Determination of Total *trans* Fatty Acids in Foods: Comparison of Capillary-Column Gas Chromatography and Single-Bounce Horizontal Attenuated Total Reflection Infrared Spectroscopy

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ABSTRACT: The total *trans* fatty acid content of 18 food products was determined, after acid hydrolysis, extraction and methylation of fatty acids, by gas chromatography with a polar 100% cyanopropylsiloxane capillary column and by single-bounce horizontal attenuated total reflection spectroscopy (SB-HATR). The *trans* fatty acid methyl esters (FAME) of 9-hexadecenoate (9*t*-16:1), 9-octadecenoate (9*t*-18:1), and 9,12-octadecadienoate (9*t*,12*t*-18:2) were identified by comparison of their retention times with those of known standards and quantitated. The isomers *c,t*- and *t,c*-18:2 were identified from their published retention times and included in the quantitation of *trans* FAME. Neat 50-μL portions of the FAME that were used for gas-chromatographic analysis also were analyzed by SB-HATR. This technique requires neither weighing nor quantitative dilution of test portions prior to spectroscopic quantitation of isolated double bonds of *trans* configuration. A symmetric 966-cm^{-1} absorption band on a horizontal background was obtained from unhydrogenated soybean oil FAME as the reference material. For 9 of 11 products with *trans* fat content >5% of total fat, results obtained by SB-HATR were higher than those obtained by gas chromatography. Results obtained by the gas-chromatographic procedure were slightly to significantly higher than those obtained by SB-HATR for the six foods in which *trans* fat content was <5% of total fat.

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KEY WORDS: Attenuated total reflection infrared spectroscopy, capillary-column gas chromatography, *trans* fatty acids.

Under Food and Drug Administration (FDA) regulations to implement the Nutrition Labeling and Education Act of 1990 (NLEA), food labels are required to list fat and saturated fat content. The new labeling regulations define "fat" or "total fat" as total lipid fatty acids (FA) expressed as triacylglycerol (TG). Food-label declarations of total fat must be expressed as the amount of TG that would provide the analytically measured amount of total lipid FA in the food (1). Partially hydrogenated fats, which are ingredients in a wide variety of foods, contain *trans* isomers of unsaturated FA. Regulations to implement the NLEA do not permit *trans* FA labeling. Currently, *trans* fats are included in the definition of total fat but are not included in definitions of mono- and polyunsaturated FA.

Trans isomers have been reported to raise serum levels of low-density lipoprotein cholesterol (2,3). Judd *et al.* (3) recently reported that, compared with oleic acid, the *trans* FA 18:1 raised low-density lipoprotein cholesterol, although somewhat less than do saturates. Recent epidemiologic and clinical studies suggest that intake of *trans* FA may be a risk factor for cardiovascular disease (4,5). The Center for Science in the Public Interest (CSPI) petitioned FDA in 1994 to include *trans* fatty acids as part of saturated FA content for nutrition labeling, and the Malaysian Palm Oil Promotion Council (MPOPC) has also requested such labeling (6). MPOPC has requested separate labeling of *trans* fats, rather than inclusion of *trans* fat with saturated fat. The agency is currently reviewing these petitions.

Continuing interest in issues of fat labeling and discussions of the nutritional significance of *trans* fatty acids have prompted efforts to optimize methods for determining *trans* FA in foods. Several methods are available for determining total *trans* content of oils by infrared (IR) spectroscopy. The IR determination measures the unique C–H out-of-plane deformation absorption at 966 cm^{-1} for analytes that contain isolated *trans* double bonds after quantitatively dissolving the analytes in carbon disulfide, a volatile and toxic solvent. Usually, the *trans* band overlaps with other features in the IR spectrum and leads to a strongly sloping background that reduces the accuracy of the quantitation, especially at *trans* levels near and below 2–5%. Although many changes, ranging from minor refinements to major modifications, have been proposed, none has been fully satisfactory (7). Recently, an attenuated total re-

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flection procedure was proposed (7). The capacity of the liquid cell used was about 1.5 mL. We investigated the potential utility of single-bounce horizontal attenuated total reflection (SB-HATR) spectroscopy because of the small test portion requirements (50 μL) and rapidity of analysis.

We report for 18 food products the results of a comparison of analysis of *trans* fatty acid methyl esters (FAME) by capillary gas chromatography (GC) with a polar 100% cyanopropylsiloxane stationary phase vs. determinations by SB-HATR spectroscopy (8,9). Use of the novel SB-HATR technique requires only about 50-μL test portions of neat concentrated FAME. It eliminates the need to weigh test portions and to quantitatively dilute them in solvent. The problem of severely sloping background was overcome by "ratioing" the single-beam spectrum of the FAME mixture under analysis against that of an unhydrogenated soybean oil FAME (7), thus exhibiting a symmetric *trans* IR band on a horizontal background.

MATERIALS AND METHODS

Preparation of food samples. Eighteen food products were purchased locally. Total fat contents (calculated from label declarations) were <4% to about 30% (w/w). Products included breakfast foods, meat-containing products, meal-type products, snack foods, and infant formula powder. Foods were composited in a dual-speed blender and stored in tightly sealed glass containers. Composites were stored refrigerated or frozen as appropriate for the individual food item. All products were extracted and analyzed before their labeled expiration dates.

Fatty acid standards. A mixture of FAME in *n*-heptane was purchased from Matreya, Inc. (Pleasant Gap, PA) (No. 4210). Standards included the following FAME: octanoate, 8:0; decanoate, 10:0; dodecanoate, 12:0; tridecanoate, 13:0; tetradecanoate, 14:0; 9-tetradecenoate, 14:1; pentadecanoate, 15:0; hexadecanoate, 16:0; 9-hexadecenoate, 16:1; heptadecanoate, 17:0, octadecanoate, 18:0; 9-octadecenoate (elaidate) 18:1*t*; 9-octadecenoate (oleate), 18:1; 9,12-octadecadienoate, 18:2; eicosanoate, 20:0; 9,12,15-octadecatrienoate, 18:3; 11-eicosenoate, 20:1; docosanoate, 22:0; and 13-docosenoate, 22:1. Reference standards that consisted of mixtures of *cis* and *trans* methyl esters of known composition, e.g., quantitative mixture no. K110 (#19050, Alltech Associates, Inc., Deerfield, IL) and gas–liquid chromatography (GLC)-426 (Nu-Chek-Prep, Inc., Elysian, MN), were also used. Mixture GLC-426 contained methyl esters of fatty acids 6:0 to 24:0. Quantitative mixture no. K110 consisted of hexadecanoate, 16:0; *cis*-9-hexadecenoate, 16:1*c*; *trans*-9-hexadecenoate, 16:1*t*; octadecanoate, 18:0; *trans*-9-octadecenoate, 18:1*t*; *cis*-9-12-octadecadienoate, 18:2*cc*; and *trans*-9,12-octadecadienoate, 18:2*tt*. Tridecanoin was used as an internal standard.

Acid hydrolysis. The method was modified from that described by Ngeh-Ngwainbi and Lin (10) for their collaborative study and has been described in detail (11,12). About 2 g of the fresh (undried) food composite was weighed to 0.0001 g into a test tube (20 × 150 mm), wetted with absolute ethanol (2 mL), and 1 mL tridecanoin internal standard (5.0 mg/mL) was added. The digestion test tube was swirled to moisten all particles of the material to prevent lumping with the addition of the acid. Then, 10 mL of 6 N HCl solution was added, and the tube was capped. Digestion was carried out for 40 min in an 80°C water bath with the tubes held in a shaker attachment set at 60 cycles/min. Immediately after removal from the water bath, 10 mL of absolute ethanol was added, and the digestate was mixed on a vortex mixer. The digestate was cooled and quantitatively transferred to a 250-mL separatory funnel. The digestion tube was rinsed sequentially with diethyl ether and petroleum ether, and the rinses were added to the digestate. The digestate was then extracted with 100 mL of a mixture of equal parts of diethyl ether and petroleum ether. The aqueous layer was extracted twice more with 60 mL of the diethyl ether–petroleum ether mixture. The ether layer was drawn through a filter, consisting of a pledget of fat-free cotton or glass wool covered with anhydrous sodium sulfate (approximately 25 g), into a 125-mL flat-bottom flask (preweighed), which contained boiling chips. The ether was evaporated slowly to near-dryness on a steam table under a stream of dry nitrogen in a ventilated hood.

Preparation of FAME. FAME were prepared as described in AOAC method 969.33 (13,14) as follows: Methanolic NaOH solution and a boiling chip were added to the extracted fat. The flask was attached to a water-cooled condenser and refluxed for 10 min. Ten mL of BF_3 reagent was added through the condenser, and refluxing was continued for 2 min. Ten mL of *n*-heptane was added through the top of the condenser, and reflux was continued for an additional 1 min. The flask was removed from the heat and allowed to cool to ambient temperature. Five to seven mL of saturated salt solution was added to the flask to float the fatty acid esters in *n*-heptane. One mL of the upper layer, containing FAME in *n*-heptane, was transferred to a gas chromatography (GC) vial, and 1 μL was injected into the GC.

GC of FAME. GC was performed in a Shimadzu GC-14A chromatograph, equipped with a flame-ionization detector (FID) and a Shimadzu CR501 Chromatopac integrator (Shimadzu Scientific Instruments, Inc., Columbia, MD). The method was described by Ngeh-Ngwainbi and Lin (10) who used a nonbonded 90% cyanopropyl, 10% phenyl siloxane capillary column, 30 m × 0.25 mm i.d., 0.2 μm film and the following temperature program: initial temperature, 120°C (hold 4 min); rate, 5.0°C/min; final temperature 230°C; final time, 5.0 min. However, we obtained better separation of isomers in our food samples by using a flexible fused-silica capillary column (100 m × 0.25 mm i.d., 0.20 μm film thickness; Supelco, Inc., Bellefonte, PA) and the following temperature program: initial temperature, 175°C (hold 14 min); then programmed to 185°C at a rate of 5.0°C/min and held at this final temperature for 50 min. Using this column and temperature program, we obtained better resolution in the complex 18:1 and 18:2 regions. The detector and injector port temperatures

were 225°C. Helium was used as the carrier gas; linear velocity, 23 cm/s at 175°C; and nitrogen was used at 40 mL/min as the make-up gas to the FID.

GC standardization/calibration. The calibration procedure used in the method described in this report required correction response factors for each FAME based on elution of an external FAME standard mixture (Matreya No. 4210). The response factors (R_i) for each FA were calculated as shown below, except that for any unknown or uncalibrated peak, the nearest calibrated FA response factors and conversion factors were used to calculate total, saturated, and unsaturated fat (10).

$$R_i = \frac{A_i \times \mathrm{wt}_{13:0}}{A_{13:0} \times \mathrm{wt}_i} \quad [1]$$

where R_i = response factor for FA i, A_i = peak area of the individual FAME i in the standard, $\mathrm{wt}_{13:0}$ = weight of 13:0 FAME in the standard, $A_{13:0}$ = peak area of 13:0 FAME in the standard, and wt_i = weight of individual FAME i in the standard.

GC calculations. The amount of each FA, F_i, in each test sample (as the corresponding methyl ester) was calculated according to the following equation:

$$F_i = \frac{A_i}{A_{13:0}} \times \frac{\mathrm{wt}_{13:0}}{R_i} \quad [2]$$

where A_i = peak area of the individual FAME i in the sample, $\mathrm{wt}_{13:0}$ = weight of 13:0 standard (ISTD) in the sample, $A_{\mathrm{C13:0}}$ = peak area of 13:0 standard (ISTD) in the sample, and R_i = response factor.

The amount of each FA, $F_{i,\mathrm{TG}}$, in each sample (as the corresponding TG) was calculated according to the following equation:

$$F_{i,\mathrm{TG}} = (F_i \times CF_{\mathrm{TG}i}) \quad [3]$$

where $CF_{\mathrm{TG}i}$ is the factor for conversion of FAME to their corresponding TG (10).

The amount of each FA, $F_{i,\mathrm{FA}}$, in each sample (as the corresponding FA) was calculated according to the following equation:

$$F_{i,\mathrm{FA}} = (F_i \times CF_{\mathrm{FA}i}) \quad [4]$$

where $CF_{\mathrm{FA}i}$ is a factor calculated as follows: $CF_{\mathrm{FA}i}$ = (molecular weight free FA i)/(molecular weight FAME i).

The amount of total fat (sum of all FA) in each test sample, expressed as TG, was calculated according to the following equation:

$$\%\ \text{total fat} = \frac{\sum F_{i,\mathrm{TG}}}{\mathrm{wt}_{\mathrm{sample}}} \times 100 \quad [5]$$

The amount of monounsaturated fat (sum of *cis* and/or *trans* FA) in the test sample as the corresponding FA was calculated individually according to the following equation:

$$\%\ \text{monounsaturated fat} = \frac{\sum \text{monounsaturated } F_{i,\mathrm{FA}}}{\mathrm{wt}_{\mathrm{sample}}} \times 100 \quad [6]$$

The amount of polyunsaturated fat can be calculated in an analogous manner. Total unsaturated fat is calculated by summing contributions from mono- and polyunsaturated FA.

The *trans* FAME 9-hexadecenoate (9*t*-16:1), 9-octadecenoate (9*t*-18:1), and 9,12-octadecadienoate (9*t*,12*t*-18:2) were identified by comparison of their retention times with those of known standards and quantitated. The isomers *c,t*- and *t,c*-18:2 were identified from their published retention times on an SP-2560 column (Supelco, Inc.) under the following experimental conditions: column temperature programmed at rate of 1°C/min from 125–175°C and held at 175°C for 25 min; detector and injector temperatures, 235 and 225°C, respectively (15). They were included in the quantitation of *trans* FAME.

SB-HATR spectroscopy. An FTS-60A Fourier transform infrared spectrometer (Bio-Rad, Digilab Division, Cambridge, MA), consisting of an SPC 3200 workstation with the IDRIS™ operating system and an optical console, was used. The optical bench included a Michelson interferometer with a quality air bearing, a potassium bromide (KBr) substrate beam splitter, and a DTGS detector. A Spectra-Tech (Shelton, CT) SB-HATR cell that requires about 50-µL test sample was used. This accessory contains a zinc selenide (ZnSe) crystal for high throughput sampling. It is pre-aligned in the factory, pre-attached to its baseplate that attaches to the floor of the Fourier transform infrared spectrometer sample compartment. Mounting holes and pegs in the baseplate ensure its proper positioning and alignment.

SB-HATR procedure. Neat test samples (50 µL) of the FAME, prepared as described above, were placed without weighing on the horizontal (face-up) ZnSe sampling surface of the SB-HATR cell. For quantitative work, it is necessary for the test sample to cover all of the horizontal surface of the ZnSe crystal. Sixty-four scans were collected at 4 cm^{-1} resolution. After a measurement was made, the surface of the crystal was cleaned by gently wiping it with a soft material to avoid scratching it.

SB-HATR calibration and quantitation. SB-HATR quantitation was based on the measurement of the integrated area under the 966-cm^{-1} absorption band between 990 and 945 cm^{-1} (7). A calibration plot of "area" vs. "percentage" *trans* isomers of octadecenoate was generated for reference mixtures in the range of 0.592–54.15% ME in methyl oleate (MO). Refined, bleached, deodorized soybean oil was used as the reference background material. The regression line parameters for the *trans* standards were 0.0021 (intercept) and 0.0186 (slope). The correlation coefficient (R) was 0.99957. For test samples, the percentage *trans* was calculated from the area of the observed absorption band at 966 cm^{-1} and the linear regression equation that describes the calibration plot. The lower limit of quantitation was about 1%.

RESULTS AND DISCUSSION

The ability of the "ratioing" procedure (7), coupled with the SB-HATR technique to measure ME, was tested by adding known

TABLE 1
Quantitation of Methyl Elaidate (18:1*t*) in Esterified Canola Oil by SB-HATR[a]

Methyl elaidate (g)	Canola oil (g)	Methyl elaidate		Measured (% of calculated)
		Calculated (%)	Measured (%)	
0.0079	1.0725	0.73	0.65	89.3
0.0067	0.7092	0.94	0.87	93.0
0.0234	0.9891	2.31	2.22	96.1
0.0234	0.7098	3.19	3.10	97.2
0.0238	0.4211	5.35	5.34	99.8
0.0216	0.2099	9.33	9.36	100.3
0.0250	0.1334	15.78	14.85	94.1
0.0424	0.0710	37.39	36.28	97.0
0.0690	0.0595	53.70	52.32	97.4

[a]Mixtures of methyl elaidate in canola oil were prepared by adding weighed amounts of methyl elaidate (*trans* 18:1) to methylated canola oil. Results of single-bounce horizontal attenuated total reflectance spectroscopy (SB-HATR) measurements of total *trans* fatty acids in the mixtures were compared to calculated values. Mean ± SD for nine determinations was 96.0 ± 3.4%.

amounts of ME to canola oil FAME and quantifying ME in the resultant mixtures (Table 1). In this experiment, the single-beam spectra of these mixtures were "ratioed" against the single-beam spectrum of canola oil FAME (reference material). The *trans* content of canola oil FAME (1.06%) did not affect the quantitation of ME in these mixtures. Recovery of ME, measured in nine such mixtures, was 96.0 ± 3.4% (mean ± SD).

Trans *fatty acids in foods.* Figure 1 represents the chromatogram obtained for FAME prepared from breaded fish fillets. Total fat content of the breaded fish fillets was 11%. Peaks of interest are identified according to the chainlength and number of double bonds present. Although the *cis* and *trans* 18:1 isomers in the food were not completely resolved, baseline resolution on the SP-2560 column was routinely achieved for mixtures of ME and MO as well as for other pairs of geometric isomers. This observation is in agreement with results of Ratnayake and Beare-Rogers (16).

The results of analysis of 18 food products by the two techniques are shown in Table 2, with the food products listed in order of increasing total fat content. Total fat content of the products, calculated from the results of GC determinations of FA content, ranged from about 3 to 30%. Total *trans* fat content determined by GC or calculated from SB-HATR values of *trans* fat as percentage of total fat ranged from about 1 to 130 mg/g in the samples. *Trans* fat content, as percentage of total fat, ranged from less than 4 to more than 40% in the products.

The *trans* isomers of 9-hexadecenoate (9*t*-16:1), 9-octadecenoate (9*t*-18:1), and 9,12-octadecadienoate (9*t*,12*t*-18:2) FAME were quantitated by GC in this study. These FA were present in foods at levels of (mg/g) 0.06 to 0.59 (9*t*-16:1); 0.96 to 105.85 (9*t*:18:1); and 0.06 to 3.38 (9*t*,12*t*-18:2) (data not shown).

In Table 3, the 18 food products are listed in order of increasing *trans* fat content, expressed as percentage of total fat. The percentage relative differences in results obtained by the two methods were calculated as follows:

$$\% = \frac{\text{GC} - \text{SB-HATR}}{\text{SB-HATR}} \times 100 \qquad [7]$$

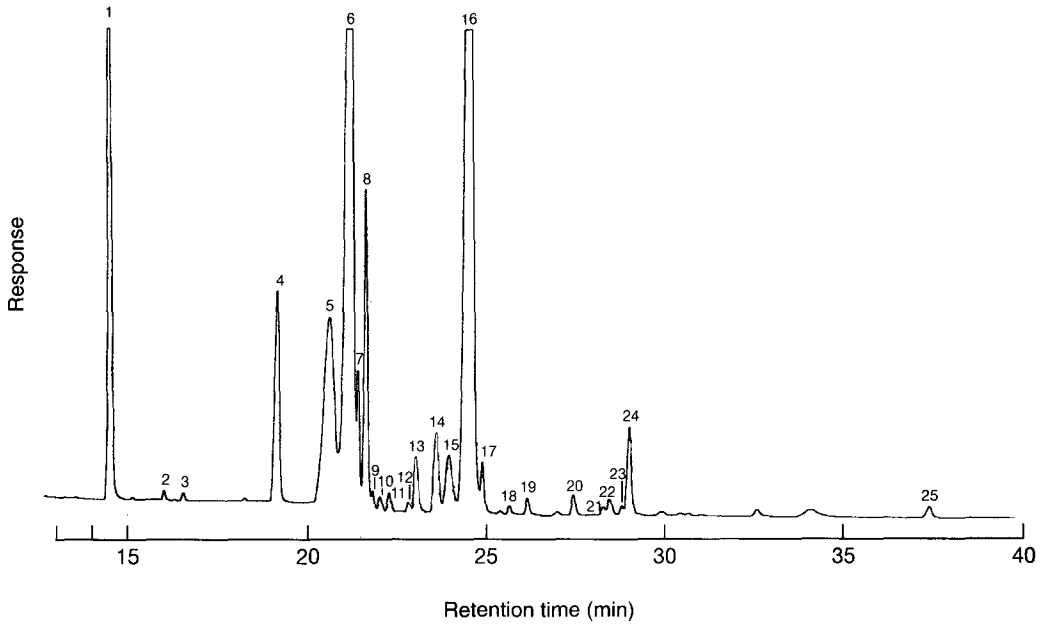

FIG. 1. The C_{16}, C_{18}, and C_{20} regions of the gas chromatogram of fatty acid methyl esters prepared from breaded fish fillets that contained 11% total fat. Analysis was performed on an SP-2560 flexible fused-silica capillary column (100 m × 0.25 mm i.d.). Numbers correspond to: 1, 16:0; 2, 16:1*c*; 3, 17:0; 4, 18:0; 5, 18:1*t*; 6–8, 18:1*c* [based on (14)]; 9, 18:1Δ12*c*; 10, 18:1Δ13*c*; 11, ?; 12, 18:2*tt*; 13, 14, 15, 18:2*ct/tc*; 16, 18:2Δ9*c*,12*c*; 17, 18:2Δ9*c*,15*c*; 18, ?; 19, 20:0; 21, 22, ?; 23, 20:1; 24, 18:3Δ9*c*,12*c*,15*c*; 25, 22:0.

TABLE 2
Comparison of Values for Total *trans* Fatty Acids in Food Products, Determined by GC and SB-HATR Procedures

Food product	Total fat[a] (%)	*Trans* fat, mg/g[b] GC[c]	*Trans* fat, mg/g[b] SB-HATR[d] (calc)	*Trans* fat, % of total fat[b] GC[c] (calc)	*Trans* fat, % of total fat[b] SB-HATR[d]
1 Fat-free margarine	2.63 ± 0.03	0.40 ± 0.08	0.16	1.52	(0.63)
2 Chicken pie	3.41 ± 0.04	8.68 ± 0.04	9.00	25.45	26.42
3 Turkey gravy dressing meal	3.61 ± 0.16	5.39 ± 0.39	6.49	13.92	17.97 ± 0.26
4 Beef ravioli with sauce	3.77 ± 0.11	1.88 ± 0.23	1.32	4.96	3.49 ± 0.21
5 Chili macaroni	4.21 ± 0.10	1.61 ± 0.11	1.19	3.82	2.82 ± 0.50
6 Cheese crackers	4.29 ± 0.06	5.48 ± 0.09	6.43	12.76	14.98 ± 0.13
7 Cereal with raisins	4.83 ± 0.39	7.26 ± 0.48	8.86	15.02	18.34 ± 1.70
8 Breaded fish fillets	11.20 ± 1.23	18.61 ± 1.98	16.41	16.62	14.66 ± 0.53
9 Taco dinner	11.55 ± 0.98	34.32 ± 1.53	38.29	29.72	33.15 ± 0.56
10 Chili without beans	12.15 ± 0.28	7.19 ± 0.78	7.72	5.92	6.36 ± 0.43
11 Toddler formula	16.48 ± 1.63	3.14 ± 0.13	1.22	1.91	(0.74)
12 Peanut butter cookies	21.53 ± 0.72	3.64 ± 0.36	1.47	1.69	(0.68)
13 Crackers	21.53 ± 0.97	30.44 ± 1.23	31.19	14.14	14.49 ± 0.04
14 Blue cheese dressing	22.06 ± 0.92	12.07 ± 0.72	11.71	5.47	5.31 ± 0.02
15 Biscotti	23.44 ± 0.44	7.36 ± 1.08	5.63	3.14	2.40 ± 0.59
16 Peanut butter cracker sandwich	29.47 ± 0.61	48.49 ± 0.95	52.77	16.45	17.91 ± 0.01
17 Chicken flavored crackers	30.55 ± 0.90	131.2 ± 1.70	132.65	42.89	43.42 ± 1.03
18 Chocolate bar with almonds	35.77 ± 4.18	n.d.	N/A	N/A	n.d.

[a]Total fat (%) was determined from gas chromatography (GC) analyses.
[b]*Trans* fat is expressed as mg/g of the food product and as percentage of total fat in the products. Abbreviations: n.d., not detected; N/A, not applicable.
[c]For GC analysis: Values for total fat and *trans* fat determined by GC are means ± standard deviations (SD) of two independent determinations. Mean values for *trans* fat as percentage of total fat were calculated from GC measurements.
[d]For SB-HATR analysis: With four exceptions, values for *trans* fat determined by SB-HATR as percentage of total fat are means ± SD of two measurements on one of the two samples prepared for GC analysis. A single measurement was made on samples of fat-free margarine, chicken pie, formula, and peanut butter and grape cookies. Mean values for *trans* fat expressed as mg/g product were calculated from SB-HATR measurements. Values in parentheses are below the lower limit of about 1% for quantitation by SB-HATR.

The greatest relative differences between results obtained by the two methods were found for products in which *trans* FA represented less than 5% of the total fat (e.g., fat-free margarine (product 1), peanut butter and grape cookies (peanut butter cookies; product 12), biscotti (product 15); chili macaroni (product 5), and beef ravioli with meat sauce (beef ravioli with sauce, product 4). For these products, results obtained by the GC procedure were significantly higher than those obtained by the SB-HATR procedure. For 9 of 11 products with *trans* fat content greater than 5% of total fat, results obtained by SB-HATR were higher than those obtained by GC.

For the IR procedure, it is desirable to use a reference background material that is as close as possible in composition to that of the hydrogenated oil under analysis. Ideally, the corresponding unhydrogenated oil should be used. It is also important that such reference materials contain no *trans* double bonds. Bleaching and deodorization of vegetable oil unavoidably produce low yet measurable levels of *trans* species. In practice, it is recommended that an appropriate reference material that contains the lowest possible level of *trans* species be used. Palm or olive oils with relatively low levels of polyunsaturation may meet this criterion. However, their FA compositions are quite different from those of soybean or corn oils, which are much more commonly used in processed foods.

Identification of a single reference material that is appropriate for the analysis of all types of foods is difficult if not impossible because the exact composition of source oils (soybean, sunflower, palm, canola, cottonseed, safflower, etc.) used for hydrogenation and their relative proportions in processed food products will not be known and will differ significantly among different types of food products.

The refined, bleached, deodorized soybean oil used as the reference background material in this study contained about 0.5% *trans* fat as determined by capillary GC (7). The presence of this level of *trans* fat in the background reference material may account for the lower *trans* values found by SB-HATR, relative to GC, for the food products in which *trans* fatty acids represented less than 5% of the total fat (Table 3). The lower limit of quantitation by SB-HATR is about 1%.

Another inherent source of error in the quantitation of total *trans* content by SB-HATR derives from the fact that all isolated *trans* double bonds in food products are assumed to be due to *trans* isomers of octadecenoate. However, the absorptivities of monounsaturated, diunsaturated, and other polyunsaturated *trans* species are different (17). Although most *trans* FA in hydrogenated fats are monoenes, important quantities of *trans* dienes are also found and may be responsible for some of the observed discrepancies between SB-HATR and GC values. The extent of such deviations will depend on the relative proportions of *trans* polyunsaturated to monounsaturated FA present in the foods under analysis.

TABLE 3
Relative Differences Between Total *trans* Fatty Acid Content of 18 Food Products Determined by GC and SB-HATR Procedures

Food product[a]	GC (*trans* fat, % of total fat)	SB-HATR[b] (*trans* fat, % of total fat)	Relative difference[c] (%)
Chocolate bar with almonds	0	0	N/A
Fat-free margarine	1.52	(0.63)	N/A
Peanut butter cookies	1.69	(0.68)	N/A
Toddler formula	1.91	(0.74)	N/A
Biscotti	3.14	2.40	+30.83
Chili macaroni	3.82	2.82	+35.46
Beef ravioli with sauce	4.96	3.49	+42.12
Blue cheese dressing	5.47	5.31	+3.01
Chili without beans	5.92	6.36	−6.92
Cheese crackers	12.76	14.98	−14.82
Crackers	14.14	14.49	−2.42
Turkey gravy dressing meal	14.92	17.97	−16.97
Cereal with raisins	15.02	18.34	−18.10
Peanut butter cracker sandwich	16.45	17.91	−8.15
Breaded fish fillets	16.46	14.66	+12.28
Chicken pie	25.45	26.42	−3.67
Taco dinner	29.72	33.15	−10.35
Chicken flavored crackers	42.89	43.42	−1.22

[a]Food products are listed in order of increasing *trans* fat content expressed as percentage of total fat. The percentage relative differences in results obtained by the two methods were calculated from the *trans* fat results as follows: % = {|(GC) − (SB-HATR)/(SB-HATR)| × 100}.
[b] Because the lower limit of quantitation by SB-HATR is about 1%, the values in parentheses are listed for comparative purposes only. Relative differences between results obtained by the two methods for such products were >140%. See Tables 1 and 2 for abbreviations.
[c]The large relative differences between methods found in products with *trans* levels below 5% may be due in part to the fact that the reference background material used in the SB-HATR procedure contains about 0.5% *trans* fat. This decreased SB-HATR measurements by about 0.5% and had the greatest impact on products with the lowest *trans* fat concentrations. N/A = not applicable.

Because of the numerous positional isomers present in partially hydrogenated vegetable oils, a satisfactory separation of 18:1-*trans* isomers (*trans* isomers of octadecenoate) as a group from the *cis* isomers is not feasible on any capillary column. For example, Molkentin and Precht (18) reported that, with use of a 100-m CP-Sil column (Chrompack), the 18:1-*t* isomers with Δ values up to 12 were separated from the 18:1-*c* isomers, but the 18:1-*t* isomers with high Δ values (i.e., Δ13–Δ16) overlapped with the *cis* isomers (18). Because of this overlap, the direct GC method underestimates total 18:1-*t* isomers in favor of the *cis* isomers (19,20). At lower levels of *trans* (near 1%), GC may be more accurate than SB-HATR or ATR for quantitation of total *trans* FA. This is in agreement with observations of Mossoba *et al.* (7).

Numerous geometric and positional FA isomers are found in the majority of food products tested. Satisfactory separation of all *trans* from *cis* isomers, particularly in the 18:1 region of the gas chromatogram, was not possible. Because of this overlap, the GC method underestimates total *t*-18:1 in favor of the *cis* isomers. This could explain some of the differences between measurements obtained by the SB-HATR and GC procedures, particularly at high *trans* levels, because all of the isolated *trans* double bonds gave rise to the 966-cm^{-1} band (7).

The SB-HATR procedure offers several advantages over the GC procedure. The analysis time is short: about 5 min are needed for the spectroscopic measurement, band area integration, and calculation of the *trans* content from a linear regression equation. Small quantiaties of test samples (about 50 μL) are required. The need for weighing and quantitatively diluting test samples with solvent is eliminated with SB-HATR. However, because of the issues discussed above, additional studies with a wider range of food types are needed to determine the applicability of this novel and rapid technique to the routine analysis of *trans* FA in foods, particularly at low *trans* levels. Alternatively, a more limited number of different food types could be used to investigate the two methods if a validated reference method (i.e., silver-ion LC–GC) were available to provide "true" values of *trans* fat for comparative purposes.

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