Trans Fatty Acid Determination in Fats and Margarine by Fourier Transform Infrared Spectroscopy Using a Disposable Infrared Card

Kangming Ma, F.R. van de Voort*, J. Sedman, and A.A. Ismail

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

ABSTRACT: The use of a disposable polyethylene infrared (IR) card as a sample carrier for the quantitative determination of *trans* content of fats and oils and margarine by Fourier transform IR spectroscopy was investigated. Standards prepared by dissolving trielaidin in a zero-*trans* oil were used to develop partial least squares (PLS) calibrations for both the IR card and a 100-μm transmission flow cell. These calibrations were then used to predict a series of gas chromatographically-preanalyzed unknowns, the *trans* predictions obtained using the card being comparable to those obtained with the transmission flow cell. Somewhat improved performance could be obtained when the spectral data from the card were normalized to compensate for inherent variations in path length and variability in sample loading. Both IR methods tracked the gas chromatographic reference *trans* values very well. A series of margarine samples was also analyzed by the card method, producing results similar to those obtained using a flow cell. For the analysis of margarines, the card method has the advantage that the *trans* analysis can be performed directly on microwave melted emulsions because moisture is not retained on the card. Overall, the disposable IR card was shown to work well and has the benefit of allowing *trans* analyses to be carried out without requiring investment in a heated flow cell or attenuated total reflectance accessory.

Paper no. J9206 in *JAOCS 76*, 1399–1404 (December 1999).

KEY WORDS: 3M IR card, disposable IR card, fats and oils, FTIR spectroscopy, margarine, *trans* analysis.

Trans fatty acids are of increasing concern due to their association with heart disease, specifically in that there appears to be evidence that the consumption of *trans* fatty acids is associated with higher plasma total cholesterol and low-density lipoprotein (LDL) cholesterol concentrations. Partially hydrogenated fats and oils are widely used in the food industry, and the U.S. Food and Drug Administration (FDA) has been petitioned to require the inclusion of *trans* fatty acids content as part of saturated fat content on food labels (1,2). As a result, both the gas chromatography (GC) and infrared (IR) spectroscopic approaches to the determination of *trans* content of fats and oils have been improved upon. In the case of GC, two official methods exist, one for fats and oils that employs a capillary column (Ce 1f-96) (3) and another for margarines that uses a packed column (Cd-17-85) (4). In capillary GC methods, the key limitation has been the incomplete separation of *trans* octadecenoic acid isomers (5), but recent reports have demonstrated that substantial improvements in separation are attainable with the use of 100-m columns and some of the newer liquid phases (6). Alternatively, accurate *trans* analyses can be achieved using argentation (Ag^+) thin-layer chromatography combined with GC or GC in combination with Fourier transform infrared (FTIR) spectroscopy (7). Although workable, these two approaches are somewhat complicated and hence not generally suitable for routine applications.

The AOCS method for the determination of *trans* content by IR spectroscopy is based on the measurement of the characteristic C–H bending vibration of isolated *trans* bonds at 10.3 μm (966 cm^{-1}) (8), and the well-known inherent limitations of the original method (9,10) led to its revision in 1995. Beyond updating the experimental protocol to reflect the data-handling capabilities of modern IR spectrometers (both FTIR and dispersive instruments), the recent modifications to the AOCS method are largely directed toward improving the accuracy of IR *trans* analysis. A significant amount of new development work has also been carried out to improve the basic IR *trans* method (11). Much of this work has been directed toward simplifying the experimental procedure, particularly by eliminating the need to use CS_2, which is both volatile and noxious, as well as increasing the accuracy of the IR *trans* determination. Methods have been proposed for the analysis of fats and oils in their neat form with the use of either a transmission flow cell (12) or an attenuated total reflectance (ATR) accessory (13). Each of these sample handling techniques has been advocated because of its own particular advantages, and each has its disadvantages, with two points of commonality: they are expensive and the accessories need to be heated to allow for the analysis of solid fats.

An alternative sample handling technique is based on the use of organic polymers such as polyethylene and Teflon as

*To whom correspondence should be addressed at McGill IR Group, Dept. of Food Science and Agricultural Chemistry, Macdonald Campus of McGill University, Box 187, 21,111 Lakeshore Rd., Ste. Anne de Bellevue, Quebec, Canada H9X 3V9. E-mail: vandevoort@macdonald.mcgill.ca

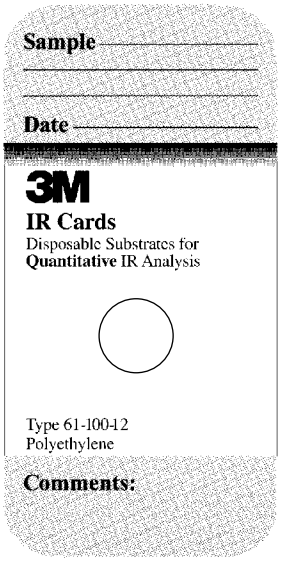

FIG. 1. 3M Quant IR card. IR, infrared.

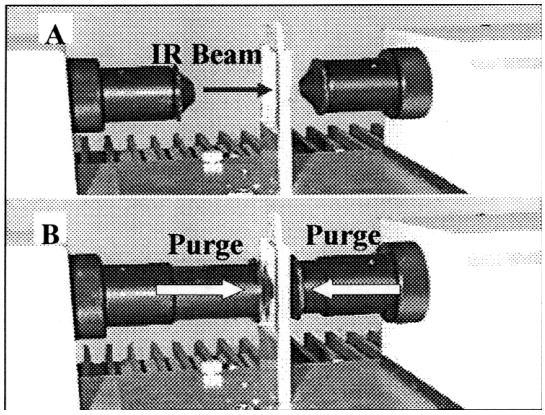

FIG. 2. Illustration of the IR card and purge system in the Bomem (Québec, Canada) spectrometer: (A) card holder, purge tubes (open position), IR beam, IR card inserted into card holder; (B) purge tubes in the closed position. See Figure 1 for abbreviation.

IR-transparent substrates (14,15). In the early 1990s, 3M (St. Paul, MN) developed a family of single-use, disposable IR cards that fit into a standard IR cell mount. These cards are available in either quantitative or qualitative versions made from either polyethylene or polytetrafluoroethylene films sandwiched between two layers of rigid cardboard (Fig. 1). The polymer films employed in the quantitative cards have microcrystalline pores capable of absorbing liquids into the polymer matrix by capillary action. These "Quant Cards" were designed to simulate a constant-pathlength transmission cell having a pathlength of 100 μm. 3M claimed that their cards were capable of handling both liquid and solid samples (applied with a solvent, which is subsequently evaporated) without difficulty (16). Applications cited include the analysis of fuel oils, lubricants, engine oils, and oils in wastewater; however, specific scientific literature detailing such applications is difficult to find. The McGill IR Group investigated the use of IR cards as a replacement for a conventional transmission cell and was able to develop a workable quantitative method to measure the peroxide value (PV) of edible fats and oils (17), a routine analysis in the edible oil sector. The objective of this research is to investigate the use of IR cards for the determination of *trans* content of fats and oils as well as margarines.

MATERIALS AND METHODS

Instrumentation. Two instruments were used in this study, a Bomem FTIR spectrometer (MB-Series, Bomem, Inc., Québec, Canada) running in the mid-IR mode under BomemGrams/386 software (Galactic Industries Co., Salem, NH) and a Nicolet Magna 550 mid-FTIR spectrometer (Nicolet Instrument, Madison, WI) running under OMNIC software. The Bomem spectrometer was used for the IR card work because of its accessible sample mount and unique purge system using two telescoping tubes that minimize the optical path to be purged (Fig. 2). The Nicolet spectrometer was used for conventional transmission spectroscopy, being equipped with a flow cell accessory (Dwight Analytical, Toronto, Canada) designed for fats and oils analysis, described in a previous publication (18). The transmission flow cell had KCl windows and a pathlength of 100 μm. The optical compartments of both instruments were continuously purged with CO_2-free dry air, supplied by a Balston dryer (Balston, Lexington, MA) to minimize spectral interferences from moisture and carbon dioxide. Spectra were collected by scanning for 1 min over the spectral range of 4,000–400 cm^{-1} (64 co-added scans on the Nicolet spectrometer and 16 co-added scans on the Bomem spectrometer); all spectra were collected at a resolution of 4 cm^{-1} using triangular apodization. For both the card and transmission cell spectra, the single-beam spectrum of the sample was ratioed against an open-beam background spectrum to produce a conventional absorbance spectrum, which was stored to disk for subsequent analysis or spectral data processing.

Materials. Disposable polyethylene "Quant" IR cards (Type 61-100-12) with a nominal pathlength of 100 μm were obtained from 3M (Industrial and Consumer Sector, St. Paul, MN). Trisun HB 95, a highly hydrogenated vegetable oil having a *trans* content of ~70%, was obtained from SVO Specialty Products (Eastlake, OH) and was diluted with a low-*trans* soybean oil to provide a working set of samples with a range of *trans* values for preliminary evaluation of the card method. A series of reference calibration standards made from trielaidin added to a certified zero-*trans* oil were obtained from Dr. M. Mossoba at the U.S. FDA. 4-Tetradecylaniline (97%) was obtained from Aldrich (Milwaukee, WI) to be used as an internal standard for normalizing the inherent variability in IR card pathlength. A set of fats and oils, supplied by a major processor and preanalyzed by GC (AOCS Method Ce 1f-96), provided validation samples. In addition, a variety of locally purchased branded margarines were evaluated.

Sample handling. Oils were delivered onto the IR card using a 200-µL micropipette; any excess sample was removed by dabbing the IR card polymer film aperture with an absorbent tissue. In the case of fats, the samples were first melted in a microwave oven and applied while warm and in the liquid state. For the margarine samples, 5 g of sample was placed into a 40-mL vial and heated in a microwave oven to melt the fat and break the emulsion. The melted fat was applied directly to the IR card. For the transmission analysis, fats and oils were first warmed in a microwave oven and then aspirated into the transmission flow cell, which was maintained at 80°C. For the margarines, the sample was heated in a microwave oven to break the emulsion, and the upper melted-fat layer was removed and vacuum filtered while hot through Whatman #1 (Clifton, NJ) filter paper to remove residual solids and moisture and then aspirated into the flow cell.

Developmental work, calibration, and validation. Preliminary IR card assessment and development work was carried out using the Trisun *trans*/soy standards. For both the card and the transmission cell method, the calibrations were developed using the trielaidin standards. For calibration of the card method, each standard was applied to two cards. Partial least squares (PLS) regression was employed to develop calibrations for both the card and the flow cell over the range of 1,012–930 cm^{-1}. The calibrations were initially validated by predicting the *trans* values of the Trisun/soy samples to assess reproducibility. As the predictive accuracy of the calibrations could not be properly assessed with these samples, the actual *trans* content of the soybean oil used in preparing them being unknown, additional validation was subsequently carried out using industrial samples preanalyzed for *trans* content by GC. A series of margarines were also analyzed using both the card and the flow cell method, with the predictions of the two methods being compared. For the margarines an internal standard was added to all samples. The standard, prepared by dissolving 5 g of 4-tetradecylaniline in 45 g of mineral oil and mixing thoroughly, was added at a level of 5% (w/w) to each sample. The peak height of the band at 1515 cm^{-1} was used to normalize the spectra of the margarines to compensate for the inherent pathlength variation associated with the IR card as well as variability in sample loading

RESULTS AND DISCUSSION

While working with the IR card, we observed that solid fats which had been melted and then applied to the card did not appear to solidify on cooling. Figure 3 illustrates three spectra of a fat having a *trans* content of ~40%. Figures 3A and 3B represent spectra from a 100-µm cell collected at 80°C and room temperature, respectively, whereas Figure 3C is the spectrum of the same sample on the IR card at room temperature. The room temperature spectrum of the fat on the IR card (Fig. 3C) is similar to that of the sample in the flow cell maintained at 80°C. The transmission spectrum of the room temperature sample, which had crystallized in the cell (Fig. 3B), is clearly different, with spectral line splitting associated with solid-state effects being quite apparent. These comparative spectra indicate that the microcrystalline polyethylene matrix of the IR card prevents or inhibits the crystallization of the fat. This apparent inhibition of crystallization could allow one to record the liquid-state spectra of relatively hard fats in their neat form at room temperature. The height of the *trans* peak

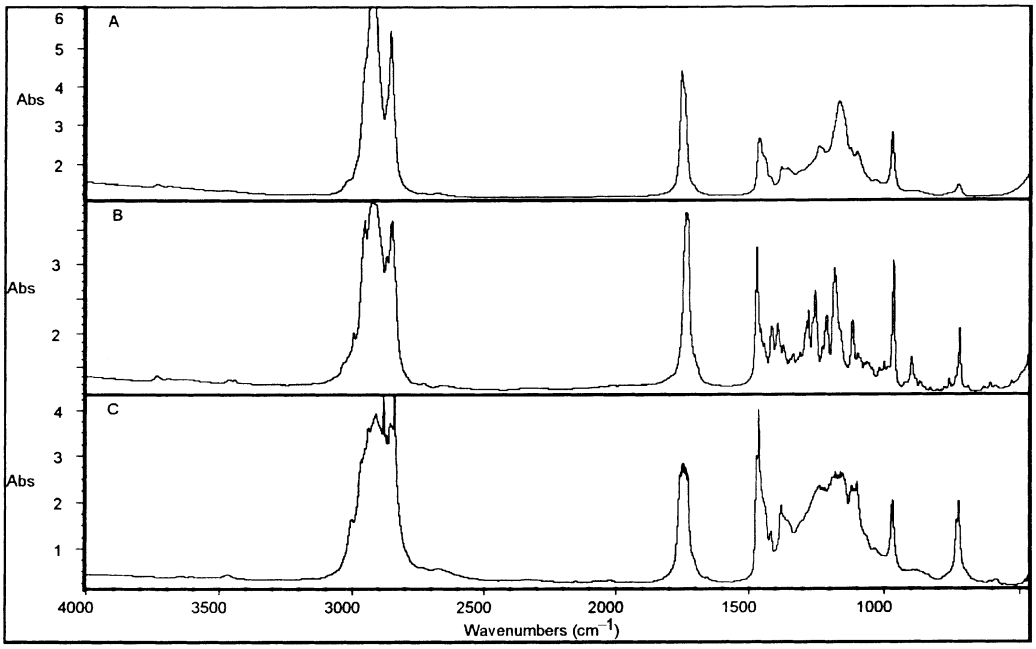

FIG. 3. Three spectra of a 40% *trans* fat (A) in a 100-µm transmission cell at 80°C; (B) in a 100-µm transmission cell at 25°C, and (C) on a 100-µm 3M card at 25°C.

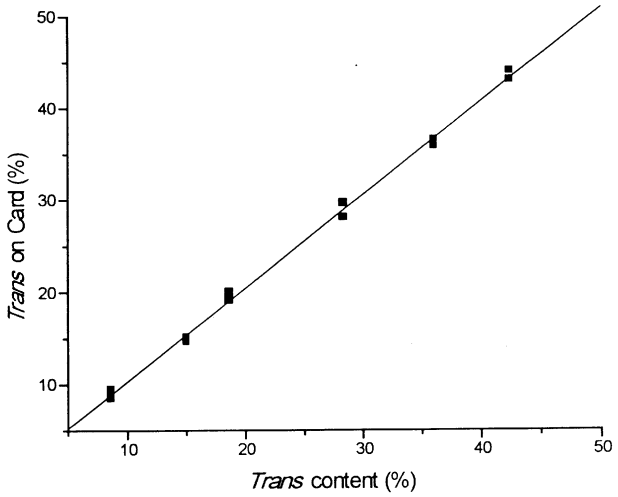

FIG. 4. Partial least squares *trans* calibration obtained for certified trielaidin standards using the infrared card.

(968 cm^{-1}) in the spectra of a series of Trisun/soy mixtures was monitored as a function of time following application of the melted sample to the IR card. In general, the peak height was stable over time, with a slight trending toward higher values for the highest *trans* sample, but no line splitting indicative of crystalization was observed in any of the spectra.

Calibration. Certified trielaidin standards were used to develop *trans* calibrations for both the IR card and transmission flow cell. A PLS calibration approach was employed because the spectra recorded from the IR cards exhibit interference fringes at longer wavelengths that would be a source of predictive error in a simple peak-height calibration but which can be mathematically compensated for by a PLS calibration model (17). For consistency and to allow a direct comparison of the card and cell predictions, a PLS calibration was also developed for the cell. Figure 4 illustrates the calibration plot obtained for the card, the calibration for the flow cell being quite similar. The respective regression equations for cross-validation of the calibrations for the card (Ca) and the cell (Ce) were:

$$\text{CaTrans} = 0.195 + 1.01\ trans \quad R = 0.998 \quad \text{SD} = 0.679 \quad [1]$$

$$\text{CeTrans} = 0.097 + 0.99\ trans \quad R = 0.999 \quad \text{SD} = 0.107 \quad [2]$$

Although the cross-validation SD for the card is not as good as that for the cell owing to the inherent pathlength variability of the cards, it is quite acceptable, being <1% *trans*.

Validation—fats and oils. The card method was initially validated using Trisun/soybean samples. Table 1 presents the predictions of *trans* content obtained for the card and transmission methods, together with the mean difference and standard deviation of the differences for reproducibility (MD_r and SDD_r) of each method. The predictions and reproducibility data obtained for both the cell and card approaches were not significantly different, and both tracked the formulated values very well. In order to validate more rigorously for accuracy, the *trans* contents of a series of industrial samples, preanalyzed by GC, were predicted using the card method. The accuracy of the IR card *trans* analysis was found to be on the order of ±0.8% *trans* relative to the GC data.

Margarine samples. Direct analysis of margarine samples presents a distinctly different challenge, as these samples are not pure fats but emulsions, containing water, emulsifiers, and some milk proteins. Our experience with the cards has been that almost any constituent with any significant vapor pressure is not retained on the IR card, particularly with the telescoping tube purge configuration of the Bomem spectrometer (Fig. 2), which directs dry air onto the card. The loss of moisture is illustrated in Figure 5, which shows a series of spectra of canola oil initially saturated with water and then heated to 100°C to remove the moisture. Spectra A–C were samples recorded in a CaF_2 flow cell over time as moisture was lost from the oil sample, whereas spectrum D was recorded immediately after application of the unheated, water-saturated oil directly onto an IR card. The progressive decrease in the intensity of the broad bands between 3,700 and 3,500 cm^{-1} in the transmission cell spectra A–C illustrates the presence and loss of moisture from the oil over time. Spectrum D taken on the card, does not show any trace of moisture in the oil sample within 10 s of its application. The dispersal of the sample onto the card, the hydrophobic nature of the polymer, and the high surface area exposed to a continuous flow of dry air favor removal of any moisture from the water-saturated oil sample within several seconds. The implication of this observation is that a product such as margarine could be analyzed directly without significant pretreatment.

To investigate this possibility, six margarine samples were analyzed by both the IR card and the flow cell method. For the IR card method, the margarine emulsion was broken by heating in a microwave oven, and a 200-μL sample was applied directly onto the card. For the flow cell method, the melted margarine was first centrifuged to remove the bulk of the solids and moisture, then the melted fat was passed through filter paper to dry the oil prior to its being introduced into the flow cell. The *trans* content of each oil was determined by the card and the transmission cell methods, and their relationship to each other was evaluated by linear regression yielding the following relationship:

$$\text{CaTrans} = 0.146 + 0.992\ \text{CeTrans} \quad R = 0.995 \quad \text{SD} = 0.970 \quad [3]$$

Equation 3 indicates that the *trans* predictions obtained from the card and flow cell methods are linearly related, have a slope of about 1.0, and an SD of ~1% *trans*. As such, the direct analysis of *trans* content of margarines is possible without prior clarification.

Normalization issues. As noted earlier, the IR card has some inherent pathlength variability due to small variations in the thickness of the polyethylene film. Another potential source of variability is the possibility of under- or overloading the sample, although we have found that consistent loading can be attained with experience. To compensate for both

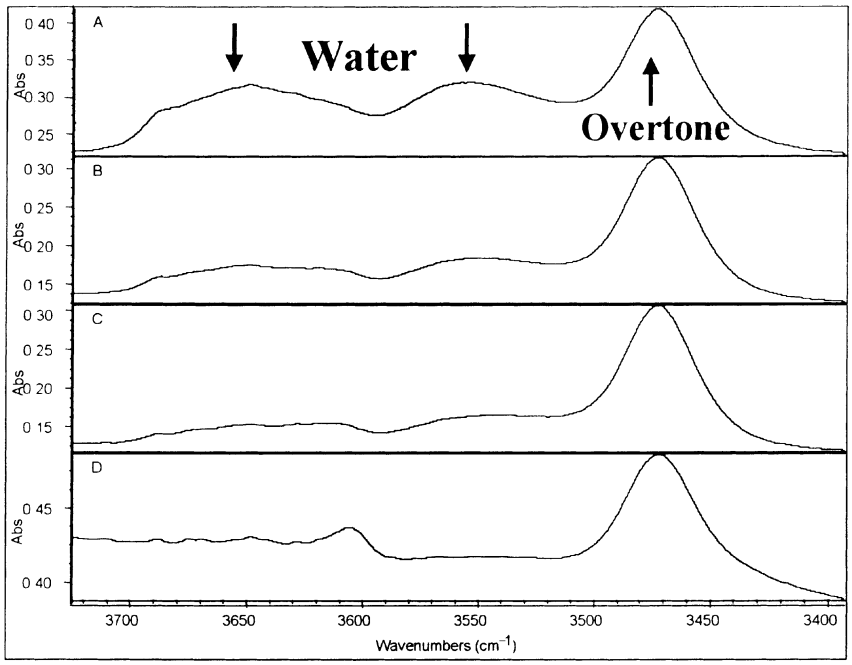

FIG. 5. Comparison of the spectra of water-saturated canola oil in a transmission cell at 100°C over time (A, 0 h; B, 2 h; C, 4 h) vs. the spectrum of the water-saturated oil scanned on the infrared card (D, 0 h).

of these effects, one can normalize the spectral data on a characteristic and essentially invariant absorption band. In our previous work on the determination of the PV of edible oils using the IR card, a normalization protocol based on the measurement of the peak height of the ester linkage carbonyl overtone band at 3,475 cm^{-1} was employed (17). As margarine samples may contain mono- or diglyceride emulsifiers exhibiting absorptions around 3,475 cm^{-1}, this normalization protocol is not suitable for these types of samples. Alternatively, an internal standard can be added to the samples provided that it meets three criteria: it must be nonvolatile, it must possess an absorption band that can be accurately measured without interference from sample absorptions, and it must not interfere with the measurement of the *trans* absorption band.

We have found that 4-tetradecylaniline, dissolved in mineral oil, meets these criteria and devised a normalization protocol based on the measurement of the absorbance of this standard at 1,515 cm^{-1}. When a solution of 4-tetradecylaniline in mineral oil was added to the GC-analyzed validation samples (Table 1) as an internal standard, the SDD_a improved from 0.79 to 0.38% *trans*. This improvement, while significant, comes at a cost in terms of increased complexity of sample preparation, requiring weighing of the sample and the standard, and spectral analysis. However, by programming of the spectrometer, the normalization procedure can be automated so that the operator is only required to do the weighings and enter the weights, the pathlength-corrected *trans* values being output by the program.

This study demonstrates that the 3M IR card has some

TABLE 1
Predictions of *trans* Content of Formulated Fats from Duplicate Analyses by the IR Card and Transmission Cell Methods

Sample	Formulated *trans* (%)	IR card		Transmission cell	
		Trans 1 (%)	*Trans* 2 (%)	*Trans* 1 (%)	*Trans* 2 (%)
1	10.30	10.84	10.93	10.40	10.59
2	17.96	17.96	18.14	18.52	18.12
3	22.32	22.48	22.48	22.59	22.39
4	33.95	36.67	37.21	34.39	34.19
5	43.25	44.71	44.84	43.34	43.34
6	50.87	51.99	52.54	50.66	50.46
	MD_r[a]	–0.24		–0.13	
	SDD_r	0.23		0.20	

[a]Mean difference for reproducibility.
[b]Standard deviation of the difference for reproducibility. IR, infrared.

unique properties making it an interesting vehicle for the analysis of the *trans* content of fats and oils as well as emulsions such as margarines. In particular, it is unique in that it inhibits the crystallization of premelted hard fats, producing an "oil" spectrum at room temperature for fats having up to 50% *trans* content. In the case of emulsions, once broken, any residual mositure left in the oil is relatively easy to remove. This is to some degree related to the manner in which the optical compartment of the spectrometer is purged, and it has not been tested with spectrometers other than the Bomem system employed in this work. The analytical performance of the card method for the determination of *trans* content compares well with that obtained with a transmission flow cell making it very convenient for monitoring *trans* content. Another practical benefit of the card approach is the ability to carry out *trans* analyses without the need to invest in heated sample accessories. The cards are disposable and simple to use, making them ideal for spot checks and monitoring. Normalization protocols using an internal standard allow one to further optimize the accuracy and reproducibility of the card; however, even without this additional step, one can attain a reproducibility on the order of ±1%.

ACKNOWLEDGMENTS

The authors would like to acknowledge the National Science and Engineering Research Council of Canada (NSERC) for financial support to undertake this study and give special thanks to 3M for supplying the IR cards used to undertake this work.

REFERENCES

1. Anon, Controversy, Three Nations Wrestle with *trans* Issue, *INFORM 6*:1148–1149 (1995).
2. Anon., Some Food-Labeling Questions Still Unresolved, *Ibid. 6*:335–340 (1995).
3. Official Method Ce 1c-89, in *Official Methods and Recommended Practices of the American Oil Chemists' Society*, edited by D. Firestone, AOCS Press, Champaign, 1993.
4. Official Method Cd 17-85, *Ibid.*
5. Ratnayake, W.M.N., R. Hollywood, E. O'Grady, and J.L. Beare-Rogers, Determination of *cis*- and *Trans*-Octadecenoic Acids in Margarines by Gas–Liquid Chromatography-Infrared Spectrophotometry, *J. Am. Oil Chem. Soc. 67*:804–810 (1990).
6. Ratnayake, W.M.N., and J.L. Beare-Rogers, Problems of Analyzing C18 *cis*- and *Trans*-Fatty Acids of Margarine on the SP-2340 Capillary Column, *J. Chromatogr. Sci. 28*:633–639 (1990).
7. Ratnayake, W.M.N., Analysis of *trans* Fatty Acids, in Trans *Fatty Acids in Human Nutrition*, edited by J.L. Sebedio and W.W. Christie, The Oily Press Ltd., Dundee, 1998, pp. 115–162.
8. Official Method Cd 14-61, in *Official Methods and Recommended Practices of the American Oil Chemists' Society,* edited by D. Firestone, AOCS Press, Champaign, 1993.
9. Firestone, D., and P. LaBouliere, P., Determination of Isolated *Trans* Isomers by Infrared Spectrophotometry, *J. Assoc. Off. Anal. Chem. 48*:437–443 (1965).
10. O'Connor, R.T., Application of Infrared Spectrophotometry to Fatty Acid Derivatization, *Ibid. 33*:1–15 (1956).
11. Firestone, D., and A. Sheppard, Determination of *trans* Fatty Acids, in *Advances in Lipid Methodology-One*, edited by W.W. Christie, The Oily Press, Dundee, 1992, pp. 273–322.
12. Sedman, J., F.R. van de Voort, and A.A. Ismail, Upgrading the AOCS Infrared *trans* Method for Analysis of Neat Fats and Oils by Fourier Transform Infrared Spectroscopy, *J. Am. Oil Chem. Soc. 74*:907–913 (1997).
13. Mossoba, M.M., M.P. Yurawecz, and E. McDonald, Rapid Determination of the Total *Trans* Content of Neat Hydrogenated Oils by Attenuated Total Reflection Spectroscopy, *Ibid. 73*:1003–1009 (1996).
14. Saver, R.W., and W.C. Krueger, Infrared Investigation of the Conformation of Proteins Deposited on Polyethylene Films, *Anal. Biochem. 212*:519–525 (1993).
15. Moeur, H.P., and R.P. Pinnell, Use of the Disposable IR Card in the Organic Chemistry Laboratory, *J. Chem. Ed. 73*:371 (1996).
16. Anon. 3M IR Card Manual, St. Paul, 1997.
17. Ma, K., F.R. van de Voort, A.A. Ismail, and J. Sedman, Quantitative Determination of Hydroperoxides by Fourier Transform Infrared Spectroscopy with a Disposable Infrared Card, *J. Am. Oil Chem. Soc. 75*:1095–1101 (1998).
18. Van de Voort, F.R., J. Sedman, and A.A. Ismail, A Rapid Automated Method for the Determination of *cis* and *trans* Content of Fats and Oils by FTIR Spectroscopy, *Ibid. 72*:873–880 (1995).

[Received April 12, 1999; accepted August 29, 1999]