reported (10, 11). Pascual et al. (11) analyzed the crude protein, true protein, and casein contents of samples of Manchega ewe milk by using a 19-filter optical instrument in the transflectance mode (liquid drawer).

NIRS instruments allow treatment of samples in different physical states (solids, powders, creams, or liquids). For this purpose, the spectrophotometer requires different sample holders that allow measurements in transflectance or reflectance mode. In transflectance mode, the energy must traverse the sample twice before it is sensed by the detector. The arrangement is less suited than that for the transmittance mode for samples with high optical densities (12). Different sample holders may be used: The aluminum cup (British cup), designed for viscous fluids, is used for liquid sugar products (13) and for fats and oils (14–16). The black plastic cup or disposable cup (Italian cup) is used frequently for butter and margarine (15) and for fermented milks.

The objectives of this study were to develop and evaluate an NIRS calibration for sheep milk and to compare the calibrations obtained for 3 sample presentations: by transflectance mode (aluminum cup), by reflectance mode (black plastic cup), and by transflectance mode with preconditioned samples (ceramic disk with homogenized milk).

Received May 13, 1998. Accepted by JL November 10, 1998.
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Table 1. Sample presentation used for near-infrared measurements

Presentation	Sample conditioning		Mode of optical measurement	Path length
	Homogenizer	Temperature, °C		
Liquid drawer	Yes	40	Transflectance	Fixed (<0.3 mm)
Plastic cup	No	20–22	Reflectance	Not fixed[a]
Aluminum cup	No	20–22	Transflectance	Fixed (0.3 mm)

[a] Reflectance measurements require samples with high absorbance.

Experimental

Sample Collection and Preparation

Milk samples were taken from individual ewes at the experimental farm (Veterinary School of the Autonoma University, Barcelona, Spain). The flock consisted of 120 ewes of Manchega and Lacaune breeds. Samples were composites of morning and evening milks taken on different milking days. They were preserved with 0.03% potassium dichromate, mixed, stored at 4°C, and then analyzed optically and chemically the day after collection.

Chemical Analysis

Crude milk protein was analyzed with the Kjeldahl method as a reference (N × 6.38; 17). Digestion and distillation were performed with a Kjeltec Auto 1030 Analyzer (Tecator, Höganäs, Sweden). The Gerber method (18) was used as the reference for fat. Total solids were determined by oven drying (19). All measurements were made in duplicate.

NIRS Analysis

The NIRS equipment consisted of an InfraAlyzer 450 spectrophotometer fitted with 19 discrete filters (Bran+Luebbe, Norderstedt, Germany) and interfaced to a personal computer. Filters covered the range from 1445 to 2348 nm.

The same NIRS instrument and the same wavelength range were used for the 3 sample presentations (Table 1).

(a) *Liquid drawer.*—Samples were heated to 40°C, placed in a high-pressure homogenizer, and applied to a cell with a ceramic disk of constant thickness. Spectra were measured twice for each sample.

(b) *Black plastic cup (Italian cup).*—A 4 mL sample of milk at room temperature (20°–22°C) was placed into a disposable black plastic cup (Bran+Luebbe), covered with a slide glass, and measured as a typical food system. Two cups were used for each milk sample.

(c) *Aluminum cup (British cup).*—A 0.5 mL sample of milk at room temperature (20°–22°C) was placed into a 0.3 mm thick aluminum sample holder (Bran+Luebbe) and covered with a slide glass. Each sample was measured twice.

The signal coming from the instrument was converted to absorbance (A = log 1/reflectance; A = log 1/transmittance), and the 2 measurements obtained were averaged before the equations were selected. Calibrations were developed with 175 samples for protein content, 168 for fat content, and 148 for total solids content; 40 samples were used for validation of parameters. Samples in the validation set were not used in the calibration set.

The software for scanning, mathematical processing, and statistical analysis came with the spectrophotometer (IACAL P01, 1987; Bran+Luebbe). Filter constants were assessed by multiple linear regression analysis (MLR). The best equation for each constituent was chosen by the optimal combination of the statistics from equation development: high R^2 (squared multiple correlation coefficient), low standard error of calibration (SEC), and high F (Fisher regression ratio) values in the calibration set. The relative importance of the wavelengths selected in each calibration was obtained according to the t-test. Calibration equations obtained were validated with 40 independent samples to test the accuracy of the equations based on high r^2 (simple correlation coefficient), low standard error of prediction (SEP), and low bias.

Results and Discussion

Table 2 shows the characteristics of the sample sets used in the study. Chemical analyses indicated a wide variation of composition among samples, and the calibration and validation sets covered similar ranges for each component. The protein content of samples had mean values (5.7%) higher than those reported (11) for milk from Manchega ewes (5.1%). The range of variation of each component was due to different breeds and lactation stages.

Table 2. Protein, fat, and total solids content of ovine milk samples obtained by near-infrared analysis

Analyte	Content for calibration set, %				Content for validation set, %			
	N	Range	Mean	SD	*n*	Range	Mean	SD
Protein	175	4.02–7.49	5.68	0.70	40	4.41–6.92	5.55	0.52
Fat	168	2.80–13.30	7.60	1.84	40	4.80–10.00	7.07	0.93
Total solids	148	12.90–23.35	18.10	2.14	40	15.14–20.85	17.65	1.22

Table 3. Near-infrared calibration statistics for protein, fat, and total solids content of ovine milk

Sample presentation	R^2	SEC[a]	CCV, %[b]	Wavelengths, nm[c]			
				1	2	3	4
Protein							
Liquid drawer	0.91	0.208	3.66	2139	2180	2336	2348
Plastic cup	0.91	0.216	3.80	2230	2190	2139	2336
Aluminum cup	0.92	0.190	3.35	2180	2100	2230	1818
Fat							
Liquid drawer	0.99	0.160	2.11	2180	2310	1722	
Plastic cup	0.97	0.300	3.95	2230	2270	2100	
Aluminum cup	0.99	0.225	2.96	1722	2190	2270	
Total solids							
Liquid drawer	0.98	0.274	1.51	2270	2230	2190	
Plastic cup	0.94	0.506	2.80	1778	1759	2270	
Aluminum cup	0.96	0.446	2.47	2270	2230	2336	

[a] Standard error of calibration.
[b] Calibration coefficient of variation.
[c] In order of importance according to the *t*-test.

For the reference methods, the repeatabilities expressed as the standard deviation of the difference between duplicates were 0.09% for protein, 0.04% for fat, and 0.05% for total solids. For NIRS, repeatabilities were 0.08% for protein, 0.09% for fat, and 0.17% for total solids.

The calibration procedure looks for the best combination of wavelengths to fit the chemical data. All possible combinations are computed, and the best combination for statistical analysis is selected. The optimal calibration equation for ovine milk samples was obtained by using 4 terms for milk protein content and 3 terms for milk fat and total solids contents. Results are shown in Table 3.

Eight wavelengths were used in the equations for milk protein calibrations. The wavelengths of 2139, 2180, 2230, and 2336 nm were common to 2 spectrophotometer cells (Table 3). Murray and Williams (20) reported that the wavelength area at 1700 nm consists of overtones of N–H, C–N, and C=O. The 2050–2300 nm region is used frequently for protein determinations. Wavelengths of 2050 and 2179 nm previously have been used to predict protein content in milk from different species (6). Kamishikiryo et al. (21, 22) reported that absorption at 2170 nm (because of peptide bonds) is the most stable and suitable signal for determining protein content in the presence of various food components. Pascual et al. (11) developed NIRS calibrations for Manchega ewe milk presented in a liquid drawer, relating the wavelengths of 1759, 1982, 2100, and 2180 nm to crude protein and those of 1734, 1759, 2139, 2180, and 2336 nm to true protein content.

The R^2 value obtained for milk protein calibration was 0.91 for the transflectance mode and the homogenizer (liquid drawer), as well as for the reflectance mode and the black plastic cup. The highest R^2 of 0.92 was obtained for the transflectance mode and the aluminum cup when the linear regression included 4 terms at 1818, 2100, 2180, and 2230 nm.

Another criterion for determining the suitability of a calibration in NIRS is the calibration coefficient of variation (CCV). The CCV (in percent) is the ratio of the standard error to the mean of the laboratory (reference) values × 100 (12). Good calibrations have CCV values of 10% or less, and the most robust calibrations have CCV values of 5% or less (12). For protein calibrations, CCV was 3.8% or less, and SEC values ranged from 0.190 for the aluminum cup to 0.216 for the black plastic cup. For bovine dried milk (23), the SEC value was 0.193. The relationship between protein content as determined by traditional chemical analysis and as predicted by NIRS with presentation in an aluminum cup is illustrated graphically in Figure 1.

Seven wavelengths were used in equations for milk fat calibrations (1722, 2100, 2180, 2190, 2230, 2270, and 2310 nm). The wavelengths of 1722 and 2270 nm were common for spectophotometer cells. Wavelengths used in the equation for the aluminum cup were 1722, 2190, and 2270 nm. To increase the range of chemical variations, Robert et al. (6) used milk samples from different species (cow, goat, ewe, cow colostrum, and cow with mastitis) and applied principal component analysis, relating the wavelengths of 1724, 1752, 2308, and 2344 nm to the fat content and those of 2050 and 2180 nm to protein content. Using the NIRS spectra of various fatty acids, Holman and Edmonson (24) showed that absorptions at 1680, 2150, and 2190 nm might be due to vibration of C–H bonds bound to *cis* unsaturation. Absorption bands around 1600–1800 nm and 2100–2200 nm are due to the straight carbon chain and *cis* double bonds, which reflect fatty acid moieties in fat molecules (15, 16).

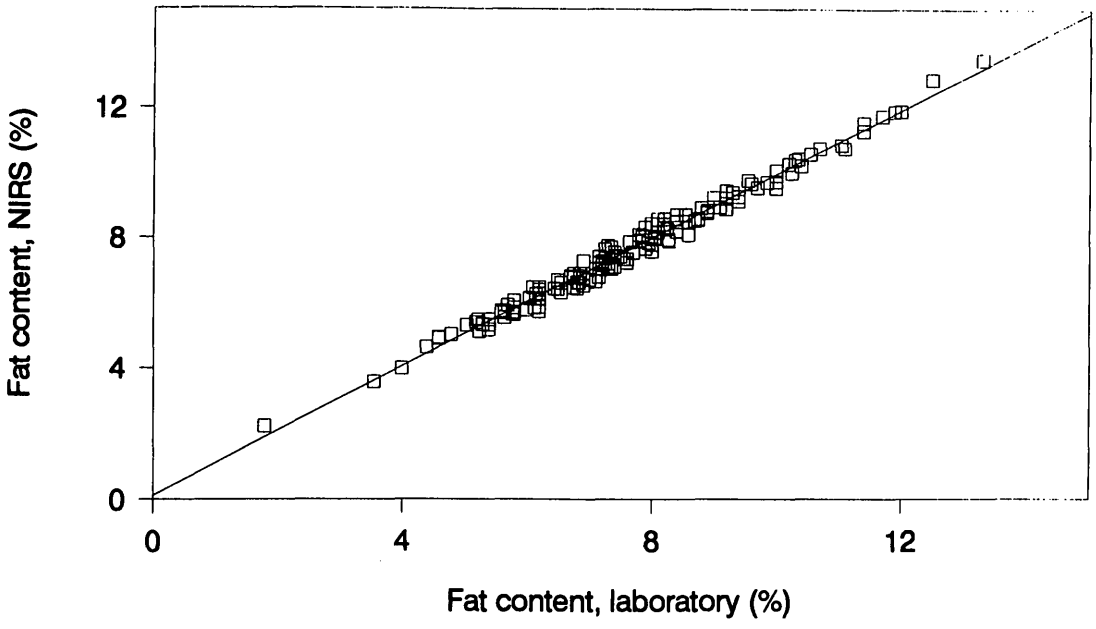

Figure 1. Relationship of protein reference method values to NIRS values for the calibration set presented in aluminum cup.

The R^2 values for milk fat calibrations (Table 3) were satisfactory for the 3 calibrations obtained. The highest R^2 value (0.99) was obtained in the transflectance mode with the homogenizer at 1722, 2180, and 2310 nm, as well as in the transflectance mode with the aluminum cup at 1722, 2190, and 2270 nm. SEC values ranged from 0.160 to 0.300, and CCV was 3.95% or less. The relationship between chemically determined and NIRS-predicted values for the aluminum cup are illustrated in Figure 2.

For calibrations of milk total solids content, 6 wavelengths were used in the equations. The wavelength of 2270 nm was common to all 3 cells, while that of 2230 nm was selected for 2 cells (Table 3). R^2 values were generally satisfactory for the 3 calibrations: 0.98 for the transflectance mode and homogenized milk, 0.96 for the transflectance mode and the aluminum cup, and 0.94 for the reflectance mode and the black plastic cup. SEC values ranged from 0.274 to 0.506, and CCV was 2.8% or less.

Statistical results from linear regression analysis comparing results of chemical analysis with those predicted by NIRS are shown in Table 4. The r^2 values obtained for protein, fat, and total solids contents, respectively, are as follows: 0.84, 0.97, and 0.92 for the homogenizer; 0.83, 0.94, and 0.84 for the black plastic cup; and 0.92, 0.95, and 0.91 for the aluminum cup.

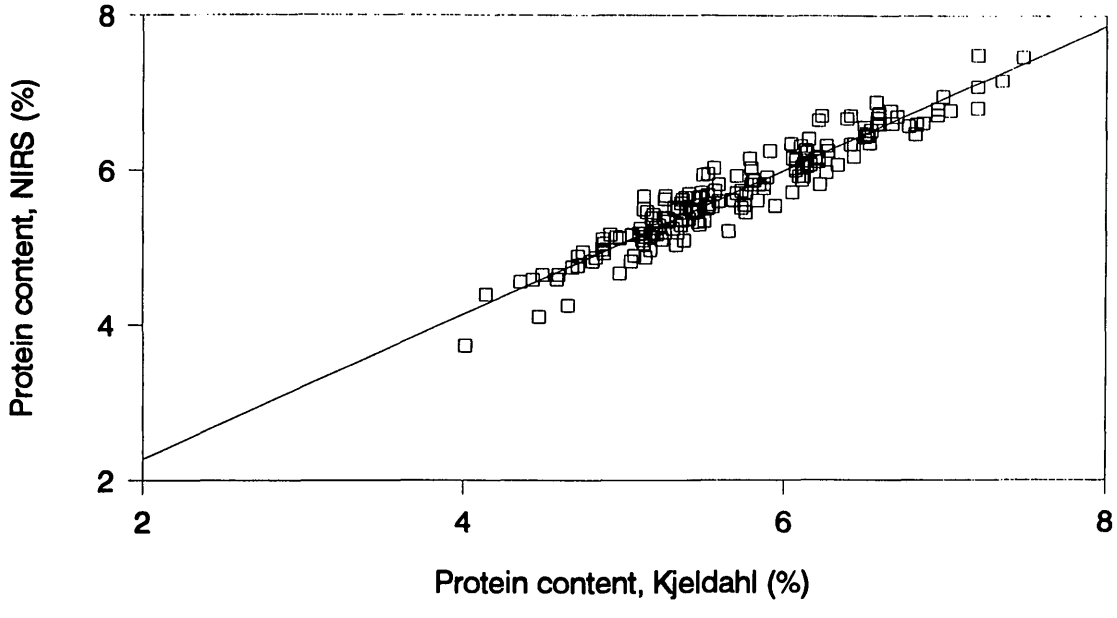

Figure 2. Relationship of fat reference method values to NIRS values for the calibration set presented in aluminum cup.

Table 4. Validation statistics for determination of protein and fat contents of samples of ovine milk by near-infrared analysis

Presentation	r^2	SEP[a]	Bias	Slope
		Protein		
Liquid drawer	0.84	0.229	−0.071	0.904
Plastic cup	0.83	0.187	0.057	1.056
Aluminum cup	0.92	0.175	0.051	1.013
		Fat		
Liquid drawer	0.97	0.202	0.014	1.078
Plastic cup	0.94	0.269	0.050	1.042
Aluminum cup	0.95	0.197	−0.014	1.025
		Total solids		
Liquid drawer	0.92	0.370	0.001	0.960
Plastic cup	0.84	0.537	−0.041	1.047
Aluminum cup	0.91	0.411	−0.004	1.003

SEP values ranged from 0.175 to 0.229 for milk protein content, from 0.197 to 0.269 for milk fat content, and from 0.373 to 0.537 to milk total solids content. For any model, the mean bias (mean difference between chemical and NIRS values) was near zero. Using the homogenizer, Pascual et al. (11) obtained SEP values of 0.112 for crude protein and 0.115 for true protein in Manchega ewe milk. Kamishikiryo et al. (21) investigated the effect of oil content on NIRS for protein in aqueous solution in a transmittance mode and obtained SEP values of 0.209 and 0.229 for determination of protein content (0–10%) in the presence of 5 and 10% oil content, respectively, using absorption at 2170 and 2306 nm. For commercial bovine milk with 2–5% fat content, the SEP value was 0.177 (22).

For all calibrations, SEP values were comparable to SEC or slightly higher, perhaps because SEP includes both the error associated with wet chemical analysis and the error associated with the NIRS equipment (25). However, SEP did not exceed SEC (Table 3) by 33% as suggested by Senk et al. (26).

Except for the total solids calibration using the black plastic cup, the wavelengths we selected had similar ranges for the 3 sample presentations used. The aluminum cup (transflectance mode and fixed path length) gave better performance than the black plastic cup (reflectance mode and unfixed path length). When comparing the aluminum cup with the liquid drawer (reflectance mode and homogenized milk), no differences are apparent for fat calibrations and total solids validations. However, the aluminum cup was better on protein validation.

Statistical results showed sufficient accuracy in predictions of protein, fat, and total solids content of sheep milk by any of the 3 spectrophotometer cells. SEC, SEP, and R^2 values for the calibration and validation sets obtained by the transflectance mode (aluminum cup or liquid drawer) were more accurate than those obtained by other modes.

Conclusions

NIRS calibrations obtained as for a typical food system with the aluminum cup (British cup) can be used quickly and accurately to evaluate the fat and protein contents of ovine milk. This sample cell can work at room temperature without any sample conditioning (homogenization) and can analyze liquid (e.g., milk), paste (e.g., fermented milk), or solid (e.g., cheese, butter, milk powder) samples.

Acknowledgment

We thank Luis Sancho (Bran+Luebbe, Madrid, Spain) for his technical assistance.

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