Rapid Determination of Total *Trans* Fat Content by Attenuated Total Reflection Infrared Spectroscopy: An International Collaborative Study

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ABSTRACT: Interest in *trans* fat labeling has prompted efforts to develop new, more efficient methods for rapidly and accurately determining *trans* fat content in foods. The lower limit of quantitation, 5% *trans* fat (as percent of total fat), of transmission infrared official methods, such as AOAC 994.14 and 965.34, for total isolated *trans* fatty acids is too high to be generally useful for the determination of low levels of *trans* fats in foods. A novel and rapid (5 min) attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopic procedure was recently developed and applied to food products. This procedure was voted official method AOCS Cd 14d-99 by the American Oil Chemists' Society in 1999 after testing in a 12-laboratory international collaborative study. The results of this study are described in this paper. Analytical ATR-FTIR results exhibited high accuracy in the range investigated, 1–40% *trans*; results tended to have <2% high bias relative to the gravimetrically determined values. The precision of this internal reflection method was found to be superior to those of transmission infrared official methods. It is recommended that the applicability of the ATR-FTIR method be limited to *trans* levels of >1% (as percent of total fat).

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Recently, the U.S. Food and Drug Administration (FDA) published proposed rules for labeling the *trans* fatty acid content of food products (1). Continuing interest in *trans* fat labeling and discussions of the nutritional significance of *trans* fatty acids have prompted efforts to optimize existing official methods and to develop new, more efficient ones for rapidly determining the *trans* fat content of foods (2).

Infrared (IR) spectroscopy is a widely used method for the determination of total fatty acids with isolated (nonconjugated) *trans* double bonds (2). The IR determination is based on the C-H out-of-plane deformation band at 966 cm^{-1} that is uniquely characteristic of isolated double bonds with *trans* configuration. These double bonds are found primarily in *trans*-monoenes, and at much lower levels in minor hydrogenation products such as methylene-interrupted and non-methylene-interrupted *trans*,*trans*-dienes, mono-*trans*-dienes, and other *trans*-polyenes. Many modifications have been proposed to improve the accuracy of the IR methods (2) including refinements recently introduced in two new transmission IR official methods adopted by the Association of Official Analytical Chemists (AOAC), namely, AOAC 994.14 in 1994 (3) and AOAC 965.34 in 1997 (4). The latter method was also approved by the American Oil Chemists' Society (AOCS) as AOCS Cd 14-95 in 1995 (5). Current procedures and official IR spectroscopic methods are not entirely satisfactory because they assume that the band at 966 cm^{-1} is isolated, when in fact it overlaps with other features of the observed IR spectrum (2). This overlap produces a strongly sloping background (Fig. 1) that reduces the accuracy of the IR quantitation, particularly at *trans* levels below 5% (1–4).

In 1996, a novel procedure (6) was proposed to eliminate the highly sloping background by "ratioing" the single beam spectrum of the hydrogenated fat against that of a *trans*-free reference background material. A symmetric IR absorption band on a horizontal background was obtained at 966 cm^{-1} (Fig. 1). The area under the *trans* band could be accurately integrated between the same limits, 990 and 945 cm^{-1}, for all the *trans* levels investigated (about 1–70%). A second modification was also proposed; instead of conventional IR transmission cells, the use of a zinc selenide (ZnSe) attenuated total reflection (ATR) IR liquid cell was used to speed up the determination (6). Time was saved because with ATR cells, neither test samples of neat (undiluted) melted fats nor fatty acid methyl esters (FAME) were weighed or quantitatively diluted in the toxic and volatile solvent carbon disulfide (CS_2).

In order to apply this procedure efficiently to the determination of *trans* fats in commercial food products (7), a 50-µL ATR cell, which required a smaller quantity of test material, was used instead of a higher capacity (1.5–2 mL) liquid ATR cell (6). By using this 50-µL cell, two collaborative studies were conducted (8) in each of five laboratories on neat tri-

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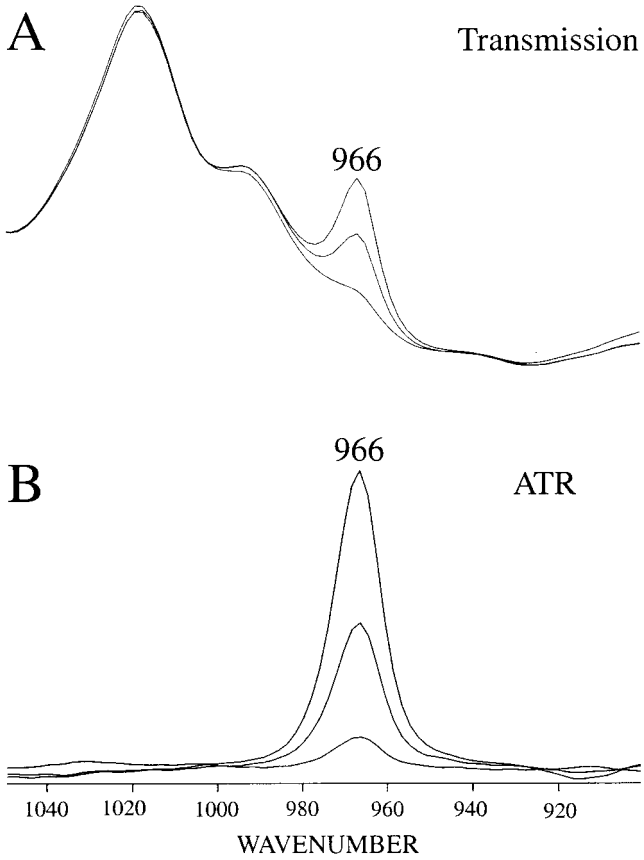

FIG. 1. (A) Transmission Fourier transform infrared (FTIR) spectra for oils containing approximately 1, 4, and 7% *trans* fat (as percentage of total fat). The spectra were obtained for carbon disulfide solutions by using official methods AOAC 965.34 (Ref. 4) and AOAC 994.14 (Ref. 3). The spectra were recorded in the range between 1050 and 900 cm^{-1} showing the 966 cm^{-1} band attributed to isolated *trans* double bonds. (B) Attenuated total reflection (ATR)-FTIR spectra for oils containing approximately 1, 5, and 10% *trans* fat (as percentage of total fat). Spectra were obtained by applying the "ratioing" and ATR-FTIR official method AOCS Cd 14d-99 (Ref. 13). Symmetric bands on a horizontal background were observed.

acylglycerols (TAG) and the corresponding neat FAME derivatives. The lower limit of *trans* quantitation was about 1% of total fat. Because it was demonstrated that the "ratioing" and ATR procedure was an acceptable alternative for the IR determination of the total *trans* fatty acid content (6–9), it was adopted as Recommended Practice Cd 14d-96 by AOCS in 1996 (10). Recently, it was used by Sedman *et al.* (11) as a reference method to validate a proposed transmission IR procedure. The ATR procedure is rapid because it requires little or no preparation of the test sample, and requires only about 5 min for spectroscopic signal averaging, band area integration, and calculation of the *trans* content from a linear regression equation. It also requires no derivatization of fats and oils to FAME (8). Recently, an attempt was made to apply the standard addition technique to enhance the accuracy of the attenuated total reflection Fourier transform infrared (ATR-FTIR) determination (12).

An ATR-FTIR 12-laboratory international collaborative study was organized recently. Its results provided the basis for approval of the ATR-FTIR procedure as Official Method AOCS Cd 14d-99 in 1999 (13). The infrared data and statistical results of this collaborative study are presented and discussed in this paper.

MATERIALS AND METHODS

Materials. Lipid standards and reagents were purchased from Nu-Chek-Prep, Inc. (Elysian, MN), Sigma Chemical Co. (St. Louis, MO), and Alltech Associates (Deerfield, IL). The primary standards trielaidin (TE) and triolein (TO) with purity of 99% were obtained from Nu-Chek-Prep, Inc.

Trans *calibration standards. Trans* calibration standards were prepared by weighing accurately to the nearest 0.0001 g, $(0.3 - x)$ g of TO and x g of TE into a 10-mL beaker, where x equals 0.0015, 0.0030, 0.0150, 0.0300, 0.0600, 0.0900, 0.1200, and 0.1500 g, in order to prepare 0.5, 1, 5, 10, 20, 30, 40, and 50% *trans* calibration standards, respectively.

Accuracy standards. Seven pairs of blind duplicate accuracy standards consisted of cold-pressed bleached degummed soybean oil and known amounts of TE. These standards were prepared gravimetrically.

Test samples. Solid fats were gently melted and mixed before sampling. Samples that appeared cloudy due to the presence of water were treated with anhydrous sodium sulfate and filtered before removing the test sample for analysis. Ten pairs of blind duplicate (unknown) test samples consisted of commercial vegetable oils or blends. Specifically, the identities of the 10 test samples listed in Table 2 were, respectively, soybean oil; rapeseed oil; hydrogenated soybean oil; palm kernel oil; a blend of soybean oil, cottonseed oil, and hydrogenated soybean oil; a blend of palm kernel oil and hydrogenated soybean oil; two blends of soybean oil and hydrogenated soybean oil; a blend of rapeseed and hydrogenated soybean oil; and sunflower oil.

FTIR. FTIR spectrometers capable of making measurements at 4 cm^{-1} resolution in the spectral range covering 1050–900 cm^{-1} were used in the 12 laboratories that participated in the ATR-FTIR international collaborative study. For instance, at the FDA laboratory, an FTS-60A Fourier transform infrared spectrometer (Bio-Rad, Digilab Division, Cambridge, MA) was used. The instrument consisted of an SPC 3200 workstation with the IDRIS™ operating system and an optical console. The optical bench included a Michelson interferometer with a quality air bearing, a potassium bromide (KBr) substrate beam splitter, and a deuterated triglycerine sulfate (DTGS) detector. A Spectra-Tech (Shelton, CT) single reflection ZnSe ATR cell with a capacity of about 50 µL was used for the internal reflection work. The ATR accessory was capable of maintaining a constant temperature of about 65 (±2)°C.

Method. Conditions employed were identical for test samples and calibration standards. In using a disposable pipet, about 50 µL were transferred without weighing to cover the entire surface of the ATR crystal. For TE/TO calibration stan-

dard mixtures, TO was used as reference background material. For accuracy standards, cold-pressed bleached degummed soybean oil was used for measuring the reference background single-beam spectrum. For unknown test samples, the refined bleached source oil for the material to be analyzed would be an appropriate reference to be used as background. In this study, the ultra-degummed bleached cold-pressed soybean oil (Owensboro Grain Co., Owensboro, KY) was used. The reference background material was placed on the horizontal (face-up) ZnSe sampling surface of the ATR cell such that the analyte *completely* covered the horizontal surface of the ZnSe crystal. The single-beam spectrum to be used as background was collected and saved. The ZnSe crystal was cleaned by wiping off the analyte with a disposable soft lint-free or low-lint tissue paper. In order to minimize contamination, a small amount (about 25 µL) of the next test sample to be analyzed was applied to the ZnSe crystal, and the crystal was cleaned again. The neat test sample was placed (without weighing) on the horizontal ZnSe crystal. The single-beam spectrum of the test sample was collected and saved. The sample single-beam spectrum was then ratioed against that of the background, converted to absorbance, and saved.

%Trans *calculations.* For each neat TE/TO standard mixture, with the absorbance spectrum wavenumber scale expanded in the region from 1050 to 900 cm^{-1}, the area under the 966 cm^{-1} band was integrated electronically between the limits 990 and 945 cm^{-1}. By using a first-order regression analysis, the slope and intercept were determined for the line which best fits the plot of the area of the *trans* band for all the *trans* standard mixtures (y axis) as a function of %*trans* expressed as percent TE in TO (x axis). Calibration curves were checked periodically to ensure that they had not shifted. By using the slope and intercept generated for *trans* standard mixtures, the %*trans* for test samples was calculated by substituting the value of the integrated area of the *trans* band into the equation: %*trans* as TE = [area – intercept]/slope. Results were reported to the nearest 0.1%.

Statistical calculations. The statistical evaluation of the collaborative study data was determined by using the AOAC AOACBUBR computer program. This program was developed by the AOAC Statistics Committee (14).

RESULTS AND DISCUSSION

All the observed ATR-FTIR data and calculated %*trans* results that were received from the 12 participating laboratories are reported and discussed in this paper.

Accuracy. In Table 1, the %*trans* values reported by the 12 laboratories for seven pairs of blind duplicate accuracy standards are compared to the true values determined gravimetrically. Analytical results typically exhibited high accuracy in the %*trans* range investigated, 0.8 to 40.0% (Table 1). The recoveries, defined as [(ATR-FTIR mean/gravimetric value) × 100], were 102.5, 97.0, 102.0, 103.2, 103.9, 103.2, and 100.3 for TE standards containing 0.8, 1.0, 5.0, 10.0, 15.0, 20.0, and 40.0 %*trans*, respectively. This indicated that, on the average, results tended to have a 1.7% high bias relative to the gravimetrically determined values.

Precision. Analysts were requested to perform single determinations on blind duplicate test materials, which consisted of standards and test samples. The terms used for the statistical parameters, also listed in Tables 1 and 2, were those given in the AOAC Harmonization Guidelines (14).

The repeatability Cochran outlier test dictated the removal of the extreme values 15.7 and 17.9 for standard Acc6/f (Table 1) from Lab 4 that showed significantly greater variability among duplicate analyses than those found for the other 11 laboratories (overall mean 20.6). The reproducibility Grubbs outlier test for removal of laboratories with extreme averages revealed a low outlier result, 0.1 and 0.1, from Lab 10 relative to the overall mean of 0.8 for standard Acc1/a (Table 1). The %*trans* data (0.7 and 0.7) for test sample TS7/d from Lab 10 were also Grubbs outliers; the overall mean was 1.5 (see Table 2).

The precision data for test samples TS1/j, TS2/i, and TS4/g (Table 2) with overall mean %*trans* levels of <1%, namely, 0.4, 0.1, and 0.1, respectively, will be discussed separately *(vide infra).*

Repeatability. All standards (Table 1) had repeatibility relative standard deviation [RSD(r)] values below about 10%. The highest RSD(r) values, 7.5 and 10.5%, were found for the 0.8 and 1.0%*trans* standards, respectively. The remaining RSD(r) values were in the range 2.2 to 4.5% for the standards with about 5 to 40 %*trans* levels.

Test samples (Table 2) had RSD(r) values between 0.6 and 7.4% for unknowns with overall means from 1.5 to 39.6%*trans.* These results are superior to those reported for test samples that were collaboratively studied among 16 laboratories, and obtained with the latest 1997 transmission infrared official method AOAC 965.34 (4). For example, for the two levels 1.5 and 4.5%*trans* obtained in the present study, the RSD(r) values were 5.9 and 2.7%, respectively. On the other hand, for test samples in the same 1 to 6% range, higher RSD(r) values, 13.9 and 3.7%, were reported (4) with official method AOAC 965.34 for two test samples with 1.3 and 6.1 %*trans*, respectively. Comparison with AOAC 965.34 was limited by the fact that no blind replicate analysis was carried out in that case; instead, only duplicate measurements of individual test materials were performed.

Reproducibility. For four test materials near 1%*trans*, namely, two accuracy standards (0.8 and 1.0%*trans*, Table 1) and two test samples (1.5 and 1.7%*trans*, Table 2), the reproducibility relative standard deviation, RSD(R), values were found to be 21.1, 29.3, 12.4, and 23.7%, respectively. A higher RSD(R) value, 32.8%, was reported (4) with official method AOAC 965.34 for the lone 1.3%*trans* test sample that fell in this range (Fig. 2).

For the 5.0 (Table 1) and 4.5%*trans* (Table 2) test materials analyzed in the present study, the RSD(R) values were 3.1 and 5.3%, respectively. On the other hand, for a test sample with 6.1%*trans* reported with official method AOAC 965.34, a much higher RSD(R) value of 9.8% was found (4). Also, for a

TABLE 1
***Trans* Levels and Statistical Data for Pairs of Blind Duplicate Accuracy Standards[a]**

	Acc1/a	Acc2/b	Acc3/c	Acc4/d	Acc5/e	Acc6/f	Acc7/g
Laboratory							
One	0.9	1.1	5.1	10.1	15.4	20.2	40.4
	0.8	1.1	5.3	10.2	15.4	20.0	43.3
Two	0.6	0.9	5.1	10.0	15.2	20.4	40.4
	0.6	0.9	5.1	10.2	15.1	20.5	40.4
Three	0.8	0.8	4.9	10.1	15.4	20.6	40.4
	0.8	0.8	4.9	10.1	15.4	20.0	40.4
Four	1.1	1.4	5.1	10.9	16.5	15.7[b]	38.1
	1.2	1.3	4.9	11.5	16.0	17.9[b]	43.6
Five	0.5	0.8	5.4	10.4	16.1	21.0	39.4
	0.5	0.7	5.4	10.8	15.9	21.0	39.4
Six	0.7	0.9	5.2	9.5	15.6	20.6	40.3
	1.0	1.2	5.3	10.2	15.2	20.5	40.3
Seven	0.9	1.2	5.0	9.8	16.1	20.8	37.5
	1.0	1.2	5.1	10.0	17.1	23.7	38.0
Eight	0.8	1.0	5.0	10.1	15.4	20.2	40.0
	0.8	1.3	5.3	10.2	15.3	20.1	40.5
Nine	0.8	1.1	5.0	9.9	15.5	19.8	38.9
	0.8	1.0	5.0	10.5	15.0	19.6	39.2
Ten	0.1[c]	0.3	5.2	10.7	16.1	20.4	41.3
	0.1[c]	0.3	5.1	9.5	15.3	20.6	39.1
Eleven	0.8	0.9	5.0	11.3	15.4	23.5	40.7
	0.8	0.9	5.4	11.2	15.7	20.3	40.4
Twelve	0.9	1.2	5.3	10.0	15.3	20.1	40.6
	0.9	1.1	5.3	10.4	15.2	20.1	39.5
TRUE[d]	0.8	1.0	5.0	10.0	15.0	20.0	40.0
XBAR	0.8	1.0	5.1	10.3	15.6	20.6	40.1
s(r)	0.1	0.1	0.1	0.4	0.3	0.9	1.4
s(R)	0.2	0.3	0.2	0.5	0.5	1.0	1.4
RSD(r)	7.5	10.5	2.3	3.6	2.2	4.5	3.5
RSD(R)	21.1	29.3	3.1	5.1	3.3	5.0	3.5
r	0.2	0.3	0.3	1.0	1.0	2.6	3.9
R	0.5	0.8	0.4	1.5	1.4	2.9	4.0

[a]The statistical parameters listed are: XBAR, the overall mean of the laboratory values; s(r), repeatability standard deviation; s(R), reproducibility standard deviation; RSD(r), repeatability relative standard deviation; RSD(R), reproducibility relative standard deviation; r, repeatability value = 2.8 s(r); R, reproducibility value = 2.8 s(R).
[b]Cochran outlier.
[c]Single Grubbs outlier.
[d]Determined gravimetrically.

test sample with 5.2%*trans* (analyzed by 12 laboratories) obtained with the official method AOAC 994.14, an unusually high RSD(R) value of 34.6% was reported (3) (see Fig. 2).

In the present study, test materials with 15.0 (Table 1) and 14.7%*trans* levels (Table 2) had RSD(R) values of 3.3 and 4.0%, respectively, as opposed to 7.4 and 6.7% for 16.1 and 16.0%*trans* test materials, respectively, obtained with official method AOAC 965.34 (4). Similarly, with official method AOAC 994.14, an even higher RSD(R) value, 11.3%, was reported for a test sample with 15.5%*trans* (3) (see Fig. 2).

Lower limit of quantitation. As discussed above, RSD(R) values generally increased as the %*trans* levels decreased to close to 1%. By contrast, the precision data in Table 2 indicated that the RSD(R) values significantly increased as the %*trans* levels decreased to well below 1%; specifically, test samples TS1/j, TS2/i, and TS4/g (Table 2) with overall mean %*trans* levels of 0.4, 0.1, and 0.1, respectively, exhibited RSD(R) values of 68.4, 133.5, and 143.2%, respectively. These high values were unsatisfactory. Thus, it is recommended that the applicability of this method be limited to %*trans* levels >1%.

The fact that no laboratory was found to be an overall outlier laboratory is remarkable. This is because the analytical data from 9 of the 12 laboratories were generated by analysts who had not used an ATR cell prior to this study. This lack of expertise had no apparent adverse effect on accuracy and precision as discussed above and indicates the ruggedness and potential of this method to be used in different laboratory environments.

The ATR-FTIR method AOCS Cd 14d-99 (13) is recommended for the determination of unsaturated fatty acids with isolated *trans* double bonds in oils, partially hydrogenated fats, or oils isolated from food products containing >1% *trans* unsaturation. Since all foods that contain *trans* fat usually

TABLE 2
***Trans* Levels and Statistical Data for Pairs of Blind Duplicate Test Samples[a]**

	TS1/j	TS2/i	TS3/h	TS4/g	TS5/f	TS6/e	TS7/d	TS8/c	TS9/b	TS10/a
Laboratory										
One	0.8	0.1	39.9	0.3	5.0	14.8	1.4	28.7	19.8	1.7
	0.7	0.2	40.1	0.4	4.8	14.9	1.5	28.6	20.2	1.8
Two	0.1	0.1	40.0	0.0	4.3	14.6	1.2	29.0	20.2	1.1
	0.1	0.1	39.9	0.0	4.3	14.7	1.2	28.9	20.2	1.0
Three	0.2	0.2	39.8	0.2	4.3	14.8	1.4	28.8	20.6	1.4
	0.2	0.2	40.4	0.2	4.3	14.8	1.4	28.8	20.6	1.4
Four	0.8	0.0	40.3	0.3	4.9	15.0	1.8	29.9	21.7	2.1
	0.8	0.0	40.5	0.4	4.5	15.2	1.8	29.1	21.8	2.1
Five	0.1	0.0	40.2	0.0	4.8	15.8	1.3	30.2	21.5	1.8
	0.0	0.0	40.9	0.0	4.7	15.7	1.3	30.4	21.3	1.8
Six	0.3	0.0	39.3	0.0	4.6	14.6	1.4	28.9	20.3	2.2
	0.4	0.0	39.2	0.0	4.7	14.5	1.6	28.9	20.5	1.8
Seven	0.6	0.7[b]	37.3	0.4	4.5	14.0	1.6	27.1	19.2	2.0
	0.6	0.7[b]	37.4	0.4	4.5	13.9	1.6	27.1	19.3	2.1
Eight	0.5	0.0	39.9	0.0	4.7	14.5	1.4	28.7	20.0	1.9
	0.5	0.0	39.8	0.0	4.5	14.4	1.4	29.0	20.1	1.9
Nine	0.4	0.0	39.0	0.0	4.4	14.2	1.4	28.1	19.8	1.9
	0.4	0.0	39.8	0.0	4.4	14.3	1.4	28.2	19.8	1.9
Ten	0.0	0.0	41.4	0.0	4.3	15.4	0.7[b]	30.4	21.6	1.0
	0.0	0.0	41.7	0.0	4.2	15.5	0.7[b]	30.4	21.4	1.0
Eleven	0.5	0.4	39.1	0.0	4.2	13.8	1.5	28.5	19.8	1.8
	0.6	0.3	39.4	0.0	4.4	13.7	1.6	27.8	19.6	2.2
Twelve	0.9	0.1	39.4	1.0[c]	4.7	14.3	1.6	29.0	18.9	1.9
	0.8	0.3	35.5	0.6[c]	4.8	14.5	1.8	25.2	18.1	2.2
XBAR	0.4	0.1	39.6	0.1	4.5	14.7	1.5	28.7	20.3	1.7
s(r)	0.1	0.1	0.8	0.0	0.1	0.1	0.1	0.8	0.2	0.1
s(R)	0.3	0.1	1.3	0.2	0.2	0.6	0.2	1.2	1.0	0.4
RSD(r)	11.1	55.9	2.1	16.3	2.7	0.6	5.9	2.8	1.1	7.4
RSD(R)	68.4	133.5	3.4	143.2	5.3	4.0	12.4	4.2	4.7	23.7
r	0.1	0.1	2.4	0.1	0.3	0.3	0.2	2.3	0.6	0.4
R	0.8	0.3	3.8	0.5	0.7	1.6	0.5	3.3	2.7	1.2

[a]The abbreviations of the statistical parameters are given in Table 1.
[b]Single Grubbs outlier.
[c]Cochran outlier.

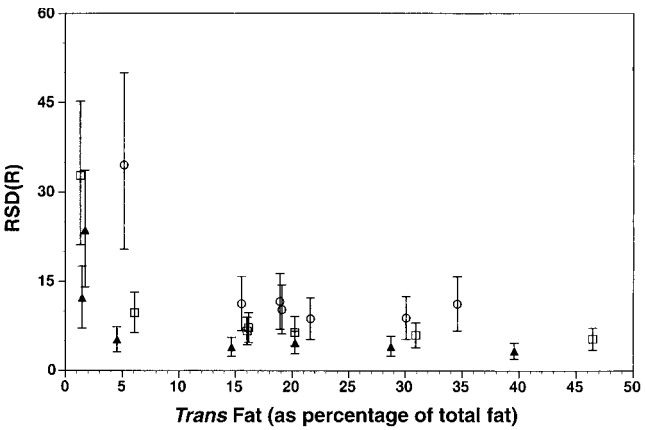

FIG. 2. Comparison of plots of reproducibility relative standard deviation [RSD(R)] against the *trans* content mean values determined by three official methods: the two transmission methods AOAC 965.34 (○) and AOAC 994.14 (□), and the ATR method (▲) AOCS Cd 14d-99. The numbers of laboratories in the corresponding collaborative studies were 12, 16, and 12, respectively. The error bars denote the upper and lower 95% confidence limits on the true RSD(R). The lowest RSD(R) values were obtained by the ATR method. See Figure 1 for other abbreviation.

contain >1% *trans* fat per serving (15), the ATR-FTIR method (13) should be applicable to all foods with a low content of *trans* fat. This method is simple because it does not require the weighing or the quantitative dilution of test materials in any solvent. It requires about 5 min for performing the IR measurement and calculation of %*trans* levels. It is accurate; results exhibited <2% high bias relative to the gravimetric values. Comparison of test materials with similar %*trans* overall means indicated that the precision of the current ATR-FTIR method was superior to those of the two most recently approved transmission infrared official methods, AOAC 965.34 (4) and AOAC 994.14 (3).

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REFERENCES

1. Department of Health and Human Services, The Food and Drug Administration, Food Labeling: *Trans* Fatty Acids in Nutrition Labeling, Nutrient Content Claims, and Health Claims; Proposed Rules, *Federal Register*, 21CFR Part 101, Docket No. 94P-0036, RIN 0910-AB66, November 17, 1999.
2. McDonald, R.E., and M.M. Mossoba, Analysis of *Trans* Fatty Acids, in *Food Lipids*, edited by C.C. Akoh and D.B. Min, Marcel Dekker, New York, 1997, pp. 137–166.
3. AOAC, Association of Official Analytical Chemists, *Official Method of Analysis*, edited by K. Helrich, Gaithersburg, 1994, Method 994.14.
4. AOAC, Association of Official Analytical Chemists, *Ibid.*, 1997, Method 965.34.
5. AOCS, American Oil Chemists' Society, *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 5th edn., edited by D. Firestone, Champaign, 1995, Official Method Cd 14-95.
6. Mossoba, M.M., M.P. Yurawecz, and R.E. McDonald, Rapid Determination of the Total *Trans* Content of Neat Hydrogenated Oils by Attenuated Total Reflection Spectroscopy, *J. Am. Oil Chem. Soc. 73*:1003–1009 (1996).
7. Ali, L.H., G. Angyal, C.M. Weaver, J.I. Rader, and M.M. Mossoba, Determination of Total *Trans* Fatty Acids in Foods: Comparison of Capillary Column Gas Chromatography and Single Bounce Horizontal Attenuated Total Reflection Infrared Spectroscopy, *Ibid. 73*:1699–1705 (1996).
8. Adam, M., M. Chew, S. Wasserman, A. McCollum, R.E. McDonald, and M.M. Mossoba, Determination of *Trans* Fatty Acids in Hydrogenated Vegetable Oils by Attenuated Total Reflection Infrared Spectroscopy: Two Limited Collaborative Studies, *Ibid. 75*:335–361 (1998).
9. Adam, M., M.M. Mossoba, M. Chew, and S. Wasserman, Comparison of Attenuated Total Reflection Infrared Spectroscopy to Capillary Gas Chromatography for *Trans* Fatty Acid Determination, *Ibid. 76*:375–381 (1999).
10. *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 5th edn., edited by D. Firestone, Champaign, 1996, Recommended Practice Cd 14d-96.
11. Sedman, J., F.R. van de Voort, A.A. Ismail, and P. Maes, Industrial Validation of Fourier Transform Infrared *Trans* and Iodine Value Analyses of Fats and Oils, *J. Am. Oil Chem. Soc. 75*: 33–39 (1998).
12. Fritsche, J., M.M. Mossoba, M.P. Yurawecz, K.D. Eulitz, J.K.G. Kramer, Y. Ku, and J.I. Rader, Application of Standard Addition to Enhance the Accuracy of *Trans* Fat Determinations in Foods by Infrared Spectroscopy, *Amer. Oil Chem. Soc. Annual Meeting Abstracts*, Orlando, May 9–12, 1999, p. S92.
13. *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 5th edn., edited by D. Firestone, Champaign, 1999, Official Method Cd 14d-99.
14. Anon., Guidelines for Collaborative Study Procedure to Validate Characteristics of a Method of Analysis, *J. Assoc. Off. Anal. Chem. 72*:95–105 (1989).
15. Exler, J., L. Lemar, and J. Smith, Fat and Fatty Acid Content of Selected Foods Containing *Trans* Fatty Acids, Special Purpose Table No. 1, [http://www.NAL.USDA.Gov//FNIC/foodcomp/data/other/trans_fa.dat].

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