At-Line Near-Infrared Spectroscopy for Prediction of the Solid Fat Content of Milk Fat from New Zealand Butter

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Near-infrared (NIR) spectroscopy calibrations that will allow prediction of the solid fat content (SFC) of milk fat extracted from butter by one measurement during manufacture were developed. SFC is a measure of the amount of the solid fraction of fat crystallized at a temperature expressed as a percentage (w/w). At-line SFC determinations are currently performed by nuclear magnetic resonance (NMR) spectroscopy, which involves a 16 h delay period for tempering of the milk fat at 0 °C prior to the SFC measurements, from 0 to 35 °C in a series of 5 °C increments. The NIR spectra (400–2500 nm) were obtained using a sample holder maintained at 60 °C. Accurate predictions for the SFC (%) were developed by principal component analysis (PCA) and partial least-squares (PLS) regression models to relate the NIR spectra to the corresponding NMR values. The independent validation samples ($N = 22$) had a standard error of prediction (SEP) of 0.385–0.762% for SFC between 0 and 25 °C, with SFC reference values ranging between 70.42 and 8.96% with a standard deviation range of 3.36–1.47. The low bias (from −0.351 to −0.025), the slopes (0.935–1.077), and the excellent predictive ability (R^2; 0.923–0.978) supported the validity of these calibrations.

KEYWORDS: Butter; milk fat; solid fat content; NIR spectroscopy; NMR

INTRODUCTION

Solid fat content (SFC) is an important parameter in the dairy industry. The SFC is a measure of the amount of the solid fraction of fat crystallized under reference conditions at a temperature expressed as a percentage (w/w) and is a good indicator of the functional characteristics of milk fat. In the dairy industry, the milk fat separated from raw milk is an important component in a range of products. Cream is an oil-in-water emulsion. When cream is subjected to agitation, the fat globule membrane can rupture and the fat will agglomerate. This process, known as churning, requires a certain crystallization pattern in the fat for optimum performance, which is created by temperature changes that may vary because of chemical differences in the triacylglycerol composition of milk. Milk fat is composed mainly of triacylglycerols, which represent about 97% of the total fat (*1*). In addition to triacylglycerols, the lipids in milk include diacylglycerols, saturated and polyunsaturated free fatty acids, and phospholipids. The large number of triacylglycerols (*2*), the fatty acids of which vary in chain length and degree of saturation, is responsible for the broad melting range of milk fat, which spans from about −40 to 40 °C. Knowledge regarding the SFC of the cream can assist the butter maker in the selection of appropriate crystallization conditions.

Crystallization in milk fat is complex because of the broad range of triacylglycerols, which leads to a wide SFC range. The SFC determines the "solids" content of milk fat as a function of temperature, and the functional characteristics, such as texture and spreadability, of cream and vegetable oil blended products derived from milk fat depend largely on this parameter.

At-line SFC determinations are currently performed by low-resolution nuclear magnetic resonance (NMR) spectroscopy (*3*) using a method (*4*) that is approved by the American Oil Chemists' Society (AOCS). The modified AOCS method used (*5*) involves a 16 h delay period for tempering of the fat at 0 °C prior to the SFC measurement, and sample analysis by NMR from 0 to 35 °C in 5 °C increments is time-consuming.

Near-infrared (NIR) analysis of fat in raw milk has been evaluated. Quantitative analysis by NIR spectroscopy in both the 1100–2500 nm region (*6*) and the short-wave (800–1100 nm) region (*7*) using partial least-squares (PLS) regression has produced reliable predicted values from the calibrations. Fat, along with moisture and solids-nonfat, in butter has been analyzed at room temperature by NIR (*8*). There has been research in the margarine industry evaluating the use of NIR for predicting the SFC profiles of vegetable oils. Calibration of NIR spectroscopy for the SFC of fat blends for margarine analysis using NMR data, and measured at room temperature (*9*) or 70 °C (*10*), has shown that NIR spectroscopy is a reliable technique and can replace NMR for SFC estimation. Butter has been evaluated by NIR, with iodine values used as the reference to discriminate between classes of edible fats and oils (*11*).

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There is scope within butter manufacturing for the development of a rapid alternative that will allow routine screening of large numbers of samples at-line with real time control of production, to check in-process SFC composition, and to meet the required functional characteristics. In response to the need for a rapid method, the Foss Analytical Fats and Oils Analyzer, on the basis of the Foss NIRS 6500 system, was used to develop accurate calibrations that allow estimation of the SFC from 0 to 35 °C in 5 °C increments by one measurement at 60 °C.

MATERIALS AND METHODS

Butter Samples. A trial was conducted in which the 76 samples selected were from manufacturing plants that were representative of the major dairying regions of New Zealand in terms of geographical distribution and volume of butter produced. Butter samples from two production seasons, 2000/2001 and 2002/2003, spring (September to November), summer (December to February), and autumn (March to May), from eight New Zealand plants were evaluated using NIR spectroscopy.

Sample Preparation. The extraction procedure for obtaining the milk fat from butter was a simple heating, centrifugation, and filtering technique (*5*). This was done in sets of two and involved heating the samples to 60 °C, which were then transferred to Duran Schott 80 mL centrifuge tubes. The tubes were then placed in 150 mL beakers and were transferred to a Harvard Trip beam balance. A 3 mL short Samco pipet was used to remove serum phase from the heavier tube until the samples were balanced. The molten samples were centrifuged at 2000 rev/min for 5 min in a Heltich universal bench top centrifuge. The liquid upper layer was poured into a filter funnel containing Whatman 185 mm diameter filter paper in a 150 mL beaker, and the filtered fat was placed in a 60 °C oven. The filtered milk fat was transferred to glass screw-capped bottles for further analysis.

NMR Reference Analysis. Reference SFC values were obtained by low-resolution pulsed NMR with a Minispec PC/120 series NMR analyzer (Bruker, Silberstreifen, Germany). Each fat sample (35 mL) was transferred to a flat-bottomed, glass NMR tube (10 mm diameter and 180 mm length) using a 3 mL Samco pipet and was placed in a DRI-Block DB-3 (Techne Inc., Cambridge, U.K.) at 67 °C for 45 min. The SFC of milk fat was measured according to the method of MacGibbon and McLennan (*5*). The tempering procedure included holding samples overnight at 0 °C followed by serial measurements in 5 °C increments from 0 to 35 °C. Two replicates of each sample were equilibrated for 45 min at each temperature (0, 5, 10, 15, 20, 25, 30, and 35 °C) prior to measurement. The mean of the two NMR values was used as the SFC reference value for the NIR calibration models. The repeatability of the mean NMR value for SFC ranges from 0.08 to 0.21 standard deviation (SD) for the NMR duplicate reference measurements.

Spectroscopic Analysis. A Foss 6500 NIR spectrometer (Foss Analytical, Hillerod, Denmark) was used for collecting the NIR spectra at Fonterra Research Centre, Palmerston North. The duplicate milk fat samples used for NMR reference determination were utilized for NIR analysis. The sample-handling accessory was a temperature-controllable single-vial holding block capable of accepting 8 mm (outer diameter, path length) transparent glass vials with a volume of 1 mL. A subsample of each NMR milk fat sample was transferred to a glass vial. The NIR spectra acquired at 60 °C were the average of 32 scans between 400 and 2500 nm, with 32 scans for air background spectra, and wavelength increments of 2 nm were used. The NIR instrument scans from 400 to 2500 nm, of which 400−780 nm is the visible and 780−2500 nm is the NIR. The sample data were recorded as log $1/R$, where R is the reflectance energy. Repeat measurements (*10*) of an individual sample were performed across the NIR wavelength range 400−2500 nm and the mean and standard deviation (SD) at each wavelength were calculated and then summed to determine the replicate instrumental error in NIR predictions. Randomized samples were analyzed over multiple days, resulting in acquisition of replicate spectra for each milk fat sample. Diagnostic tests for wavelength (0.5 ± 0.02) and bandwidth

(10.00 ± 1.00) were performed every 2 days. FOSS WinISI III V 1.6 software (FOSS, Hillerod, Denmark) was used to develop calibration equations.

Calibration Development. The spectral data for the development of the calibration were imported into FOSS WinISI III V 1.6 software. An initial PLS assessment identified spectral global outliers (GHs) and showed that only the region from 540 to 2250 nm contributed to the prediction models; thus, the wavelength regions 400−540 nm and 2250−2500 nm were eliminated. The milk fat spectra ($N = 149$) were evaluated by principal component analysis (PCA) and two outliers with GH > 3, spectra that were very different from the average spectrum, were purged. The file was randomly split into calibration samples ($N = 52$) and validation samples ($N = 22$) with the same SFC variation. Mathematical preprocessing treatments of the log $1/R$ spectral data involved standard normal variate transformation (SNV) (*12*) and detrend (D) scatter correction, which removes or reduces linear and quadratic curvature of each spectrum for subsequent PCA. The first-derivative mathematical transformation was applied, with a gap of 4 (number of data points over which the derivative is calculated), and the factors for data smoothing were 4 and 1 (hence, 1,4,4,1 mathematical treatment). The second derivative of the spectra was calculated using a subtraction gap of 6 (number of data points over which the derivative is calculated) and smoothing factors for 6 and 2 data points (hence, 2,6,6,2 mathematical treatment). Additional mathematical pretreatments, such as preprocessing with the multiplicative scatter correction (MSC) followed by first-derivative mathematical transformation, were investigated.

The mean was calculated and the distances of spectral outliers were determined. The population boundary for PCA was set to 3 SDs from the mean spectrum of the calibration set using a Mahalonobis distance method to generate a scores file. Spectra with GH > 3 were purged; one such outlier was identified. The calibration models for SFC were developed from PCA of selected spectra with modified PLS regression (*13*), which used both principal components and laboratory reference data. Modified PLS is a procedure where the residuals obtained after each factor are calculated and standardized (divided by the mean residual value) before calculating the subsequent factor. The same mathematical treatments used for developing the scores were used in the modified PLS regression and resulted in 213 wavelengths being used in the calibration equation. The critical "*T*" value for eliminating outliers was fixed at 2.5. The T value in the software is the student T statistical value. The cross-validation method predicts each sample in the calibration, as it leaves out samples according to segment size, that is, 4, which calibrates three-quarters of the samples and validates one-quarter of the samples. The optimum number of PLS factors for calibration was also determined by cross-validation.

Calibration Performance. Statistics used to assess the PLS models were the coefficient of determination (1-VR), the root mean standard error of calibration (RMSEC), and the root mean standard error of cross-validation (RMSECV). The best scatter correction and mathematical treatment to enhance the spectral data were selected by the models that provided the lowest SECV during regression modeling of the calibration data. The independent validation samples ($N = 22$) contained the same broad range of butter samples, in terms of geographical location and season of production, as the calibration samples ($N = 52$). The models were tested with independent validation samples and performance was reported as the standard error of prediction (SEP) and the coefficient of determination (R^2). The SEP is the best measure of the calibration model's performance and is calculated as

$$SEP = \sqrt{\sum (x_i - y_i)^2 / N}$$

where x_i is the laboratory reference value determined by NMR, y_i is the predicted value from NIR, and N is the total number of milk fat samples; the values shown are uncorrected for bias.

Another statistic used was the coefficient of variation (CV) for the predicted values from cross-validation, that is, the ratio of the mean to the SD multiplied by 100. The CV for the NIR predicted SFC values and the CV for the NMR reference SFC values were calculated for the validation samples.
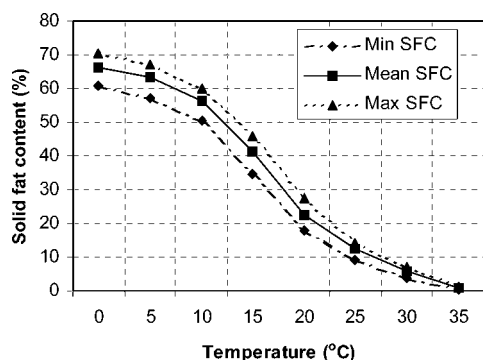
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Figure 1. Profile of the mean, maximum, and minimum SFC (%, w/w) as a function of temperature (from 0 to 35 °C) for the calibration and validation samples of milk fat extracted from New Zealand butters, determined by low-resolution pulsed NMR.

Table 1. Range, Mean, and Standard Deviation of the SFC (%, w/w) of Milk Fat Extracted from New Zealand Butters in the Reference NMR Mean Data Calibration and Validation Sets[a]

		calibration				validation		
	N	range	mean	SD	N	range	mean	SD
0 °C	104	60.84–70.42	66.30	2.70	43	61.40–69.89	65.87	2.99
5 °C	104	56.97–67.07	62.73	2.82	43	57.82–66.47	62.16	3.18
10 °C	104	50.06–60.17	55.49	3.01	43	50.01–59.37	54.88	3.53
15 °C	104	34.04–45.87	40.04	3.36	43	33.78–45.41	39.46	4.09
20 °C	104	17.71–27.61	21.81	2.55	43	17.41–26.48	21.61	2.98
25 °C	104	8.96–14.62	11.92	1.47	43	9.03–14.11	11.65	1.76
30 °C	104	3.71–7.32	5.43	0.98	43	3.57–7.05	5.35	1.19
35 °C	96	0.00–1.68	0.70	0.37	40	0.01–1.57	0.70	0.43

[a] N, number of spectra; SD, standard deviation.

The individual residual values for the validation set ($N = 22$) were determined as the difference between the mean NMR measured and NIR predicted SFC (%) value for a milk fat sample at each temperature from 0 to 35 °C.

RESULTS AND DISCUSSION

Factors Affecting the SFC. The range of SFC (**Figure 1**) values for the milk fat samples, as a function of temperature (0−35 °C), indicated that the region of greatest change in SFC was from 10 to 20 °C. At 0 °C, there were significant differences between the milk fats from butters in terms of the maximum SFC and the minimum SFC. The contrast in the SFC values at the higher temperatures (>30 °C) was less.

The composition of milk fat depends on the composition of the feed (*14−16*), the stage of lactation (*5, 17*), and the plane of nutrition of the cow (*18, 19*). In typical New Zealand factory supply milk, the fat content is 4.4−4.8% at the beginning of the season in July and rises to 5.8−6.0% at the end of the season in April. During the spring, pasture growth and the onset of lactation contribute to higher concentrations of unsaturated fats and short-chain fatty acids and hence lower SFC and softer butter. As the pasture matures, more saturated fats appear and the SFC and the butter hardness increase. In autumn, fresh pasture growth leads to a higher unsaturated fatty acid content and a lower SFC (but not as low as in early spring). The composition of late lactation milk in May tends to be very variable. Data from individual seasons (*5*) indicated that the composition varies from season to season (*17*), depending on the climatic conditions and the rate of grass growth, as there is little supplementary feeding in New Zealand. Butter hardness shows a steep rise through the spring, a plateau in the summer and a gradual decline in the autumn (*5*). The NMR reference

values for the SFC of the calibration and validation milk fat samples are given in **Table 1**.

Bands in the NIR Spectrum of Milk Fat Extracted from Butter. The spectra of the milk fat samples ($N = 76$) for the development of the SFC calibrations are shown in **Figure 2A**. Bands were observed (**Figure 2A**) at 1425, 1910, 1930, 2270, and 2350 nm. These were in similar regions to those reported and identified by Hermida et al. (*8*) in butter samples: at 1210, 1450, 1940, 2310, and 2350 nm. In raw milk, Sasic and Ozaki (*7*) proposed that the assignment of bands attributed to fat in short-wave NIR spectra on the basis of regression coefficients and loading values. The spectra were dominated by a broad feature at 970 nm because of vibration of water, also observed in **Figure 2A**.

PLS Calibration Models for SFC Prediction. The range, mean, and SD of SFC values (**Table 1**) of milk fat samples at different temperatures in the reference NMR calibration and validation data sets were well distributed. The SDs in **Table 1** show the similarity of the reference NMR method calibration and validation data sets. The magnitude of the SD at 30 and 35 °C across a narrow range may pose issues for subsequent PLS regression modeling. The instrumental error across the NIR wavelength range 400 and 2500 nm for a single milk fat sample analyzed 10 times was determined to be $0.023 \times$ SD of the mean log $1/R$ at 60 °C. The effects of a variety of mathematical preprocessing treatments and derivatives of the log $1/R$ spectra of milk fat on the subsequent cross-validation, RMSECV, results (**Table 2**), a guide to the average modeling error, were evaluated. The lowest SECV values at each temperature are shown in bold. The SNV and D scatter correction preprocessing with the first-derivative transformation (**Figure 2B**) was the chosen mathematical treatment for development of the modified PLS regression models. The RMSECV values for the second-derivative transformation at different temperatures with SNV and D scatter correction or MSC preprocessing with the first-derivative transformation did not appear to differ greatly (**Table 2**).

The calibration statistics RMSEC, a measure of fit, RMSECV, a measure for the cross-validation samples, and 1-VR for the SFC of milk fat are given in **Table 3**. The RMSECV (**Table 3**) represented the average difference between the NMR values (laboratory reference) and the NIR values (predicted) of milk fat samples. High correlations (1-VR > 0.941, except at 35 °C) were obtained for cross-validation of the calibration samples ($N = 52$) predicted by NIR. The RMSEC values were only about $30 \pm 2\%$ lower than the RMSECV values, and the 1-VR values were between 0.94 and 0.99, with the exception of the SFC at 35 °C where the RMSEC equaled the RMSECV and the 1-VR value was poor at 0.35. Few PLS factors ($n = 6$ in **Table 3**) were required to account for the SFC measured in the temperature range from 0 to 35 °C. This was comparable with the statistical values found by Hermida et al. (*8*) for regression modeling of the fat in butter; the SEC values were only about 30% lower than the SECV values, and the R^2 values were between 0.90 and 0.94. These figures can be considered to be acceptable, bearing in mind that butter is a product with a very narrow range of composition variation, because of the high level of standardization in butter factories. For fat in raw milk, improved values were obtained by Sasic and Ozaki (*7*), with SEC values between 0.102 and 0.070; SECV between 0.083 and 0.119; and R^2 values between 0.99 and 1.00, differing in the number of PLS factors employed, 4 and 6, respectively.

The validation results presented (**Table 3**) and particularly the SEP values (0.385−0.762%) showed that the calibrations developed are effective for SFC prediction between 0 and
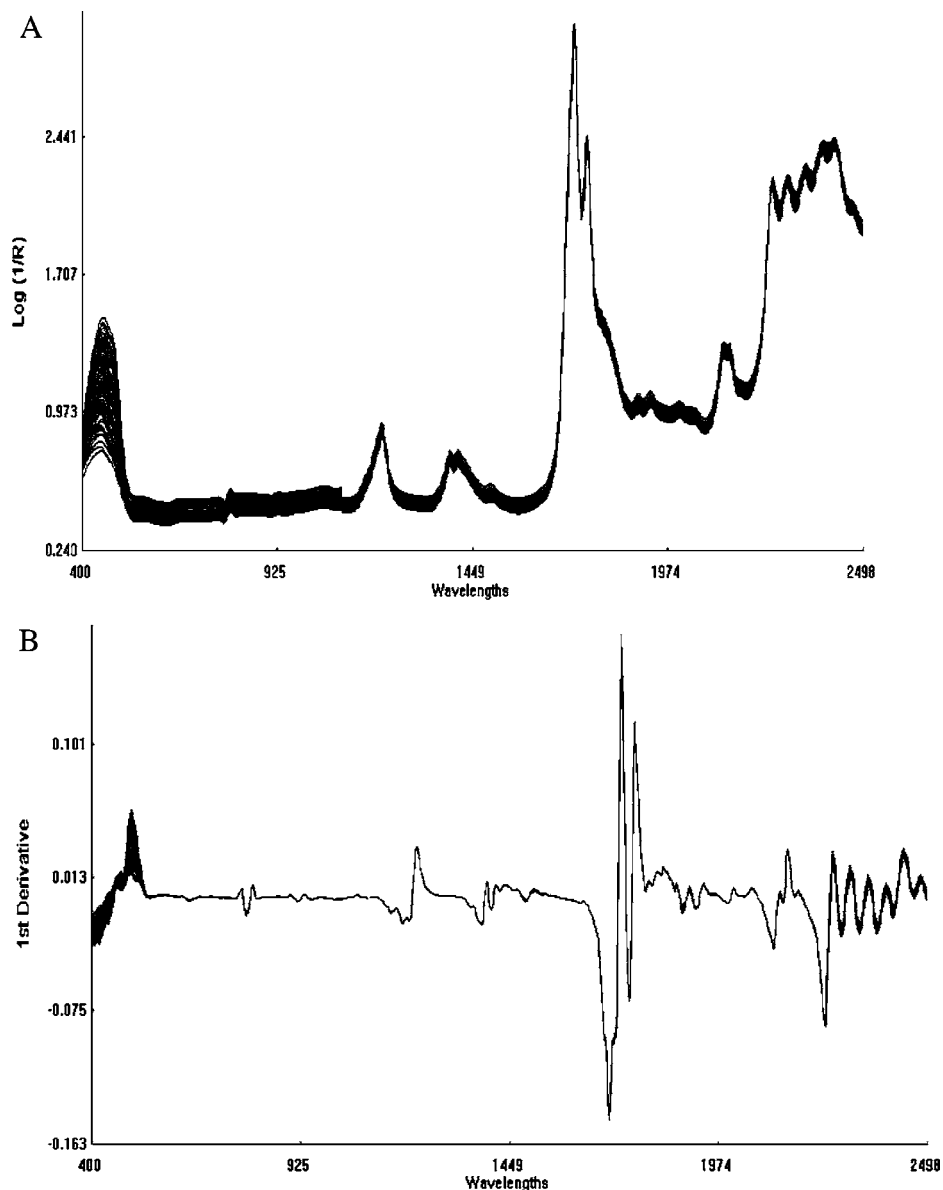
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Figure 2. Spectra acquired for calibrations. **A**: Transmission-based NIR log 1/*R* spectra for the region from 400 to 2500 nm. **B**: Standard normal variate transformation (SNV) and first-derivative transformation with detrend (D) scatter correction of the milk fat extracted from New Zealand butter samples.

Table 2. Effects of Mathematical Preprocessing Treatments and Derivative Mathematical Transformation of the log 1/*R* Spectra of Milk Fat Extracted from Butter on the Subsequent Cross-Validation (RMSECV) Results[a]

mathematical treatment derivative	SNV first	SNV second	MSC first	none first
0 °C	**0.398**	0.439	0.398	0.447
5 °C	**0.400**	0.413	0.404	0.412
10 °C	0.545	0.521	**0.505**	0.552
15 °C	0.678	**0.648**	0.752	0.687
20 °C	0.580	0.576	**0.502**	0.633
25 °C	**0.296**	0.310	0.307	0.325
30 °C	0.314	**0.258**	0.273	0.279
35 °C	0.275	0.276	**0.254**	0.274

[a] RMSECV, root mean standard error of cross-validation; SNV, standard normal variate transformation; MSC, multiplicative scatter correction; first, first derivative; second, second derivative.

25 °C. The SEP value at 20 °C of 0.622, when used with the mean SFC value of the calibration set, 21.81% determined by NMR, would predict an SFC value between 20.60 and 23.03%.

The SEP value at the higher temperature of 30 °C, was lower, that is, 0.338; when used with the mean SFC value of the calibration set, 5.43% determined by NMR, this SEP value would predict an SFC value between 4.75 and 6.11%. The bias is representative of the difference between the reference and predicted means for the validation set. The low bias (from −0.351 to −0.025), good slopes (0.935−1.077), and high R^2 values (0.923−0.978), which were taken as a measure of the good agreement between the two methods, supported the validity of these calibrations. The modified PLS regression models for the SFC of milk fat at 30 and 35 °C had a limited range, and this contributed to the poorer predictive capability.

The results in **Table 4** compare the mean NMR reference method with the predicted values determined with NIR models in terms of the mean of the SFC range at each temperature, SD for reproducibility, and CV for the SFC in the validation samples. The CVs (**Table 4**) give an indicative "difference" in the SFC values by the measured and predictive techniques for the calibration models.

In determining the performance of a calibration with a validation set, what is important is a comparison of the NIR
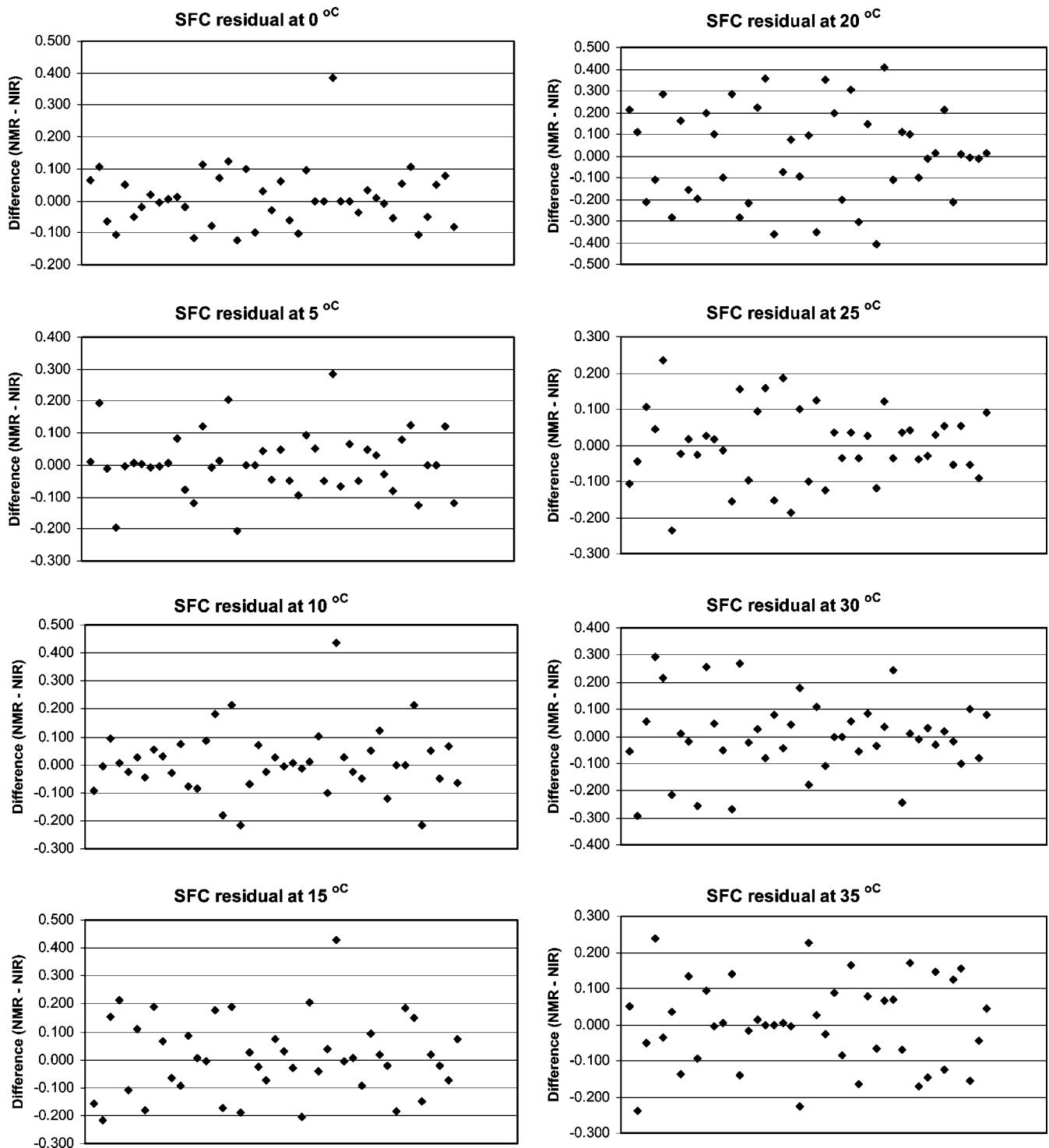
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Figure 3. Graphs for the validation set ($N = 22$) showing the residuals for the difference between NMR measured and NIR predicted SFC (%, w/w) duplicate values in milk fat extracted from New Zealand butter samples at 0–35 °C.

measurement with the mean reference value for each sample. The graphs in **Figure 3** show each individual residual determined as the difference between the mean NMR measured and NIR predicted SFC (%) value for the validation set using the modified PLS models at 0−35 °C. At 0 °C and again at 10 °C and 15 °C, one sample appears as an outlier with a residual at approximately 0.4% compared with the validation set where the residual differences are between ±0.2%. At 20 °C, a greater spread in the residual differences is observed between ±0.4%.

Nearly a decade after Rodriguez-Otero et al.'s (*20*) review of NIR spectroscopy for dairy products, there remains scope

for the introduction of new at-line NIR methodology for predictive results for the SFC of milk fat extracted from butter in dairy manufacture.

In conclusion, sample preparation for the determination of the SFC of milk fat from butter by NMR includes melting the extracted fat, cooling, and tempering under constant conditions for 16 h. When performing SFC analyses, the NMR method requires that measurements be made after stabilization for 30−35 min. In contrast, the NIR measurement can be made immediately after transfer from the heating block to the instrument sample drawer, both of which are maintained at

Table 3. Cross-Validation and Prediction Results for the SFC (%, w/w) of Milk Fat Extracted from New Zealand Butters[a]

		calibration					validation			
	N	RMSEC	1-VR	RMSECV	PLS	N	SEP	R^2	bias	slope
0 °C	100	0.278	0.989	0.398	6	43	0.675	0.949	−0.086	1.032
5 °C	101	0.291	0.989	0.400	6	43	0.514	0.978	−0.216	1.020
10 °C	99	0.375	0.985	0.545	6	43	0.669	0.974	−0.351	1.031
15 °C	100	0.488	0.979	0.678	6	43	0.762	0.972	−0.259	1.060
20 °C	100	0.390	0.975	0.580	6	43	0.622	0.960	−0.030	0.935
25 °C	100	0.206	0.980	0.296	6	43	0.385	0.955	−0.090	1.034
30 °C	100	0.235	0.941	0.314	6	43	0.338	0.923	−0.025	1.077
35 °C	92	0.263	0.351	0.275	6	40	0.400	0.141	0.012	0.766

[a] N, number of spectra; 1-VR, coefficient of determination; RMSEC, root mean standard error of calibration; RMSECV, root mean standard error of cross-validation; PLS, partial least-squares regression factors; SEP, standard error of prediction; R^2, coefficient of determination.

Table 4. Validation Statistics for the SFC (%, w/w) in Milk Fat Extracted from New Zealand Butter Samples Determined by the Mean NMR Reference Value and NIR Predicted Value Using Modified PLS Models at 0–35 °C[a]

	mean NMR value SFC (%)			predicted NIR value SFC (%)		
	mean	SD	CV (%)	mean	SD	CV (%)
0 °C	65.87	2.99	4.5	65.95	2.82	4.3
5 °C	62.16	3.18	5.1	62.37	3.09	4.9
10 °C	54.88	3.53	6.4	55.23	3.38	6.1
15 °C	39.46	4.09	10.4	39.72	3.81	9.6
20 °C	21.61	2.98	13.8	21.64	3.12	14.4
25 °C	11.65	1.76	15.1	11.74	1.66	14.2
30 °C	5.35	1.19	22.3	5.37	1.06	19.8
35 °C	0.70	0.43	62.3	0.68	0.21	30.4

[a] CV, coefficient of variation; PLS, partial least-squares regression; SD, standard deviation; SFC, solid fat content.

60 °C. The Foss instrument is currently being evaluated at-line in a production environment to determine if the required accuracy and precision will be delivered by NIR spectroscopy, to endorse the expected improvement in the turnaround time of SFC results, which would assist in control of butter production.

LITERATURE CITED

(1) MacGibbon, A. K. H.; Taylor, M. W. Composition and structure of bovine milk lipids. In *Advanced Dairy Chemistry*, 3rd ed.; Fox, P. F., McSweeney, P. L. H., Eds.; Springer: New York, 2006; Volume 2, pp 1−42.

(2) Robinson, N. P.; MacGibbon, A. K. H. The composition of New Zealand milk fat triacylglycerols by reversed-phase high-performance liquid chromatography. *J. Am. Oil Chem. Soc.* **1998**, *75*, 993−999.

(3) Gribnau, M. C. M. Determination of solid/liquid ratios of fats and oils by low-resolution pulsed NMR. *Trends Food Sci. Technol.* **1992**, *3*, 186−190.

(4) American Oil Chemists' Society. AOCS Official Method Cd 16b-93. Solid fat content (SFC) by low-resolution magnetic resonance − the direct method. In *Official and Tentative Methods of the American Oil Chemists' Society*; Link, W. E., Ed.; American Oil Chemists' Society: Champaign, IL, 1998.

(5) MacGibbon, A. K. H.; McLennan, W. D. Hardness of New Zealand patted butter: seasonal and regional variations. *NZ J. Dairy Sci. Technol.* **1987**, *22*, 143−156.

(6) Laporte, M. F.; Paquin, P. Near-infrared analysis of fat, protein, and casein in cow's milk. *J. Agric. Food Chem.* **1999**, *47*, 2600−2605.

(7) Sasic, S.; Ozaki, Y. 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. *Anal. Chem.* **2001**, *73*, 64−71.

(8) Hermida, M.; Gonzalez, J. M.; Sanchez, M.; Rodríguez-Otero, J. L. Moisture, solids-non-fat and fat analysis in butter by near infrared spectroscopy. *Int. Dairy J.* **2001**, *11*, 93−98.

(9) Rodrigues, J. C.; Nascimento, A. C.; Alves, A.; Osório, N. M.; Pires, A. S.; Gusmão, J. H.; da Fonseca, M. M. R.; Ferreira-Dias, S. Calibration of near infrared spectroscopy for solid fat content of fat blends analysis using nuclear magnetic resonance data. *Anal. Chim. Acta* **2005**, *544*, 213−218.

(10) Chang, T.; Lai, X.; Zhang, H.; Søndergaard, Ib.; Xu, X. Monitoring lipase-catalyzed interesterification for bulky fat modification with FT-IR/NIR spectroscopy. *J. Agric. Food Chem.* **2005**, *53*, 9841−9847.

(11) Yang, H.; Irudayaraj, J.; Paradkar, M. M. Discriminant analysis of edible oils and fats by FTIR, FT-NIR and FT-Raman spectroscopy. *Food Chem.* **2005**, *93*, 25−32.

(12) Barnes, R. J.; Dhanoa, M. S.; Lister, S. J. Standard normal variate transformation and de-trending of near-infrared diffuse reflectance spectra. *Appl. Spectrosc.* **1989**, *43*, 772−777.

(13) *WinISI software,* version 1.6; FOSS: Hillerod, Denmark.

(14) Palmquist, D. L.; Beaulieu, A. D.; Barbano, D. M. Feed and animal factors influencing milk fat composition. *J. Dairy Sci.* **1993**, *76*, 1753−1771.

(15) Mackle, T. R.; Petch, S. F.; Bryant, A. M.; Auldist, M. J.; Henderson, H. V.; MacGibbon, A. K. H. Variation in the characteristics of milkfat from pasture-fed dairy cows during late spring and the effects of grain supplementation. *NZ J. Agric. Res.* **1997**, *40*, 349−359.

(16) Couvreur, S.; Hurtaud, C.; Lopez, C.; Delaby, L.; Peyraud, J. L. The linear relationship between the proportion of fresh grass in the cow diet, milk fatty acid composition, and butter properties. *J. Dairy Sci.* **2006**, *89*, 1956−1969.

(17) Auldist, M. J.; Walsh, B. J.; Thomson, N. A. Seasonal and lactational influences on bovine milk composition in New Zealand. *J. Dairy Res.* **1998**, *65*, 401−411.

(18) Ashes, J. R.; Gulati, S. K.; Scott, T. W. Potential to alter the content and composition of milk fat through nutrition. *J. Dairy Sci.* **1997**, *80*, 2204−2212.

(19) Hurtaud, C.; Delaby, L.; Peyraud, J. L. Evolution of milk composition and butter properties during the transition between winter-feeding and pasture. *Grassl. Sci. Eur.* **2002**, *7*, 574−575.

(20) Rodriguez-Otero, J. L.; Hermida, M.; Centeno, J. Analysis of dairy products by near-infrared spectroscopy: a review. *J. Agric. Food Chem.* **1997**, *45*, 2815−2819.