# Near infrared spectroscopy for biomonitoring: cow milk composition measurement in a spectral region from 1,100 to 2,400 nanometers

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# Near infrared spectroscopy for biomonitoring: Cow milk composition measurement in a spectral region from 1,100 to 2,400 nanometers[1]

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**ABSTRACT:** The potential of near infrared spectroscopy (NIRS; 1,100 to 2,400 nm) to measure fat, total protein, and lactose content of nonhomogenized milk during milking and the influence of individual characteristics of each cow's milk on the accuracy of determination were studied. Milk fractions were taken during milking, twice per month, for 6 mo. Samples were taken every 2nd and 4th wk at the morning and the evening milkings. Teatcups were removed at each 3 L of milk yield as determined with a fractional sampling milk meter. A total of 260 milk samples were collected and analyzed with an NIRSystem 6500 spectrophotometer with 1-mm sample thickness. Partial least squares (PLS) regression was used to develop calibration models for the examined milk components. The comparison with the reference method was based on standard error of cross validation (SECV). The obtained SECV varied from .107 to .138% for fat content, from .092 to .125% for total protein, and from .066 to .096% for lactose content, and the accuracy of the reference method (AOAC, 1990, method No 972.16) was .05% for all measured milk components. The obtained models had lower SECV when an individual cow's spectral data were used for calibration. The reduction of SECV for each cow's individual calibration, when compared with SECV for the set of all samples, differed with the different constituents. For fat content determination, the reduction reached 22.46%, for protein 26.40%, and for lactose 31.25%. This phenomena was investigated and explained by principle component analysis (PCA) and by comparing loading of PLS factors that account for the most spectral variations for each cow and the measured milk components, respectively. The results of this study indicated that NIRS (1,100 to 2,500 nm, 1-mm sample thickness) was satisfactory for nonhomogenized milk compositional analysis of milk fractions taken in the process of milking.

Key Words: Milk, Fat, Protein, Lactose, Spectroscopy

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## Introduction

Daily measurement of milk composition for each cow could be used to improve animal selection and management schemes. Milk fat content could be used to regulate the diet forage:concentrate ratio (Hawke and Taylor, 1995), and the protein content could be used as an indicator of adequate dietary energy supply (Svennersten-Sjaunja et al., 1997). Lactose content could be used to detect mastitis (Atkinson et al., 1995). However, routine analytical methods used for milk testing are destructive and expensive, as well as time- and labor-consuming.

The modern management of dairy farms involves automated systems that control the production process and collect on-line data for each cow. The use of near infrared spectroscopy (**NIRS**) for on-line milk analysis on the farm could provide additional information to managers to be used to increase efficiency (Tsenkova et al., 1994, 1995). The advantages of NIRS to the present methods include speed and simultaneous, nondestructive measurement of a number of milk constituents as well as great potential for on-line analysis.

Although NIRS has been used to measure the content of various constituents in milk, the samples have been dry milk (Baer et al., 1983), homogenized milk (Sato et al., 1987; De Boever et al., 1990; Pascual et al., 1996), or dried milk on glass fiber filter disks (Díaz-Carrillo

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et al., 1993). Only a few authors have reported analysis of nonhomogenized milk samples (Schmilovitch et al., 1992; Tsenkova et al., 1992, 1994, 1995).

The purpose of this study was to investigate the potential of NIRS (1,100 to 2,400 nm) to measure milk fat, total protein, and lactose content of nonhomogenized milk samples taken in the process of milking and to investigate the influence of individual characteristics of each cow's milk on the accuracy of determination.

## Materials and Methods

### *Milk Samples*

A total of 260 individual milk samples were collected from three Holstein cows (97 samples from cow No. 1, 84 samples from cow No. 2, and 79 samples from cow No. 3). The samples were collected twice per month, for a 6-mo period, in each 2nd and 4th wk, at morning and evening milkings. Teatcups were removed after 3 L of milk were produced as determined with a fractional sampling milk meter (True-Test; Alfa Laval Agri, Tumba, Sweden). The milk fractions were analyzed shortly after collection. The experiment was carried out between mo 2 and 8 of the second lactation. All cows were 3 yr old and fed the same diet. The experiment was carried out from May to October.

Milk fat, total protein, and lactose content of the examined samples were analyzed with spectroscopic technology in the midinfrared region, using a MilkoScan instrument (N. Foss-Electric A/C, Hillerød, Denmark), which has been accepted as a reference method (AOAC, 1990, method No. 972.16).

### *Near Infrared Spectra*

Transmittance (**T**) spectra of 1-mm-thick milk samples were obtained with an NIRSystem 6500 spectrophotometer (FOSS NIRSystems, Silver Spring, MD) using quartz cuvettes with 1-mm-thick walls. Spectra were measured in the wavelength range from 1,100 to 2,400 nm at a 2-nm intervals and were recorded in the linked computer as absorbance that is log (1/T). Prior to spectral analysis, each sample was warmed to 40°C in a water bath.

### *Near Infrared Data Treatment*

A commercial program (Pirouette Version 2.0; Infometrics, Woodinville, WA) for multivariate spectral data analysis was used to process the data and to develop models for fat, total protein, and lactose content determination.

Data were analyzed in four sets. One set included all samples, and each of the other sets included data from only one cow.

Prior to calibration, spectral data were mean-centered and transformed as second derivatives using 25 data-point windows. The derivative transformations were based on Savitzky-Golay polynomial filter. Preliminary data analysis showed small differences or no benefit from other mathematical pretreatments.

Calibration for measured milk components was performed using partial least squares (**PLS**) regression. The PLS used both the spectral response and the respective reference data for the examined samples to determine latent variables (PLS factors) in the data set. In the development of all calibration models, 10 PLS factors were set up as a maximum number to work with. The optimum number of PLS factors used in the models was determined with a cross-validation method. In the cross-validation procedure, five samples were temporarily removed from the data set to be used for validation. With the rest of the samples, a PLS model was developed and applied to predict the measured constituent of each milk sample in the group of five samples. The results were compared to their respective reference values. This procedure was repeated until a prediction for all samples was obtained. Performance statistics were accumulated for each group of removed samples. The validation errors were combined into a standard error of cross-validation (**SECV**), which was accepted as a measure of accuracy of determination. The optimum number of PLS factors in each model was defined to be the one that corresponded to the lowest SECV.

Qualitative analysis of milk spectra was carried out with a principal component analysis (**PCA**) to determine whether the individual characteristics of each cow had an influence over the respective milk spectra. Loading plots of respective PLS factors that are responsible for most of the variations in the spectra were analyzed for each investigated milk component and each cow. Absorbance bands that have an impact in developing calibration models for each measured milk component and for each cow were discussed.

## Results and Discussion

From a spectroscopic point of view, NIRS data used for a calibration model of a certain trait have to cover as wide a range as possible to minimize the error of prediction (Schenk et al., 1992). The ranges of examined milk components measured by standard chemical analysis are presented in Table 1. The experiment was designed to cover milk composition changes that occurred during milking (Figure 1) and in one lactation. Collected samples varied widely, especially in fat content, which changed the baseline of milk spectra (Figure 2) and had to be removed by spectral data treatment (Sato et al., 1987).

The changes in milk fat, total protein, and lactose content during an afternoon milking (seven milk fractions) of one of the cows (cow No. 3) are presented in Figure 1. The respective spectra of the seven milk samples are shown in Figure 2. Due to absorbance by O-H groups in water, two bands around 1,440 and 1,950 nm dominated the spectra. The characteristic absorption bands of fat and other milk components such as protein

**Table 1.** Fat, protein, and lactose concentrations in milk samples from three cows determined with a midinfrared method, the AOAC method No. 972.16

| Sample set | Min. | Max. | Mean | SD |
|---|---|---|---|---|
| | | Fat, % | | |
| All samples | .34 | 10.41 | 3.505 | 1.921 |
| Cow No. 1 | .77 | 8.83 | 3.838 | 1.973 |
| Cow No. 2 | .69 | 7.44 | 3.303 | 1.663 |
| Cow No. 3 | .34 | 10.41 | 3.316 | 2.105 |
| | | Protein, % | | |
| All samples | 2.60 | 3.99 | 3.132 | .232 |
| Cow No. 1 | 2.83 | 3.99 | 3.132 | .224 |
| Cow No. 2 | 2.60 | 3.68 | 3.028 | .226 |
| Cow No. 3 | 2.81 | 3.71 | 3.230 | .199 |
| | | Lactose, % | | |
| All samples | 3.94 | 4.74 | 4.399 | .163 |
| Cow No. 1 | 3.94 | 4.63 | 4.362 | .148 |
| Cow No. 2 | 3.98 | 4.59 | 4.327 | .132 |
| Cow No. 3 | 4.14 | 4.74 | 4.521 | .146 |

and lactose were very weak in comparison with the water bands and were difficult to see. The spectrum baseline shifted upward with increased fat content. Computation of second derivatives of NIRS allowed the resolution of overlapping peaks and removal of baseline variation. The second derivatives of the same spectra are presented in Figure 3. Noticeable spectral features in the second derivative spectra, besides absorption of water, occurred at 1,160, 1,210, 1,726, 2,308, and 2,354 nm due to C-H bond absorption; at 2,110 nm due to O-H bond; and at 1,992, 2,054, and 2,280 nm due to N-H absorption. These spectral ranges could be used for determination of fat, protein, and lactose content, respectively.

### *Milk Composition Analysis*

The results for milk fat, total protein, and lactose content determination using milk spectral data were very successful for all sample sets (Table 2). The obtained SECV were from .107 to .138% for fat content, from .092 to .125% for total protein, and from .066 to .096% for lactose content, respectively. This accuracy was close to the accuracy of the reference method, which was .05% for milk fat, protein, and lactose, respectively.

For fat content determination, the correlation coefficients obtained from calibration and cross-validation procedures were greater than .997. Relatively low correlation coefficients for protein and lactose determination might be explained by the small total variation of protein and lactose content in milk, especially for the data sets that included milk samples from individual cows. For example, calibration coefficients, R and CVr of the model for total protein determination, based on milk samples from cow No. 1 were lower than the respective value of R and CVr for the data set of all samples. The SEC and SECV for the set of all samples were higher.

The results for protein determination were close to those reported by Pascual et al. (1996) for ewe's milk. The standard error of calibration was .089% and standard error of prediction was .112%.

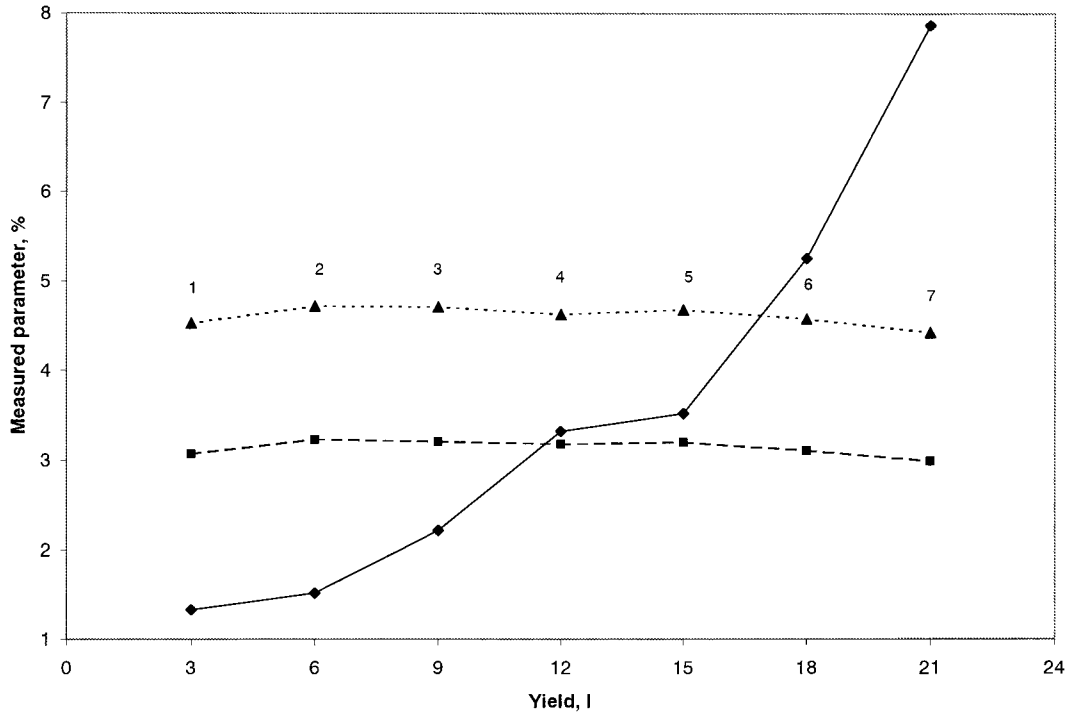


**Figure 1.** Changes in milk fat, total protein, and lactose content during one afternoon milking of cow No. 3 (◆ = fat, ■ = total protein, ▲ = lactose).

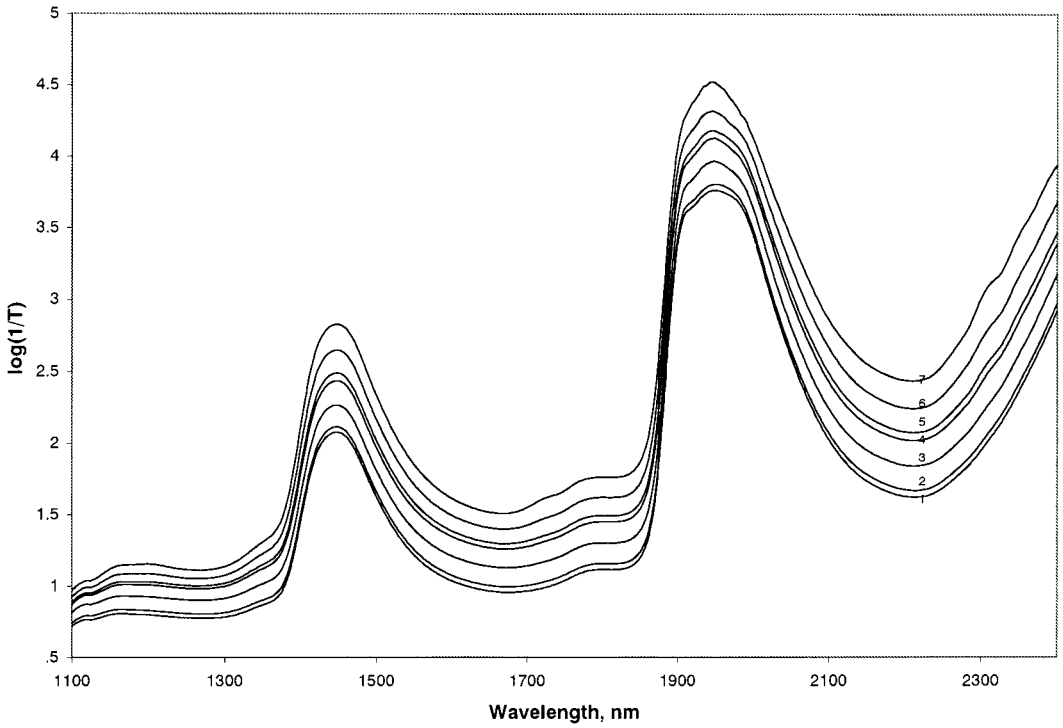


**Figure 2.** Near-infrared spectra of milk samples from one afternoon milking of cow No. 3.

For all measured constituents, the accuracy of the calibration models derived from milk samples of each cow was higher than the accuracy of models derived from all milk samples. The comparison was based on SECV. The reduction of SECV for each calibration, when compared with SECV for the set of all samples, differed with the different constituents. For fat content determination, the reduction reached 22.46% (cow No. 3), for protein 26.40% (cow No. 3), and for lactose 31.25% (cow No. 2).

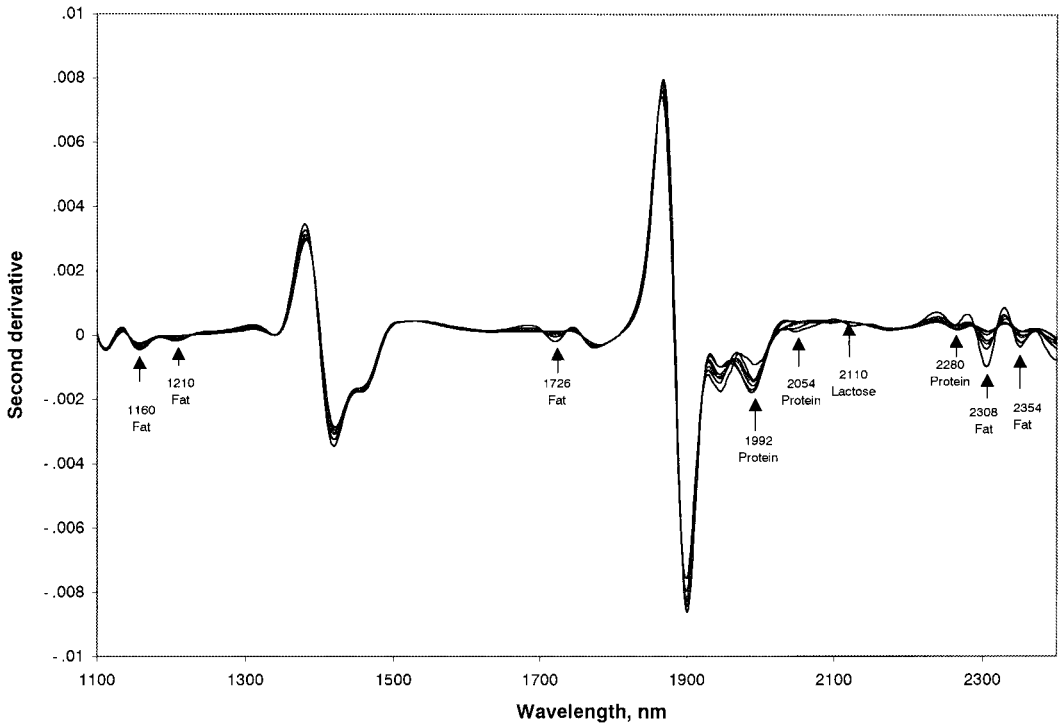


**Figure 3.** Second derivative transformation of spectra of milk samples from one afternoon milking of cow No. 3.

**Table 2.** Near infrared spectroscopy calibration and cross-validation statistics for fat, total protein, and lactose content determination in milk samples from three cows

| Sample set | PLS[a] factors | SEC[b] | R[c] | SECV[d] | CVr[e] | CV[f],% | % change[g] of SECV |
|---|---|---|---|---|---|---|---|
| | | | Fat | | | | |
| All samples | 8 | .123 | .998 | .138 | .998 | 3.94 | — |
| Cow 1 | 10 | .077 | .999 | .116 | .998 | 3.02 | 15.94 |
| Cow 2 | 9 | .092 | .999 | .123 | .997 | 3.72 | 10.87 |
| Cow 3 | 9 | .067 | .999 | .107 | .999 | 3.23 | 22.46 |
| | | | Total protein | | | | |
| All samples | 9 | .112 | .798 | .125 | .760 | 3.49 | — |
| Cow 1 | 5 | .095 | .783 | .106 | .700 | 3.38 | 15.20 |
| Cow 2 | 8 | .094 | .840 | .123 | .678 | 4.06 | 1.60 |
| Cow 3 | 9 | .062 | .934 | .092 | .807 | 2.85 | 26.40 |
| | | | Lactose | | | | |
| All samples | 9 | .087 | .810 | .096 | .783 | 2.18 | — |
| Cow 1 | 9 | .066 | .846 | .082 | .720 | 1.88 | 14.58 |
| Cow 2 | 9 | .053 | .892 | .066 | .809 | 1.53 | 31.25 |
| Cow 3 | 8 | .055 | .881 | .079 | .719 | 1.75 | 17.71 |

[a]Number of factors in the calibration model. PLS = partial least squares.
[b]SEC = standard error of calibration.
[c]R = coefficient of multiple correlation.
[d]SECV = standard error of cross-validation.
[e]CVr = cross-validation correlation coefficient.
[f]CV,% = Coefficient of variation – (SECV/mean value) × 100.
[g]Percentage change of SECV for individual calibration in comparison with SECV of general calibration.

*Milk Spectral Data Analysis*

To investigate the reason for obtaining different accuracy of the calibration models when individual cow data sets were used, qualitative analysis of milk spectra was performed with PCA and PLS (Williams and Norris, 1987). The PCA projects the spectra into new axes: principal components (**PC**). They represent the main variations of the samples into each PC. The spectra of the three cows were clearly separated in the score space of Factors 2 and 4. In Figure 4, a two-dimensional score plot (on the x-axis is Factor 2 and on the y-axis is Factor 4) represents the spectra of milk samples of all cows, taken from one morning milking. The same phenomena occurred for the rest of the milkings.

The percentage of variations, described by each PLS factor, included in the models for fat, total protein, and lactose determination are presented in Table 3. Loading plots of respective PLS factors that are responsible for most of the variations in the spectra were analyzed and compared for each milk component and each individual cow.

The first PLS factor in the models for fat determination of individual cow milk accounted for 64.3% of the total spectral variations for cow No. 1, 69.0% for cow No. 2, and 75.2% for cow No. 3. The loading plot shows similar spectral patterns for all cows (Figure 5). The peaks of the three loadings were at the same wavelengths in the areas significant for fat determination, such as peaks at 1,726, 1,760, 2,308, and 2,348 nm.

**Table 3.** Percentage of total spectral variations accounted for by each partial least squares (PLS) factor included in the models for milk fat, total protein, and lactose determination

| PLS factor | Fat | | | Total protein | | | Lactose | | |
|---|---|---|---|---|---|---|---|---|---|
| | Cow 1 | Cow 2 | Cow 3 | Cow 1 | Cow 2 | Cow 3 | Cow 1 | Cow 2 | Cow 3 |
| 1 | 64.33 | 69.03 | 75.19 | 40.08 | 11.86 | 51.36 | 58.82 | 62.68 | 74.58 |
| 2 | 3.38 | 4.34 | 5.62 | 29.45 | 58.40 | 26.17 | 16.41 | 13.11 | 6.40 |
| 3 | 8.44 | 11.24 | 6.76 | 8.78 | 13.66 | 6.46 | 4.37 | 7.94 | 4.98 |
| 4 | 6.83 | 2.41 | .86 | 3.45 | 1.35 | 4.36 | 5.69 | 2.13 | 1.82 |
| 5 | 2.29 | 1.13 | 1.24 | 5.94 | 2.44 | 1.19 | 3.22 | 1.82 | 1.12 |
| 6 | 1.45 | 1.29 | 1.13 | 1.95 | 1.18 | 1.33 | .87 | .76 | 1.83 |
| 7 | 1.83 | 1.16 | 1.52 | .70 | 1.59 | 1.32 | 1.17 | 1.41 | 1.11 |
| 8 | .94 | .51 | .95 | 2.20 | .75 | 1.00 | 1.79 | .99 | .95 |
| 9 | .79 | 1.46 | .64 | .57 | 1.22 | .73 | 1.00 | 1.27 | .96 |
| 10 | .63 | .72 | .57 | .28 | .68 | .59 | .65 | .74 | .73 |
| Total | 94.21 | 93.29 | 94.48 | 93.40 | 93.13 | 94.51 | 93.99 | 92.85 | 94.48 |

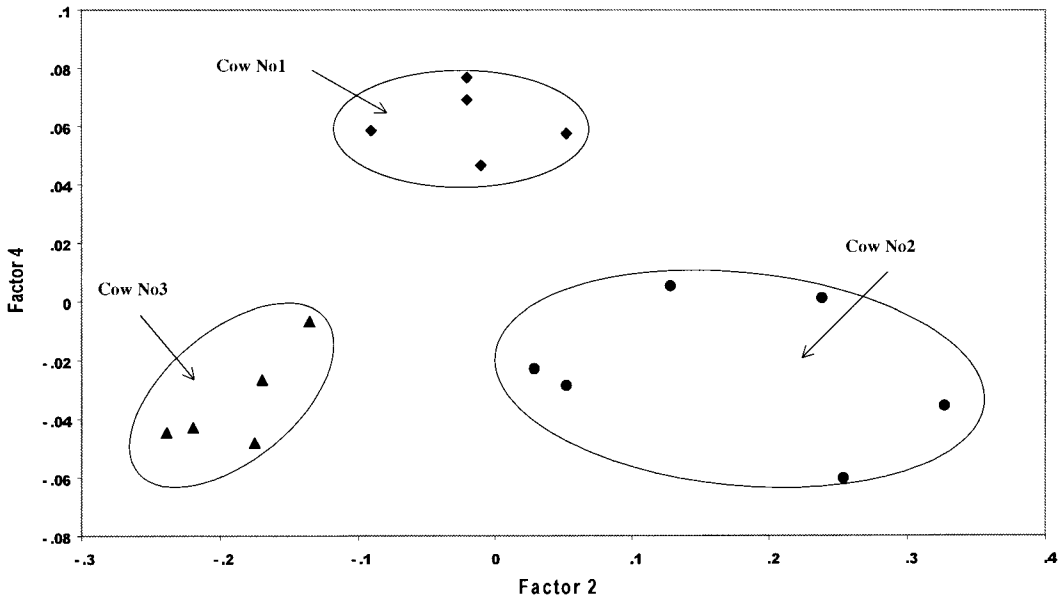


**Figure 4.** Score plot of principal component analysis (PCA) Factors 2 and 4 for milk spectral data from one morning milking.

Small differences were observed in the region of 1,925 to 2,000 nm and 2,030 to 2,160 nm. The regions from 1,980 to 2,070 nm are dominated by absorption of N-H bands of proteins. The observed differences in the PLS loadings in these regions might be explained by the influence of milk proteins on milk fat determination. Proteins are included in the native fat globule membrane that covers the lipid droplets (Keenan et al., 1988). A possible reason for the differences between the PLS loadings for each cow could be the individual differences in the milk proteins.

The PLS factors that account for the biggest percentage of the total spectral variations in the models for total protein content determination were the first factor for cow No. 1 (40.1%), the second factor for cow No. 2 (58.4%), and the first factor for cow No. 3 (51.4%). A plot of the loadings of these factors is shown in Figure 6. The spectral patterns for the three cows were different in the regions around 1,132 nm and in the area from 1,440 to 1,520 nm, from 1,920 to 2,180 nm, and from 2,340 to 2,400 nm. The area around 1,450 and 1,940 nm is dominated by the absorption of water. The differences in these areas were probably connected with the influence of the water-soluble proteins in milk from individual cows that caused different shifts in absorption of O-H groups in water. The spectral features at

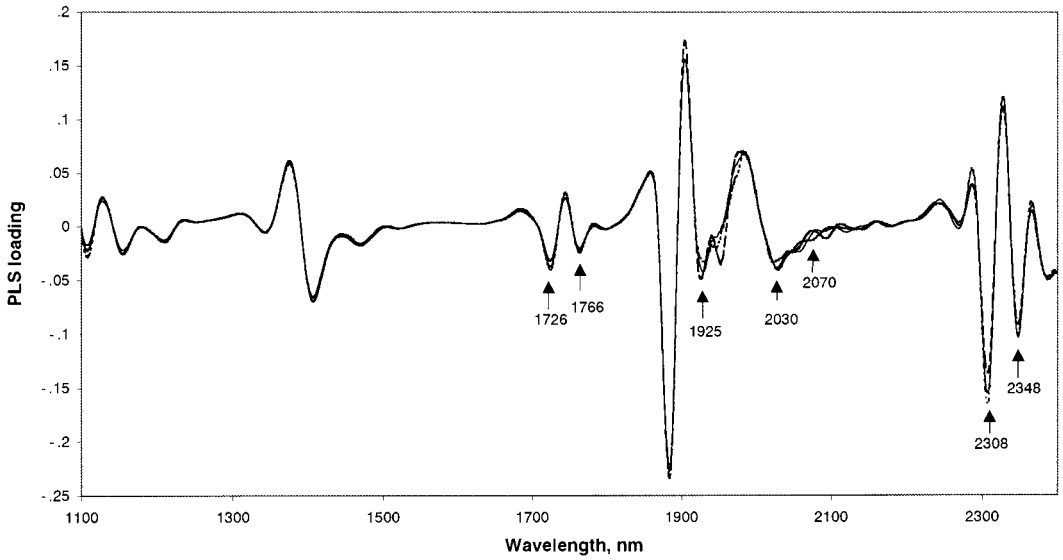


**Figure 5.** Plot of partial least squares (PLS) loadings used for milk fat content determination with models for each cow (— Cow No. 1, – – – Cow No. 2, - - - - Cow No. 3).

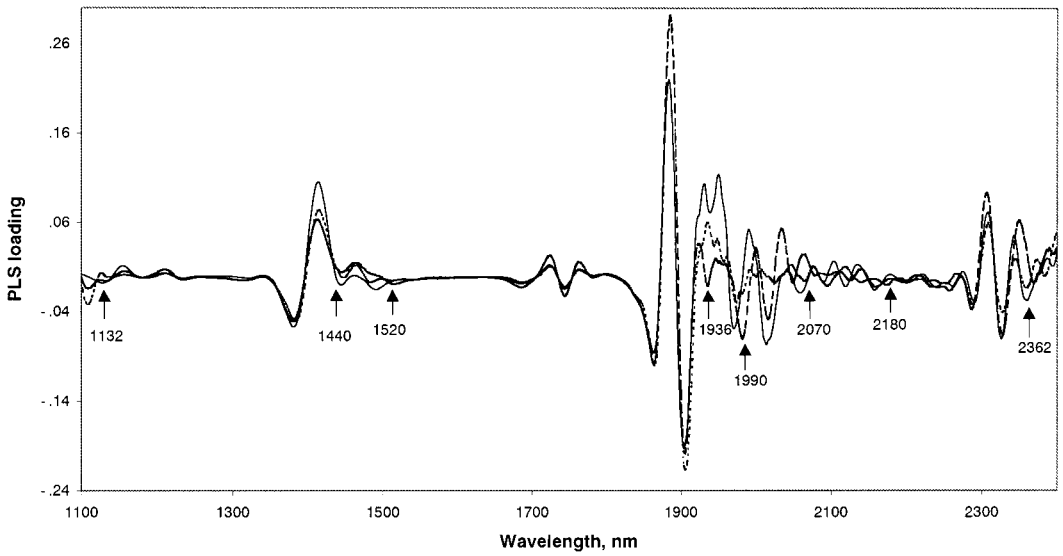


**Figure 6.** Plot of partial least squares (PLS) loadings used for milk total protein content determination with models for each cow (— Cow No. 1, – – – Cow No. 2, - - - - Cow No. 3).

1,132 nm, from 1,460 to 1,520 nm, 1,980 to 2,070 nm, and 2,170 to 2,180 nm, respectively, were due mainly to the absorption of proteins. Some of the most significant differences were observed at wavelengths connected with the absorption of N-H groups in protein, such as 1,460, 1,490, 1,520, 1,990, 2,030, and 2,070 nm (Schenk et al., 1992). The total protein content of milk, estimated using the MilkoScan, consisted of true proteins (e.g., casein and albumin) as well as nonprotein nitrogenous compounds. Differences in the PLS loading for each cow might be explained by differences in the content or physical structure of true proteins and differences in the ratio of true protein and nonprotein nitrogen in the milk samples collected from different cows.

The first PLS factor in the models for lactose determination of individual cow milk accounts for 58.8% of the total spectral variations for cow No. 1, 62.7% for cow No. 2, and 74.6% for cow No. 3. The loading of these factors shows differences in the position of the peak around 1,406 nm; in the regions 1,438 to 1,450 and 1,460 to 1,500 nm, around 1,860 nm, and from 1,920 to 2,120 nm (Figure 7). The differences around 1,406, 1,450, and 1,920 nm could be explained by a shift of the water absorption band due to differences in lactose content. Sodium, potassium, and chloride ions are negatively correlated to lactose and maintain osmotic equilibrium of milk with blood (Atkinson et al., 1995). They are present in milk almost entirely as free ions. The

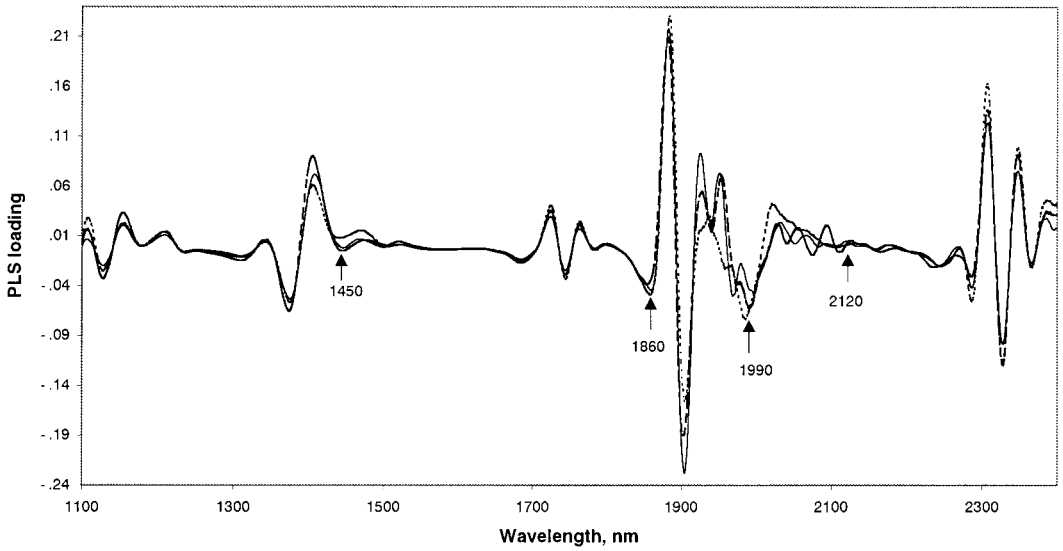


**Figure 7.** Plot of partial least squares (PLS) loadings used for lactose content determination with models for each cow (— Cow No. 1, – – – Cow No. 2, - - - - Cow No. 3).

changes in concentration of these ions in accordance with the changes of lactose content of milk could shift the position of water absorbance bands (Binette and Buijs, 1996; Molt et al., 1998). The differences in the 1,950 to 2,100 nm range could be explained by the influence of nitrogenous components in the milk.

## Implications

Near infrared spectroscopy (NIRS) determination of milk fat, total protein, and lactose for nonhomogenized cow's milk in the region from 1,100 to 2,400 nm, with 1-mm sample thickness, had an accuracy close to a reference method. More accurate results were obtained when individual calibration models for each cow were developed. The differences in the accuracy between the general calibration model based on all milk samples and the models for milk samples from individual cows were explained by the differences in chemical composition and physical structure of the milk from each cow. The NIRS method allowed rapid, simple, and simultaneous determination of various components of an individual cow's milk. This may be of great economic importance for dairy farm management.

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