Determination of Solid Fat Index by Fourier Transform Infrared Spectroscopy

F.R. van de Voort*, K.P. Memon, J. Sedman, and A.A. Ismail

Department of Food Science and Agricultural Chemistry, Macdonald Campus of McGill University,
McGill IR Group, Ste. Anne de Bellevue, PQ, H9X 3V9 Canada

ABSTRACT: A unique and rapid Fourier transform infrared (FTIR) spectroscopic method for the determination of solid fat index (SFI) of fats and oils was developed, which is capable of predicting the SFI profile of a sample in approximately two minutes, without the need for tempering. Hydrogenated soybean oil samples ($n = 72$), pre-analyzed for SFI by dilatometry, were melted and their FTIR spectra acquired using a 25 μm NaCl transmission flow cell maintained at 80°C. Approximately half the samples were used for calibration, with the balance used as validation samples. Partial least squares (PLS) calibrations were developed from selected spectral regions that are associated with the *cis, trans*, ester linkage and fingerprint regions of the spectrum and related to the dilatometric SFI values obtained at 50, 70, 80, and 92°F. The calibrations were initially optimized and cross-validated by using the "leave one out" approach, with the accuracy and reproducibility of the calibration models assessed by predicting the validation samples. The overall cross validation accuracy of the PLS calibration models was in the order of ±0.71 SFI units over the four temperatures. Week-to-week validation accuracy and reproducibility was determined to be ±0.60 and ±0.38 SFI units, respectively, the reproducibility being within the specifications associated with the dilatometric reference method. To facilitate routine "on-line" FTIR analyses, a Visual Basic program was written to drive the spectrometer, prompt the user to load the sample, calculate, and print the SFI values determined from the PLS calibrations. As structured, the FTIR method has the potential to serve as a viable substitute for the traditional dilatometric SFI method, with the elimination of the tempering step reducing analysis time from hours to minutes. The FTIR approach should also be applicable to the determination of solid fat content if calibrated against solids data obtained by nuclear magnetic resonance.
JAOCS 73, 411–416 (1996).

KEY WORDS: Dilatometry, edible oil analysis, Fourier transform infrared spectroscopy, FTIR spectroscopy, SFC, SFI, solid fat content, solid fat index.

Solid fat index (SFI) is an important quality control parameter in the edible fats and oils industry (1). Its main purpose is to determine the "solids" content of a lipid system as a function of temperature, which has an important bearing on the functional characteristics of margarines, shortenings, and other fat blends. SFI determinations have traditionally been based on dilatometry which provides a measure of the change in the specific volume of the fat as a function of temperature, which is related to its solids content. The SFI determination is an empirical method, and obtaining accurate results is strongly dependent on rigorously following a standardized procedure. Solid fat content (SFC), based on nuclear magnetic resonance (NMR spectroscopy) (2), is another commonly used means of determining solids, which has recently been approved as an official method by the AOCS (3). Differential scanning calorimetry (4) is also used for selected applications; however, SFI and SFC are the dominant methods used in the industry. Both of these methods are quite tedious: the lipid sample must be taken through a series of set temperatures, and a long tempering step is required at each temperature to obtain reproducible values. Although these methods do not necessarily produce identical solids profiles (5), each provides a reproducible means by which solids data can be obtained for formulating products in terms of their functional characteristics. Both SFI and SFC determinations are troublesome, tedious, and expensive to carry out, but no simple, practical alternatives to them exist at the present time.

The McGill infrared (IR) group has been actively engaged in the development of rapid methods of edible oil analysis through the use of Fourier transform infrared (FTIR) spectroscopy (6). Our research has concentrated on the development of methods that could replace traditional chemical methods, including the determination of free fatty acids (7), iodine value (IV) and saponification number (SV) (8), peroxide value (9), *cis/trans* content (10), plus fundamental IR spectroscopic work related to the monitoring of oil oxidation products (11). These methods are designed to produce analytical results within 1–2 min per sample and are based on the spectral information obtained from neat fats and oils in the mid-IR portion of the electromagnetic spectrum. Our approach includes the integration of sample handling, spectral acquisition, calibration development, and programming to produce analytical systems suitable for routine "at line" analysis. Nicolet Instrument (Madison, WI), a major manufacturer of FTIR instrumentation, presently markets an IV/SN/*cis/trans* package based on these concepts, allowing these four measures to be obtained in less than two minutes. The FTIR approach has many benefits, including speed, reliability, and the elimination of noxious solvents and hazardous reagents that are associated with tradi-

---

*To whom correspondence should be addressed.

Copyright © 1996 by AOCS Press

tional chemical methods. In this paper, we describe the development of an automated FTIR method that is capable of determining a standard four-temperature SFI profile within two minutes, without the need for tempering.

MATERIALS AND METHODS

Seventy-two samples of soybean oil, pre-analyzed for SFI (3) by the AOCS dilatometric method (Cd10-57), were obtained courtesy of a major U.S. vegetable oil processor. The samples were comprised of 11 lots of soybean oil, hydrogenated to various degrees, with SFI data for 50, 70, 80, 92, 100, and 104°F.

Instrumentation/sample handling. IR spectroscopy was carried out with a Nicolet "Impact 400" FTIR spectrometer interfaced to a 486/33MHz PC operating under Windows based Nicolet Omnic 2.1® software (12). The instrument and sample compartment were purged with dry air from a Balston dryer (Balston, Lexington, MA) to minimize water vapor and CO_2 interferences. The spectrometer was equipped with a custom-designed oil analysis sample-handling accessory, fitted with a heated transmission flow cell and input/output lines (Dwight Analytical Solutions Ltd., Toronto, Ontario, Canada), that was capable of handling preheated fats and oils (5) in their neat state. All components of the accessory were maintained at 80 ±0.2°C to ensure that the samples flowed readily and to avoid sample crystallization in either the lines or the cell. Samples were loaded by aspiration, first through a bypass line to flush out the bulk of the previous sample, and then through a 25-μm NaCl cell, thus reducing both cross-contamination and the sample volume passing through the narrow cell.

Spectral acquisition/calibration. Fat samples, preheated in a water bath to 80°C, were aspirated into the cell in their neat form, and single-beam spectra were collected and ratioed against an air background to produce an absorbance spectrum for each sample. Of the samples, approximately half were used as calibration standards, with the balance serving as validation samples. A total of 256 scans were co-added for the calibration standards and 128 scans for the validation samples. The accumulated calibration spectra were input together with the corresponding SFI data into the Grams 386 partial least squares (PLS) chemometrics package (Galactic Industries Corp., Salem, NH) (13) to develop calibrations. Correlation spectra, which relate spectral changes to the value of the variable of interest, were generated and examined to identify spectral features that correlated with the SFI data for each temperature. Calibrations were derived and optimized to generate predictive models for four temperatures (SFI50, SFI70, SFI80, SFI92); each was cross-validated by the "leave one out" procedure, and the optimal number of factors was selected on the basis of the predicted residual error sum of squares (PRESS) test. The final calibration models were reproduced with Nicolet QuantIR software so that calibrations developed on the Grams software could be implemented on the Nicolet spectrometer for routine analysis. These calibrations were subsequently revalidated by reanalyzing 15 of the validation samples twice, one week apart, and comparing the predictions to the dilatometric SFI values. The accuracy and reproducibility of these data were assessed in terms of mean difference (MD) and standard deviation of the differences (SDD) according to the method of Youden (14). To facilitate routine analyses, a Visual Basic program was written to drive the spectrometer, prompt the user to load the sample, and calculate and print the SFI values determined from the PLS calibrations.

RESULTS

Preliminary results/methodology rationale. In a preliminary study, the 11 lots of soybean oil were initially analyzed by FTIR to determine their degree of unsaturation, molecular weight, *cis* and *trans* content (6,10). It is well known that these parameters are related to the physical state of a fat; i.e., increasing unsaturation (IV) reducing the melting point, with the relative amounts of *cis* and *trans* forms having opposing effects, while decreasing the weight average molecular weight (higher SN) tends to reduce the melting point if the *cis/trans* ratio is constant. Using the IV/SN/*cis*/*trans* analytical package developed for Nicolet Instrument, an attempt was made to relate these parameters to SFI *via* multiple regression. Excellent linear relationships could be obtained for any single lot of hydrogenated soybean oil ($r^2 > 0.99$; SE < 1.5), but the multiple regression model did not hold when the data of two or more lots were combined ($r^2 < 0.85$; SE > 6.0). From this analysis, it was concluded that although IV, SN, *cis,* and *trans* data in combination are clearly related to SFI, no simple quantitative relationship could be formulated for the more general case. These results imply that there is insufficient information to account for other variables affecting the SFI measure, most likely fatty acid composition and distribution.

It was postulated that the IR spectrum of a liquid fat, measured at a constant temperature, could still serve as a basis for the prediction of an SFI profile, as it represents a superposition of all the mid-IR molecular vibrational contributions of the individual triglycerides that define the lipid system. The underlying principle is that the SFI profile is defined by the molecular makeup of the fat, and that the amount of solids crystallizing out at any given temperature is a function of both its fatty acid composition and distribution. Such information may be extracted from the mid-infrared spectrum through the use of sophisticated multivariate analysis techniques, such as PLS (15), which allow one to correlate both gross and subtle spectral variations to changes in solids behavior as a function of temperature, as measured by dilatometry. Being able to develop predictive models of this nature has the advantage of accounting for a broader range of variables contributing to SFI and, if an appropriate model is developed, it eliminates the need for the tedious tempering steps associated with conventional SFI or SFC methods.

Spectroscopy. Figure 1 illustrates five overlayed FTIR absorbance spectra of samples having SFI values of 4.0, 8.5, 14.7, 18.2, and 23.6 at 50°F. On the scale shown, the only noticeable spectral differences are in the CH *cis* region

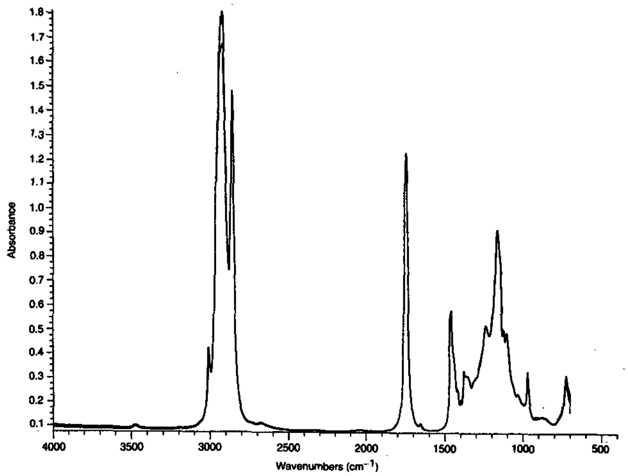

FIG. 1. Overlayed spectra of five samples of soybean oil with solid fat index values of 4.0, 8.5, 14.7, 18.2, and 23.6 at 50°F as measured by dilatometry.

(3015–3005 cm^{-1}). Figure 2 illustrates the overall variance spectrum for all 72 samples analyzed. The variance spectrum relates the gross spectral variation relative to the composite mean spectrum of all samples and clearly shows the frequency regions where spectral variations exist to a significant degree. These include the *cis* C-H stretching vibration (3015–3005 cm^{-1}), the CH_2/CH_3 stretching vibrations (3000–2850 cm^{-1}), the ester linkage carbonyl band (1750–1740 cm^{-1}), portions of the fingerprint region (1550–1050 cm^{-1}), the *trans* (980–960 cm^{-1}) and *cis* (750–730 cm^{-1}) C=CH bending absorptions. Although the magnitudes of the variations are small, they are readily measured, and this information provides a starting point for chemometric analyses. Correlation spectra were generated by PLS from the variance spectrum using the dilatometric SFI data for each temperature as an aid in searching for useful spectral regions that could be used to develop a predictive PLS model. The optimization of spectral regions was based on the "leave one out" cross validation technique, with the F-statistic as a guide to selecting the number of factors to be used in the model. Although many combinations and permutations of wavelength regions can provide fairly reasonable results, the regions determined to be optimal for the soybean samples were: the CH-*cis* (3135–2992 cm^{-1}), the high frequency side of the carbonyl ester linkage (1790–1759 cm^{-1}), the low frequency side of the carbonyl ester linkage (1731–1620 cm^{-1}), which includes the weak C=C stretching *cis/trans* band, plus the isolated *trans* (992–926 cm^{-1}) and *cis* (769–702 cm^{-1}) bands. The intense CH_2/CH_3 and carbonyl bands, which also correlate, were not used because they were near the limit of the linear range of the detector.

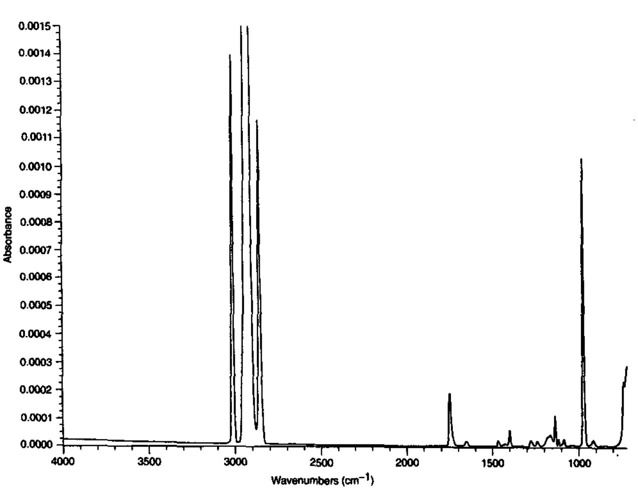

FIG. 2. The variance statistical spectrum obtained for 72 soybean oil samples, illustrating the spectral regions where spectral variation occurs relative to the overall mean spectrum.

Calibration/validation. The calibration statistics obtained for the PLS models derived are presented in Table 1. The PRESS plots obtained were smooth, and cluster analysis indicated that there were no spectral outliers in the calibration set. Relatively few factors (n = 6–10) were required to account for the variation in the SFI data for 50, 70, 80, and 92°F, and the average root mean square error for the four temperatures was ±0.71 SFI units, slightly higher than the generally accepted value for the reproducibility of the dilatometric method (~ ±0.50). Figure 3 presents the "leave one out" cross validation plot obtained from the calibration model for the SFI data at 50°F. This plot illustrates a good linear relationship between the FTIR predictions obtained from the PLS model and the dilatometric data, with the plots for 70 and 80°F being similar.

The SFI50-80 data sets all had measurable solids, while over half of the samples in the SFI92 data set had no measurable solids. It is not possible to model zero SFI data by PLS because one would be forced to relate varying spectral information to a constant. As a consequence, the PLS calibration modeling was done with only spectra that are associated with measurable solids values. On this basis, the SFI92 data set could be calibrated, and the subsequent predictions obtained for samples with a dilatometric SFI of zero ranged from ~0 to −3.0 SFI. Such negative SFI predictions can be interpreted to be "apparent" SFI values that are less than or equal to the lowest positive SFI value (0.1 SFI) in the calibration model. Although meaningless in physical terms, these negative values indicate that one can unambiguously consider negative PLS predictions to be equivalent to zero SFI. No calibrations could be derived for SFI100 and SFI104 because there was an insufficient number of samples with measurable solids to develop a robust PLS calibration model. The overall validation results obtained for SFI50-92 data sets are presented in Table 2, and they are similar to the cross validation results obtained for the calibration samples. Figure 4A–D presents the validation plots obtained for the validation samples for SFI50-92, respectively; they illustrate the linear contraction of the SFI values as a function of temperature.

To determine the reproducibility of the method, 15 validation samples were subsequently run twice, one week apart,

TABLE 1
"Leave One Out" Cross Validation Data for the Solid Fat Index Calibrations Derived for 50, 70 80, and 90°F

Calibration	# Spectra	R^2	RMSE[a]	# PLS Factors[c]
SF150	34	0.984	±0.905	8
SF170	34	0.973	±0.627	5
SF180	34	0.944	±0.580	10
SF192[b]	22	0.910	±0.730	6

[a] Residual mean square error from the predicted residual error sum of squares test.
[b] Calibration based on non-zero solid fat index data only.
[c] PLS refers to partial least squares.

TABLE 2
Validation Predictions Relative to the Dilatometric Solid Fat Index Values

Calibration	# Spectra	R^2	SEP[a]
SF150	38	0.982	0.696
SF170	38	0.964	0.686
SF180	38	0.926	0.721
SF192	50	0.940	0.720

[a] Standard error of prediction.

and the results were compared in terms of MD and SDD for reproducibility (Table 3). The reproducibility statistics indicate that, on average, the mean differences between duplicates measured one week apart were close to zero and had an average overall SDD_r of ±0.38 SFI. Regression of the duplicate predictions against each other produced good linear plots, with an average standard error of ~0.38 SFI units, similar to the SDD_r. Both of these measures are within the precision range (±0.64 to ±0.25 SFI) that is normally associated with the dilatometric method (3) for margarine-type oils. The original accuracy was also maintained; regressions of the means of the weekly replicate FTIR predictions vs. the dilatometric SFI data produced SE values of 0.63, 0.44, 0.61, and 0.26 for SFI50-92, respectively. Although not elaborated on here, this degree of stability was achieved through the use of a spectral compensator and a calibration maintenance algorithm, which accounts for baseline drift and other instrument changes over time, thus allowing the calibration to be used indefinitely.

DISCUSSION

The results presented above clearly indicate that FTIR spectroscopy provides sufficient spectral information to develop a robust SFI calibration. The calibration is largely based on spectral regions that contain information related to triglyceride weight-average molecular weight and the degree and type of unsaturation of the fatty acids that make up the triglyceride. Other more subtle but less defined spectral features, related to fatty acid composition and the distribution of the fatty acids within the triglycerides, may also play a part. The power of PLS lies in the fact that one does not necessarily need to identify specific absorption bands as being related to the measure in question, unlike conventional calibration approaches based on peak height/area measurements. As long as a representative calibration set is formulated and spectral correlations are evident, one can derive a valid and robust calibration that will predict well for subsequent samples with similar characteristics. On the other hand, it is imperative that substantial effort go into the design of the calibration set to ensure that it is representative of the samples to be analyzed. As noted earlier, it is crucial that zero SFI samples not be part of the calibration model; the upper temperature limit of SFI calibration development is restricted by the need to have an adequate number of samples available with a measurable solids content.

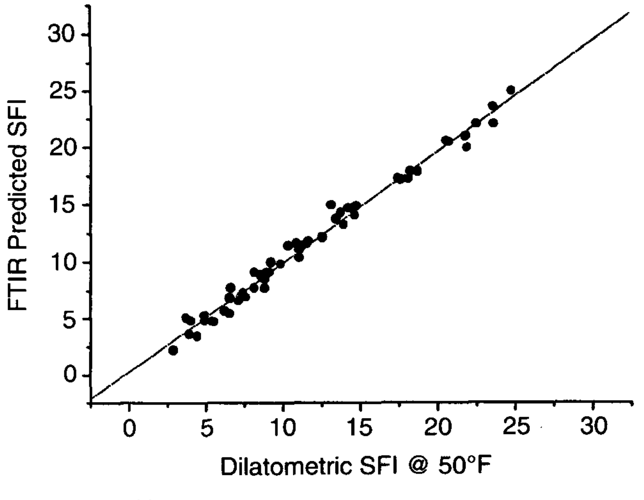

FIG. 3. Partial least squares analysis cross validation plot, showing the Fourier transform infrared (FTIR) predicted solid fat index (SFI) data vs. the dilatometric SFI data for 50°F.

One of the limitations to the FTIR approach for the determination of SFI is that the calibrations developed are only applicable to samples with similar characteristics as the standards used to derive the calibration (i.e., hydrogenated soybean oils) and as such would not be suitable for other oils and/or blends (e.g., cottonseed or cottonseed/soy); they require the development of separate calibrations. Another common limitation and concern associated with any secondary method, such as FTIR spectroscopy, is calibration stability. There is a tendency for a calibration to drift over time due to changes in instrument performance, which lead to an ongoing need to recalibrate the system. This crucial problem has been overcome by the McGill IR group by using a proprietary calibration maintenance algo-

TABLE 3
Reproducibility Statistics for 15 Validation Samples, Analyzed One Week Apart

Statistic	SFI50	SFI70	SFI80	SFI92
MD_r[a]	–0.092	–0.075	–0.116	–0.102
SDD_R[b]	0.491	0.235	0.500	0.307
R^2[c]	0.997	0.997	0.984	0.987
SE[c]	0.450	0.243	0.518	0.318

[a] Mean difference for reproducibility.
[b] Standard deviation of the differences for reproducibility.
[c] Obtained by linear regression of validation data obtained for week 1 vs. week 2.

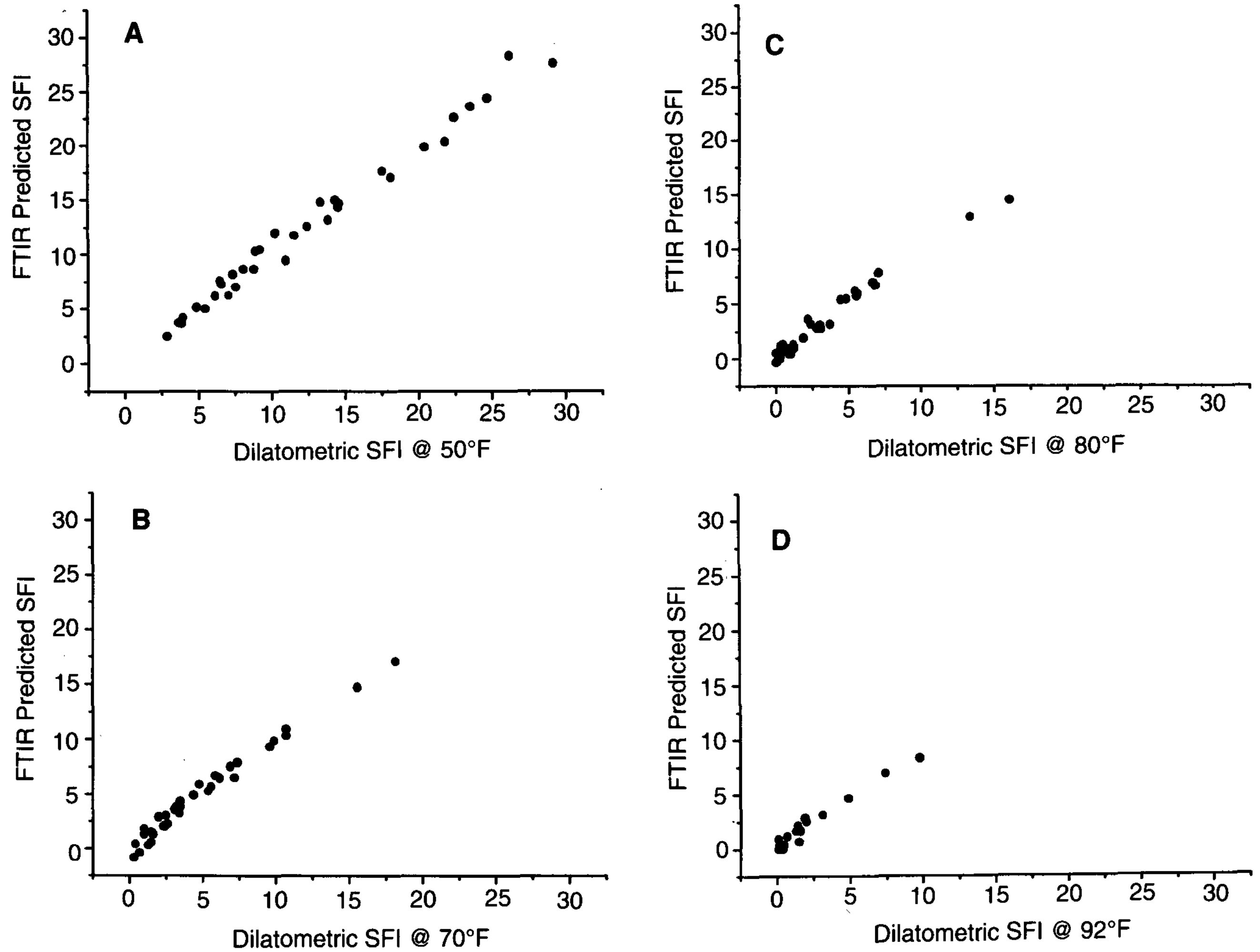

FIG. 4. A–D: Validation plots of Fourier transform infrared predicted solid fat index vs. dilatometric SFI obtained for soybean oils measured at 50, 70, 80, and 92°F. See Figure 3 for abbreviations.

rithm, which allows a PLS calibration to be used indefinitely once it has been developed, including the transfer of a calibration between instruments without deterioration in performance. The implementation of such an algorithm ensures that the time and effort invested in developing calibrations are not wasted and eliminates the need for continual preparation and re-analysis of calibration standards.

We conclude that good approximations of dilatometric SFI data can be obtained by FTIR spectroscopy and that in all likelihood SFC could also be determined by calibrating against NMR solids data. The unique advantage of the FTIR approach over SFI or SFC is that no time-consuming tempering steps are required for routine analysis and that an SFI profile can be obtained in a single sample analysis. With the FTIR spectrometer configured with an appropriate heated sample-handling accessory and the spectrometer programmed to automate the spectral data acquisition and to predict the SFI values, an operator need only load and scan the sample, an operation which takes approximately two minutes. Similarly configured FTIR analytical systems incorporating a calibration maintenance algorithm are now in routine use for quality control applications. Based on the results of this study and our previous experience in developing oil analysis systems (6–11), FTIR spectroscopy clearly has potential as an alternate, routine, and rapid quality control method for the determination of the solids content in fats and oils.

ACKNOWLEDGMENTS

The authors thank Nicolet Instrument Corp. for providing the FTIR instrumentation and Dwight Analytical Solutions for designing the sample-handling accessory used in this work. Financial support for this study was provided through a Strategic grant from the Natural Sciences and Engineering Research Council of Canada (NSERC), whose assistance is gratefully acknowledged.

REFERENCES

1. Mills, B.L., and F.R. van de Voort, The Determination of the Solid Fat Index of Fats and Oils Using the Anton Paar Density Meter, *J. Am. Oil Chem. Soc. 58:*618–622 (1981).
2. Mills, B.L., and F.R. van de Voort, Comparison of the Direct and Indirect Wide-Line NMR Methods for the Determination of Solid Fat Content, *Ibid. 58:*776–778 (1981).
3. *Official Methods and Recommended Practices of the American*

Oil Chemists' Society, 4th edn., American Oil Chemists' Society, Champaign, 1989, Methods Cd10-57; Cd16-81.

4. Cebula, D.J., and K.W. Smith, Differential Scanning Calorimetry of Confectionery Fats. Pure Triglycerides: Effects of Cooling and Heating Rate Variation. *J. Am. Oil Chem. Soc. 68:*591–595 (1991).
5 Rossel, J.B, in *Analysis of Oils and Fats*, edited by R.J. Hamilton and J.B. Rossel, Elsevier, 1985. pp. 77–88.
6. van de Voort, F.R., FTIR Spectroscopy in Edible Oil Analysis, *INFORM 5:*1038–1042 (1994).
7. van de Voort, F.R., J. Sedman, G. Emo, and A.A. Ismail, Rapid and Direct Iodine Value and Saponification Number Determination of Fats and Oils by Attenuated Total Reflectance/Fourier Transform Infrared Spectroscopy, *J. Am. Oil Chem. Soc. 69:* 1118–1123 (1992).
8. Ismail, A.A., F.R. van de Voort, G. Emo, and J. Sedman, Rapid Quantitative Determination of Free Fatty Acids in Fats and Oils by FTIR Spectroscopy, *Ibid. 70:*335–341 (1993).
9. van de Voort, F.R., A.A. Ismail, J. Sedman, J. Dubois, and T. Nicodemo, The Determination of Peroxide Value by Fourier Transform Infrared Spectroscopy, *Ibid. 71:*921–926 (1994).
10. van de Voort, F.R., A.A. Ismail, and J. Sedman, A Rapid Determination of *Cis* and *Trans* content of Fats and Oils by FTIR Spectroscopy, *Ibid. 72*:873–880 (1995).
11. van de Voort, F.R., A.A. Ismail, J. Sedman, and G. Emo, Monitoring the Oxidation of Edible Oils by FTIR Spectroscopy, *Ibid. 71:*243–253 (1994).
12. Anon., Omnic Operating Manual, Nicolet Instrument Corp., Madison, Wisconsin, 1993.
13. Geladi, P., and B. Kowalski, Partial Least Squares Regression: A Tutorial. *Anal. Chim. Acta. 185:*1–17 (1986).
14. Youden, W.J., and E.H. Steiner, *Statistical Manual of the AOAC*, Association of Official Analytical Chemists, Arlington, Virginia, 1975.
15. Fuller, M.P., G.L. Ritter, and C.S. Draper, Partial Least Squares Quantitative Analysis of Infrared Spectroscopic Data. Part I. Algorithm Implementation, *Appl. Spectrosc. 42:*217–227 (1988).

[Received December 12, 1994; accepted December 13, 1995]