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Comparison of capillary column gas chromatographic and AOAC gravimetric procedures for total fat and distribution of fatty acids in foods

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There is increasing interest in the fatty acid composition, including levels of *trans* fatty acids, of foods. The *trans* fatty acid content of American diets is increasingly studied because of reported adverse effects of *trans* fatty acids on risk of coronary heart disease. In this study, total fat content and fatty acid composition of 43 food products were determined after acid hydrolysis by gas chromatography using an SP-2560 flexible fused silica capillary column. Total fat content determined by the gas chromatographic method was compared with fat content determined by AOAC gravimetric method 922.06 for all food products. Total fat, saturated fat and unsaturated fat content of the foods determined by the gas chromatographic method ranged from 0.9 to 96.7, 0.2 to 16.8 and 0.5 to 89.3%, respectively. *Trans* fatty acids hexadecenoate (*t*-16:1), elaidic (*t*-18:1), and octadecadienoate (*t,t*-18:2) were identified by comparison of their retention times with those of known standards and quantitated. These fatty acids were present in foods at levels of 0.25 to 1.50 (*t*-16:1), 0.87 to 268.32 (*t*-18:1), and 0.23 to 7.92 (*t,t*-18:2) mg/g. Published by Elsevier Science Ltd

INTRODUCTION

Food and Drug Administration (FDA) regulations implementing the Nutrition Labeling and Education Act of 1990 (NLEA) define 'fat' or 'total fat' as total lipid fatty acids expressed as triglycerides. Food label declarations of total fat must be expressed as the amount of triglyceride that would provide the analytically measured amount of total lipid fatty acids in the food (CFR, 1995). The new labeling regulations define 'saturated fat' as the sum of all fatty acids containing no double bonds. The new food labels are required to list fat and saturated fat content, while polyunsaturated (i.e., *cis*, *cis*-methylene-interrupted polyunsaturated fatty acids) and monounsaturated (i.e., *cis*-monounsaturated fatty acids) fat may be declared voluntarily under defined conditions (CFR, 1995).

There is considerable interest in the fatty acid composition of dietary fats and foods and in the health effects of *trans* fatty acids and of *cis*- and *trans*-monounsaturates (e.g., *c*-18:1, *t*-18:1). A number of *cis* and *trans* isomers of $C_{18:1}$ or $C_{18:2}$ acids are formed when vegetable oils are partially hydrogenated to produce dietary fats with improved texture, stability, and other economically desirable properties (Mensink *et al.*, 1992; Zock & Katan, 1992). These isomeric fatty acids constitute quantitatively an important part of the diet in industrialized countries. Margarines, cakes, cookies, corn chips, crackers, donuts and potato chips may contribute substantial amounts of *trans* fatty acids (Ratnayake *et al.*, 1991, 1993).

Regulations implementing the NLEA do not permit *trans* fatty acid labeling. The Center for Science in the Public Interest and the Malaysian Palm Oil Promotion Council have requested changes in the current regulations (Comment to Docket No.94P-0036, 1994). The agency is currently reviewing these petitions.

Continuing interest in issues of fat labeling has prompted efforts to improve methods for determining isomeric fatty acids in foods. In this report we describe an acid hydrolysis method for extraction of fatty acids from 43 food products followed by capillary column gas chromatography (GC) that addresses the new definitions of total fat and saturated fat in the NLEA. We also

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report a comparison for all foods analyzed of the results for fat obtained by the acid hydrolysis-capillary column GC methodology with results obtained by an AOAC direct gravimetric method. Finally, the *trans* fatty acids *t*-16:1, *t*-18:1, and *t,t*-18:2 were analyzed and quantitated under the same GC conditions.

MATERIALS AND METHODS

Preparation of food samples

Forty-three food products were purchased locally. Total fat contents (calculated from label declarations) were < 1-97% (w/w). Products included breakfast foods (e.g. dry cereals, powdered breakfast drink, fat-free waffles), meat-containing products (e.g. ravioli, fish fillets, chicken), meal-type products (e.g. taco dinner, turkey dinner), snack foods (e.g. cookies, snack crackers, chocolate bar), baby food, powdered infant formula, margarines and margarine-like products, mayonnaise, and canola oil. 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 labelled expiration dates.

Fatty acid standards

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

Standard reference materials

Two Standard Reference Materials (SRM) were included in the study. SRM 1548 (total diet) was purchased from NIST (Gaithersburg, MD). The certified fat value for this material (20.6 ± 2%) was determined by three gravimetric methods (i.e., AOAC direct, Folch-CH_3Cl/CH_3OH, and Weibull–Soxhlet, petroleum ether) (NIST, 1991). SRM 1846, a milk-based infant formula reference material, was produced in large quantity as a liquid, spray-dried and packaged for the FDA in 1991. The resultant dry material was analyzed by Analytical Systems Corp., Indianapolis, IN. The Certificate of Analysis provided by Analytical Systems Corp. included values for total fat (27.7%) as well as values for saturated and unsaturated fatty acids.

Reagents

All solvents and reagents were of analytical grade. Methanolic sodium hydroxide solution (0.5N) was prepared fresh. Boron trifluoride-methanol (14%) reagent was purchased from Sigma Chemical Company (St. Louis, MO). Petroleum ether (boiling range, 30–60 °C) was purchased from Baxter (Muskegon, MI).

Internal standard

Tridecanoic was purchased from Nu-Check Prep., Inc. (Elysian, MN) and prepared at a concentration of 5.019 mg/ml in chloroform.

Ether extract

Fat (crude) was determined gravimetrically on 2 or 4 g portions of each food composite or reference material by AOAC method 922.06 (Official Methods of Analysis, 1990*a*).

Fatty acid determination

Acid hydrolysis

The method was modified from that described by Ngeh-Ngwainbi & Lin (1994) for their collaborative study. About 2 g of the fresh (undried) food composite or reference material was accurately weighed to 0.0001 g into a test tube (20×150 ml), wetted with absolute ethanol (2 ml), and 1 ml tridecanoin internal standard 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 6N HCl solution was added and the tube was capped. No significant differences in results were found when 8N HCl was used in place of 6N HCl in the extraction phase (Rader *et al.*, 1995). 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 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 packed just firmly enough in the stem of a funnel to allow free passage of the ether into a 125 ml flat-bottom flask (preweighed) containing 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 fatty acid methyl esters (FAMEs)

FAMEs were prepared as described in AOAC method 969.33 (Official Methods of Analysis, 1990*b*). One ml of the upper layer containing FAMEs in *n*-heptane was transferred to a GC vial and 1 μl was injected into the GC.

Gas chromatography of (FAMEs)

Gas chromatography (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 column used was a SP-2560 flexible fused silica capillary column (100 m $\times$0.25 mm i.d., 0.20 μm film thickness; Supelco, Inc., Bellefonte, PA). The column was held at 175 °C for 14 min and then programmed to 185 °C at a rate of 5 °C/min and held at this final temperature for 50 min. Operating conditions were modified as needed to obtain optimum separation of isomeric fatty acid methyl esters. The detector and injector port temperatures were 225 °C. Helium was used as the carrier gas with a flow rate 0.7 ml/min and nitrogen was used at 40 ml/min as the make-up gas to the FID.

Standardization/calibration

The calibration procedure used in the method described in this report used 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 fatty acids were calculated as shown below, except that for any unknown or uncalibrated peaks, the nearest calibrated fatty acid response factors and conversion factors were used to calculate total, saturated, and unsaturated fat (Ngeh-Ngwainbi & Lin, 1994).

$$R_i = \frac{A_i \times Wt_{C13:0}}{A_{C13:0} \times Wt_i}$$

where R_i = Response factor for fatty acid i; A_i = Peak area of the individual FAME *i* in the standard; $WtC_{13:0}$ = Weight of C13:0 FAME in the standard; $A_{C13:0}$ = Peak area of $C_{13:0}$ FAME in the standard; Wt_i = Weight of individual FAME i in the standard.

Calculations

The amount of each fatty acid, F_i, in each test sample (as the corresponding methyl ester) was calculated according to the following equation:

$$\text{Amt. Fatty Acid}_i,\ F_i = \left\{\frac{A_i}{A_{C13:0}}\right\} \times \left\{\frac{Wt_{C13:0}}{R_i}\right\}$$

where A_i = Peak area of the individual FAME *i*; in the sample; $Wt_{C13:0}$ = Weight of $C_{13:0}$ standard (ISTD) in the sample; $A_{C13:0}$ = Peak area of $C_{13:0}$ standard (ISTD) in the sample; R_i = Response factor

The amount of each fatty acid, $F_{i,TG}$ in each sample (as the corresponding triglyceride) was calculated according to the following equation: Fatty $Acid_i$, $F_{i,TG} = (F_i \times CF_{TGi})$ where CF_{TGi} is the factor for conversion of fatty acid methyl esters to their corresponding triglycerides (Carpenter *et al.*, 1993).

The amount of each fatty acid, $F_{i,FA}$ in each sample (as the corresponding fatty acid) was calculated according to the following equation: Fatty $Acid_i$, $F_{i,FA} = (F_i \times CF_{FAi})$where CF_{FAi} is a factor calculated as follows:

$$CF_{FAi} = \frac{\text{Mol. wt. free fatty acid}_i}{\text{Mol. wt. fatty acid methyl ester}_i}$$

The amount of total fat (sum of all fatty acids) in each test sample expressed as triglycerides was calculated according to the following equation:

$$\%\text{Total Fat} = \left(\frac{\Sigma F_{i,TG}}{Wt_{sample}}\right) \times 100$$

The amount of saturated fat (sum of saturated fatty acids) in the test sample as the corresponding fatty acids was calculated according to the following equation:

$$\%\text{Sat. Fat} = \left(\frac{\Sigma \text{Sat. } F_{i,FA}}{Wt_{sample}}\right) \times 100$$

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

$$\%\text{Monounsat. Fat} = \left(\frac{\Sigma \text{Monounsat. } F_{i,FA}}{Wt_{sample}}\right) \times 100$$

The amount of polyunsaturated fat was calculated in an analagous manner. Total unsaturated fat was calculated as the sum of mono- and poly-unsaturated fatty acids.

Table 1. Determination of total fat by acid hydrolysis–capillary column GC and AOAC gravimetric methodologies

Product	GC method		AOAC grav. method	
	Fat, %	CV, %	Fat, %	CV, %
Waffles, fat-free	0.70 ± 0.04	6.1	1.05 ± 0.03	3.0
Apples/ham baby food	0.85 ± 0.01	1.1	1.21 ± 0.00	0.0
Chicken enchilada suiza	1.64 ± 0.04	2.6	1.64 ± 0.07	4.3
Melba toast, fat-free	1.96 ± 0.04	2.2	2.47 ± 0.21	8.5
Breakfast drink (powder)	1.99 ± 0.04	2.1	2.90 ± 0.23	8.0
Crackers, fat-free	2.11 ± 0.05	2.4	2.67 ± 0.07	2.6
Margarine, fat-free (1)	2.53 ± 0.05	2.0	2.48 ± 0.30	11.0
Spread, fat-free	2.56 ± 0.07	2.7	2.86 ± 0.06	1.9
Margarine, fat-free (2)	2.62 ± 0.02	0.8	2.55 ± 0.32	12.0
Chicken pie	3.41 ± 0.04	1.0	3.12 ± 0.27	8.6
Turkey/gravy/dressing meal	3.61 ± 0.16	4.3	3.61 ± 0.23	6.3
Beef ravioli in meat sauce	3.77 ± 0.11	3.0	4.13 ± 0.37	8.9
Infant formula (liquid)	4.08 ± 0.03	0.7	3.48 ± 0.06	1.8
Chili macaroni	4.21 ± 0.10	2.4	3.68 ± 0.33	8.8
Cheese crackers	4.29 ± 0.06	1.3	5.23 ± 0.24	4.5
Cereal with raisins	4.83 ± 0.39	8.1	6.88 ± 0.26	3.8
Oat cereal	6.24 ± 0.02	0.3	8.58 ± 0.10	1.2
Graham crackers, cinnamon	9.91 ± 1.11	11.2	11.55 ± 0.32	2.8
Potted meat product	10.86 ± 0.74	6.8	10.60 ± 0.38	3.6
Breaded fish fillets	11.20 ± 1.23	11.0	11.76 ± 0.29	2.4
Taco dinner	11.55 ± 0.98	8.4	11.55 ± 0.14	1.2
Chili without beans	12.15 ± 0.28	2.3	11.47 ± 0.11	0.9
Chocolate chip cookies (1)	13.22 ± 0.03	0.2	12.77 ± 0.12	0.9
SRM 1548, Total Diet	14.43 ± 0.48	3.3	19.94 ± 0.24	1.2
Infant formula powder (1)	16.44 ± 1.69	10.3	19.85 ± 0.09	0.4
Crackers	21.53 ± 0.97	4.5	22.03 ± 0.27	1.2
Blue cheese dressing	22.06 ± 0.92	4.2	20.19 ± 0.15	0.7
Peanut butter/grape crackers	22.28 ± 0.09	0.4	22.39 ± 0.12	0.5
Biscotti	23.44 ± 0.41	1.7	25.47 ± 0.45	1.8
Chocolate chip cookies (2)	23.56 ± 1.97	8.4	26.51 ± 0.57	2.1
SRM 1846, Infant Formula	25.82 ± 1.81	7.0	26.85 ± 0.18	0.7
Infant formula powder (2)	26.70 ± 1.98	7.4	26.04 ± 0.28	1.1
Peanut butter crack. sand.	29.47 ± 0.61	2.1	27.72 ± 0.71	2.6
Chicken-flavored crack.	30.55 ± 0.90	2.9	29.99 ± 0.02	0.1
Margarine, low-fat	31.61 ± 0.52	1.7	30.92 ± 0.82	2.7
Chocolate bar/almonds	36.04 ± 0.42	1.8	36.32 ± 0.18	0.5
Peanut butter, reduced fat	37.31 ± 0.65	1.7	32.56 ± 0.12	0.4
Margarine, light	43.98 ± 0.91	0.4	40.73 ± 0.04	0.1
Vegetable oil spread	63.92 ± 0.85	1.3	68.47 ± 1.55	2.3
Mayonnaise	78.32 ± 9.93	12.7	81.30 ± 0.46	0.6
Margarine	79.58 ± 2.25	2.8	80.22 ± 0.10	0.1
Margarine blend w butter	82.29 ± 4.14	5.0	77.35 ± 1.88	2.4
Canola oil	97.46 ± 4.71	4.8	96.72 ± 0.43	0.4

Values for % fat by acid hydrolysis–capillary column GC and AOAC gravimetric methodologies are means ± SD of two independent replications. Coefficient of variation (CV), % = (SD/mean)x100. Relative difference (RD), % can be calculated by the following formula: RD, % = [(GC)−(AOAC direct)/(AOAC direct)] x (100). The designations (1) and (2) for fat-free margarines, chocolate chip cookies, and infant formula powder are used to distinguish products from different manufacturers.

RESULTS AND DISCUSSION

Total fat in foods determined by the capillary column-GC method and an AOAC direct gravimetric method

The fat content of food products has traditionally been determined by gravimetric methodologies and existing databases for the fat content of many foods are based on such results. Before the new NLEA definition of fat, there was no requirement to quantitate fatty acids in food for labeling purposes. Hence, values for fat on food labels were also primarily determined by gravimetric measurement of ether extracts. For these reasons, data are needed that compare results based on the new definition of fat with results based on fat content obtained by traditional methodologies.

Table 1 shows the values for total fat in foods obtained by the capillary column-GC method and by AOAC direct gravimetric method 922.06 (Official Methods of Analysis, 1990*a*). Food products are listed in order of increasing total fat content as determined by the capillary column-GC method. The designations (1) and (2) for fat-free margarines, chocolate chip cookies and infant formula are used to distinguish products

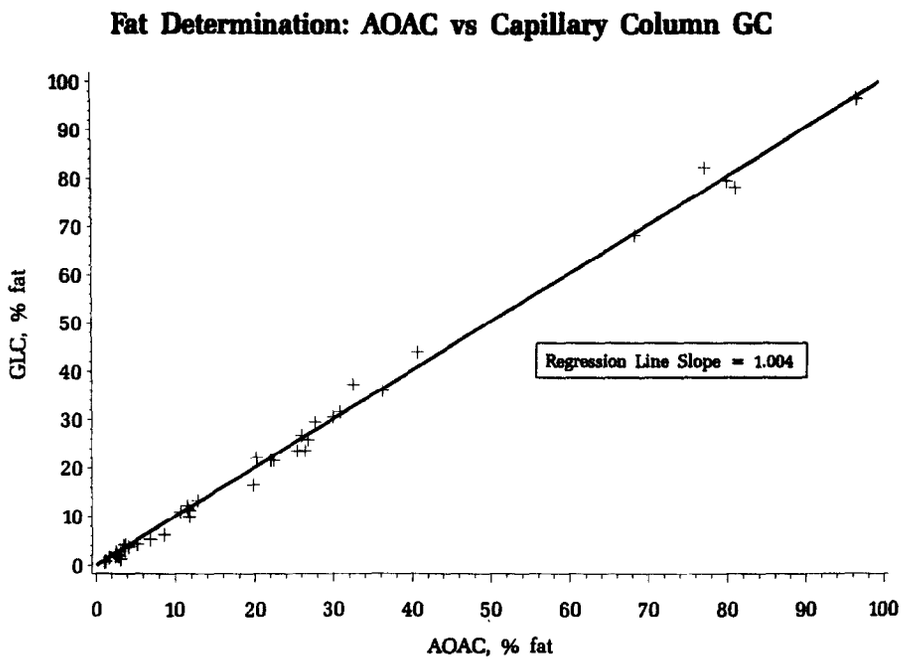

Fig. 1. Values for total fat determined by the acid-hydrolysis–capillary column GC methodology were regressed on values for crude fat obtained by AOAC gravimetric method 922.06. The resulting line, forced through the origin, had a slope of 1.004. Each value represents the mean of two independent determinations.

from different manufacturers. For some products (e.g. waffles, fat-free Melba toast, breakfast drink (powder)), total fat content determined by the GC method was about 20–30% lower than total fat content of the same food samples determined by AOAC gravimetric method 922.06. Relative differences between the two methods [i.e., relative difference, % = [(GC)-(AOAC direct)/(AOAC direct) ×(100)] declined markedly with increasing fat content, and for many foods containing < 4% fat (w/w), the relative differences were < 6%.

With the exception of the fat-free waffles and the cereal product with raisins, coefficients of variation (CV) for duplicate determinations by the GC method in foods containing less than 5% fat were < 5%. CVs for determination of total fat by the AOAC method in most foods containing less than 5% fat were > 5% (Table 1).

Duplicate analysis by the AOAC method for the two fat-free margarine products gave CVs of 11–12%, values that were higher than those observed for other products of lower fat content (Table 1). However, duplicate analysis by the acid hydrolysis-GC method for the same products gave CVs of 1–2%.

Although the relative differences between the two methods were very large for products of low fat content (1–3% fat by weight), the absolute differences were quite small. Expressing the data as relative percent differences emphasizes the differences between the methods.

The values obtained by the two methodologies for all food products were subjected to linear regression analysis (Fig. 1). The slope of the resulting regression line was 1.004.

Figure 2a,b are typical chromatograms obtained for FAMEs prepared from a fat-free low-calorie spread and a light margarine, analyzed on the 100 m SP-2560 fused silica capillary GC column after acid hydrolysis, extraction, and methylation. Figure 2a shows the profile of FAMEs prepared from the fat-free-low-calorie spread. Total fat content of the product determined by the GC method was 2.56%. Figure 2b shows the profile of FAMEs prepared from the light margarine product which contained about 44% total fat. The complexity of the area around *c*-18:1 and *t*-18:1 in this product is apparent.

Figure 3a,b represent chromatograms obtained for FAMEs prepared from fat-free crackers and breaded fish fillets, respectively. Total fat contents of the fat-free crackers and breaded fish fillets were 2 and 11%, respectively. Peaks of interest are identified according to the chain length and number of double bonds present in the FAMEs. Although the *cis*- and *trans*-18:1 isomers in samples shown in Fig. 2b and Fig. 3b were not completely resolved, baseline resolution on the SP-2560 column was routinely achieved for mixtures of methyl elaidate (*t*-18:1) and methyl oleate (*c*-18:1) as well as for other pairs of geometric isomers. This observation is in agreement with results of Ratnayake & Beare-Rogers (1990).

The conversion of FAMEs to their triglyceride equivalents for purposes of calculating total fat and saturated fat content according to the new NLEA definition is straightforward, since the summation of all peaks represents total fat and the summation of all saturated fatty acid peaks represents saturated fat.

Saturated fatty acids

The total saturated fat content (% saturated fat as fatty acids (w/w)) and distribution of these saturated fatty acids (expressed as mg fatty acid/g product) in the food

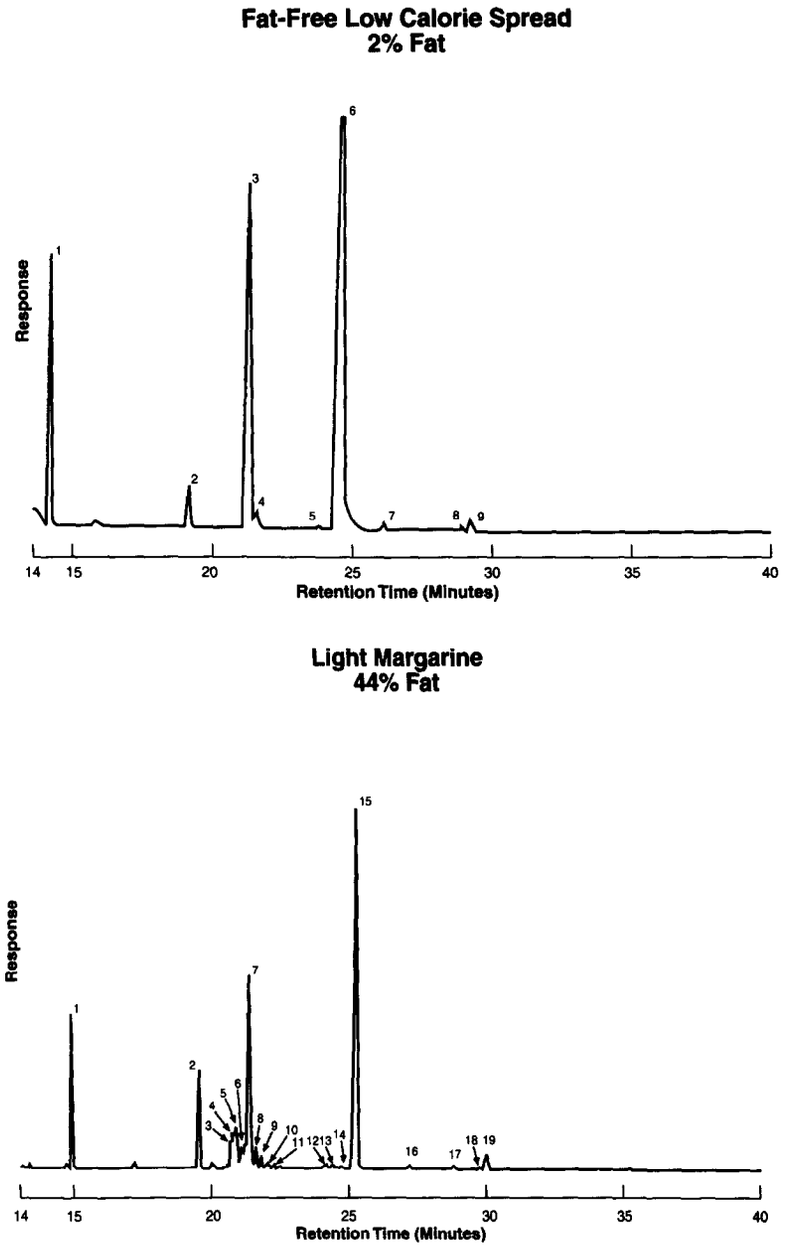

Fig. 2. The C_{16}, C_{18}, and C_{20} regions of the gas chromatograms (SP-2560 flexible fused silica capillary column, 100 m ×0.25 mm i.d.) of (a: top) fat-free low calorie spread (about 2% fat). Numbers correspond to 1, 16:0; 2, 18:0; 3, *c*-18:1; 4, *c*-18:1Δ10; 5, 18:2Δ9*c*,12*t*; 6, *c*-18:2; 7, 20:0; 8, *c*-20:1; 9, *c*-18:3; (b: bottom) light margarine (about 44% fat). Numbers correspond to 1, 16:0; 2, 18:0; 3, 18:1Δ6-8*t*; 4, *c*-18:1Δ9*t*; 5, 18:1Δ10*t*; 6, 18:1Δ11*t*; 7, 18:1Δ9*c*; 8, 18:1Δ10*c*; 9, 18:1Δ11*c*; 10, 18:1Δ12*c* +18:1Δ15*t*; 11, 18:2*tt*; 12, 13, 14, 18:2*tc*/*ct*; 16, 20:0; 17,?; 18, 20:1; 19, 18:3.

samples are shown in Table 2. Saturated fat is defined for food labeling purposes as the sum of all fatty acids containing no double bonds expressed as fatty acids. The sums of individual saturated fatty acids may differ from the value found in the % saturated fat column because of the presence in some products of unknown fatty acids for which standards are not available. In general, the fatty acids found in the products were consistent with fats or oils identified in their ingredients lists.

The simplest distribution of saturated fatty acids was that found in the fat-free waffles. The product contained 0.19% saturated fat present only as C14:0 (1.82 mg/g) and C18:0 (0.16 mg/g) fatty acids. The highest saturated fat content (16.64%) was found in a chocolate bar with almonds. The predominant fatty acids were C16:0 and C18:0 found at levels of 76.69 and 84.54 mg/g, respectively.

Fatty acid C6:0 was found only in the peanut butter/grape crackers and in one type of chocolate chip cookie (chocolate chip cookies (2), Table 2) at levels of 4.66 and 6.50 mg/g, respectively. Coconut and butter fat, rich sources of C6:0, are included in ingredient lists for these products.

Fatty acids C10:0 and C12:0 were found more commonly in the products than were fatty acids C6:0 and

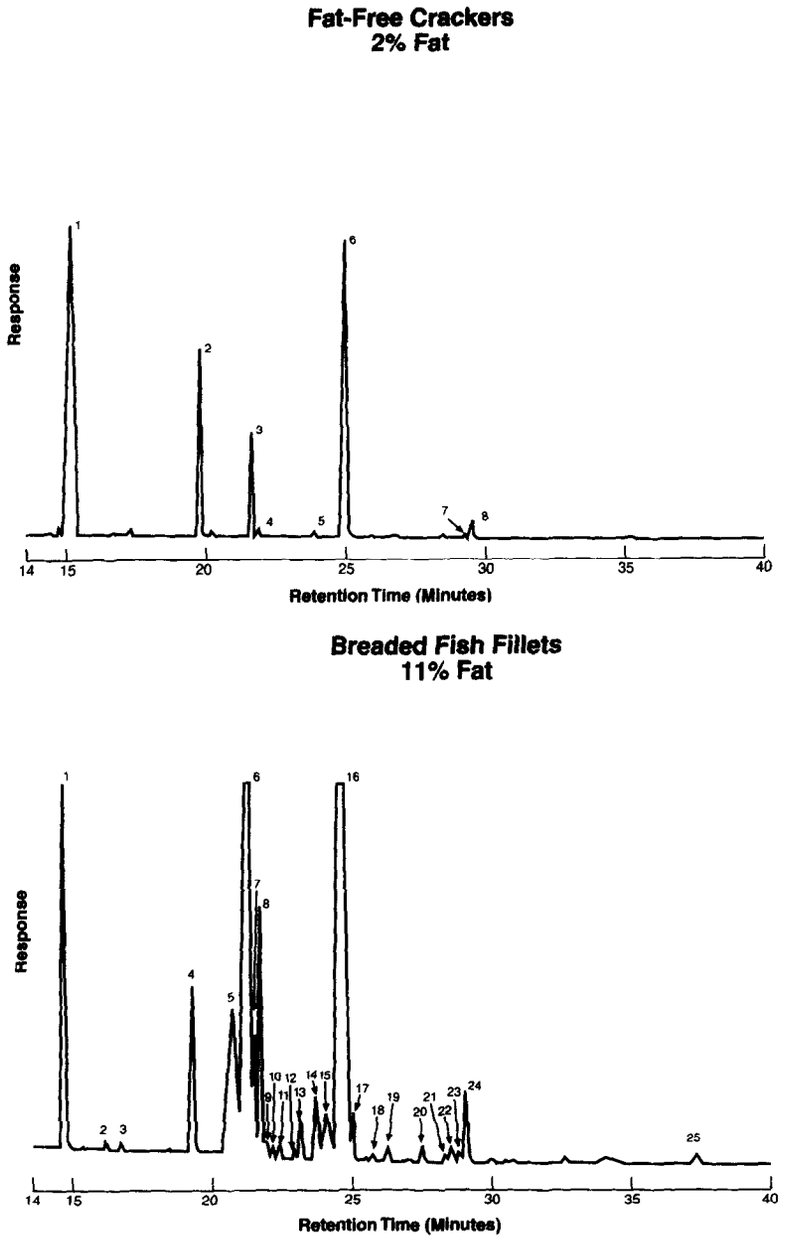

Fig. 3. The C_{16}, C_{18}, and C_{20} regions of the gas chromatograms (SP-2560 flexible fused silica capillary column, 100 m ×0.25 mm i.d.) of (a: top) fat-free crackers (about 2% fat). Numbers correspond to 1, 16:0; 2, 18:0; 3, 18:1*c*; 4, *c*-18:1Δ10*c*; 5, 18:2*tc*/*ct*; 6, 18:2Δ9*c*; 7, 20:1; 8, 18:3Δ9*c*,12*c*,15*c*; (b: bottom) breaded fish fillets (about 11% fat). Numbers correspond to 1, 16:0; 2, 16:1*c*; 3, 17:0; 4, 18:0; 5, 18:1*t*; 6, 18:1*c*; 7, 18:1Δ10*c*; 8, 18:1Δ11*c*; 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; 20, 21, 22,?; 23, 20:1; 24, 18:3Δ9, 12*c*,15*c*; 25, 22:0.

C8:0. The ingredients butter fat, coconut oil, and palm oil are likely sources of these fatty acids. Fatty acids C10:0 and C12:0 were present at levels of 0.07 to 5.48 and 0.09 to 50.50 mg/g, respectively. Ingredient lists for two products (infant formula powder (1) and crackers) included coconut and palm oils. The crackers contained 5.48 and 50.50 mg/g of C10:0 and C12:0, respectively, compared with levels of 2.04 and 17.09 mg/g of C10:0 and C12:0, respectively, in infant formula powder (1).

Fatty acid C14:0 was found in low-fat margarine and crackers at levels of 0.08 and 20.96 mg/g, respectively. Coconut oil and soybean oil, good sources of C14:0 and are included in the ingredient lists for these products.

Fatty acid C15:0 was found at levels of 0.05 to 1.26 mg/g in 14 of 43 food products examined. This fatty acid is widely distributed in animal fat products such as butterfat, lard, and tallow (beef and mutton) (White, 1992) which were identified in the products' ingredient lists.

Fatty acids C16:0, C18:0 and C20:0 were found in all food products except the fat-free waffle, which did not contain fatty acids C16:0 and C20:0. The levels of fatty acids C16:0, C18:0, and C20:0 in the products tested were 1.98 to 91.78 mg/g (C16:0), 0.16 to 84.54 mg/g

Table 2. Distribution of saturated fatty acids in food products[a]

Food	Sat. fat %	6:0 mg/g	8:0 mg/g	10:0 mg/g	12:0 mg/g	14:0 mg/g	15:0 mg/g	16:0 mg/g	17:0 mg/g	18:0 mg/g	20:0 mg/g	22:0 mg/g	24:0 mg/g
Waffles, fat-free	0.19	—	—	—	—	1.82	—	—	—	0.16	—	—	—
Apple/ham baby food	0.32	—	—	—	—	0.12	—	1.98	0.16	0.97	0.11	—	—
Chicken enchilada suiza	0.60	—	—	0.14	0.19	0.75	0.05	3.72	0.15	1.24	0.05	—	—
Melba toast, fat-free	0.50	—	—	—	—	—	—	4.42	—	0.78	0.04	—	—
Breakfast drink (powder)	0.99	—	—	0.08	0.11	0.33	—	4.77	0.07	4.96	0.16	—	—
Crackers, fat-free	0.98	—	1.27	0.28	—	—	0.17	4.15	0.28	3.88	0.12	0.18	—
Margarine, fat-free (1)	0.32	—	—	—	—	—	—	2.16	—	1.02	0.10	—	—
Spread, fat-free	0.37	—	—	0.33	—	—	—	2.86	—	0.55	0.19	—	—
Margarine, fat-free (2)	2.46	—	—	0.07	0.12	0.40	0.17	11.16	—	13.86	0.12	—	—
Chicken pie	0.91	—	—	—	—	0.11	—	4.84	0.08	4.46	0.12	—	—
Turkey/gravy/dressing meal	0.93	—	—	—	—	0.22	—	6.02	0.11	3.39	0.08	—	—
Beef ravioli/meat sauce	1.74	—	—	—	0.09	1.27	0.23	10.21	0.56	5.55	0.05	—	—
Infant formula (liquid)	2.03	—	6.20	1.15	6.34	2.62	—	3.80	—	1.57	0.09	—	—
Chili macaroni	1.44	—	—	—	—	0.51	—	7.14	0.29	4.09	0.10	—	—
Cheese crackers	1.61	—	—	0.73	0.54	1.87	0.21	10.15	0.16	3.38	0.10	0.11	—
Cereal with raisins	1.27	—	0.40	0.18	1.40	0.75	—	8.93	0.03	1.52	0.10	—	—
Oat cereal	1.27	—	—	—	—	0.16	—	11.73	0.02	0.88	0.09	—	—
Graham crackers, cinnamon	1.87	—	—	—	—	0.09	—	11.56	—	7.24	0.42	0.30	—
Potted meat product	4.27	—	—	—	—	1.77	0.24	26.06	0.74	14.62	0.19	0.18	–
Breaded fish fillets	2.01	—	—	0.58	0.13	0.14	—	12.86	0.13	5.31	0.35	—	0.58
Taco dinner	1.91	—	—	—	—	0.13	—	10.37	0.13	8.57	0.40	0.29	0.22
Chili without beans	5.18	—	—	—	—	4.09	0.68	30.22	1.56	16.44	0.11	0.09	—
Chocolate chip cookies (1)	4.87	—	—	—	—	0.18	—	23.83	0.22	25.63	0.85	0.37	—
SRM 1548, Total Diet	8.04	—	4.23	1.45	1.63	6.23	0.80	42.67	1.15	22.32	0.52	0.37	0.28
Infant formula powder (1)	7.19	—	2.92	2.04	17.09	7.53	—	38.85	0.13	5.84	0.45	0.25	0.19
Crackers	11.25	—	7.28	5.48	50.50	20.96	—	23.67	0.12	11.05	0.46	—	0.13
Blue cheese dressing	4.16	—	—	0.93	0.46	1.62	—	27.25	—	12.56	0.73	—	—
Peanut butter/grape crack.	9.63	4.66	1.11	2.43	3.18	10.60	1.26	42.18	0.81	21.82	2.72	7.65	2.10
Biscotti	9.81	—	0.92	1.72	2.28	8.73	0.92	50.06	0.78	35.65	0.96	0.17	—
Chocolate chip cookies (2)	13.62	6.50	2.71	3.64	11.70	14.41	1.15	54.09	0.95	44.82	1.10	0.25	0.18
SRM 1846, Infant Formula	12.39	—	17.88	4.87	36.97	15.67	—	28.17	0.22	27.03	0.76	0.24	0.20
Infant formula powder (2)	11.56	—	15.08	3.62	30.64	15.97	0.49	35.52	1.10	19.29	0.67	—	—
Peanut butter crack. sand.	6.57	—	—	0.28	0.41	1.70	0.20	33.99	0.41	21.81	1.95	3.66	2.75
Chicken-flavored crack.	6.47	—	—	—	—	—	—	34.89	—	30.46	1.22	1.11	—
Margarine, low-fat	4.17	—	—	—	—	0.08	—	17.83	0.08	13.81	1.87	0.50	0.40
Chocolate bar/almonds	16.64	—	—	2.40	1.53	5.63	0.59	76.69	0.87	84.54	2.48	—	—
Peanut butter/reduced fat	7.80	—	—	—	—	—	—	36.80	—	16.47	5.71	13.64	9.22
Margarine, light	6.72	—	—	—	—	0.35	—	59.41	0.96	31.84	1.78	—	—
Vegetable oil spread	11.67	—	—	—	—	—	—	57.88	0.54	55.60	2.19	3.34	1.61
Mayonnaise	11.50	—	—	—	—	0.58	—	77.57	0.85	33.52	2.52	2.74	1.15
Margarine	13.93	—	—	—	—	0.60	—	84.23	0.87	55.88	2.45	2.55	—
Margarine blend w butter	16.40	—	—	1.28	1.51	5.45	—	91.78	1.34	64.84	2.57	2.79	—
Canola oil	8.90	—	—	—	—	—	—	37.96	—	17.45	20.51	3.01	—

[a]Values are means of 2 determinations. Saturated fat was calculated as fatty acids and expressed as percent by weight of product. Individual fatty acids are expressed as mg fatty acid/g product. The sum of individual fatty acids may differ from the value found in the 'sat. fat, %' column because of the presence in some products of unknown fatty acids for which standards are not available. The designations (1) and (2) for fat-free margarines, chocolate chip cookies, and infant formula powder are used to distinguish products from different manufacturers.

(C18:0), and 0.04 to 20.51 mg/g (C20:0). Biscotti, chocolate chip cookies (2), chocolate bar with almonds, and margarine blend with butter were high in C16:0 and C18:0, with ingredients cocoa butter and butter fat the likely sources.

Fatty acid C22:0 was found in the range of 0.11–13.64 mg/g in 22 of the 43 products. Sources of fatty acid C22:0 are peanut oil and (possibly) soybean oil (White, 1992). C22:0 was present at levels of 7.65 and 13.64 mg/g in the peanut butter/grape crackers and reduced fat peanut butter, respectively. The vegetable oil spread, which contained sunflower, partially hydrogenated soybean and cottonseed oils, contained C22:0 at a concentration of 3.67 mg/g.

Fatty acid C24:0 was found in 14 of 43 products. Like fatty acid C22:0, C24:0 is found at high levels in peanut oil (White, 1992). Fatty acid C24:0 was present at levels of 0.13 and 9.22 mg/g in the cracker product and in reduced-fat peanut butter, respectively.

Table 3. Distribution of unsaturated fatty acids in food products[a]

Food	Unsat. fat%	14:1 mg/g	15:1 mg/g	16:1		17:1 mg/g	18:1		18:2		18:3 mg/g	20:1 mg/g	22:1 mg/g
				c	t		c	t	cc	tt			
				mg/g			mg/g		mg/g				
Waffles, fat-free	0.52	—	—	—	—	—	1.02	—	3.73	—	0.23	—	—
Apple/ham baby food	0.55	—	—	0.32	—	—	3.62	—	1.03	—	—	—	—
Chicken enchilada suiza	1.05	0.07	—	0.33	—	—	5.42	—	4.11	—	0.32	—	—
Melba toast, fat-free	1.47	—	—	0.16	—	—	0.19	2.97	10.25	—	0.82	0.08	—
Breakfast drink (powder)	1.01	—	—	0.07	—	—	5.95	—	2.96	—	0.43	—	—
Crackers, fat-free	1.15	—	—	—	—	—	1.87	—	8.20	—	0.53	—	—
Margarine, fat-free (1)	2.22	—	—	—	—	—	7.56	—	11.46	—	2.56	—	—
Spread, fat-free	2.20	—	—	—	—	—	6.34	—	15.03	—	0.31	—	—
Margarine, fat-free (2)	0.18	—	—	—	—	—	—	0.40	0.14	—	—	—	—
Chicken pie	2.56	—	—	0.07	—	—	9.07	7.11	3.08	0.61	0.22	—	—
Turky/gravy/dressing meal	2.69	—	—	0.58	—	—	10.46	4.74	7.37	0.24	0.42	0.07	0.26
Beef ravioli/meat sauce	2.31	0.29	—	1.24	0.11	0.73	14.49	1.60	2.32	0.06	0.26	0.11	—
Infant formula (liquid)	2.07	—	—	—	—	—	5.46	—	11.91	—	1.55	—	—
Chili macaroni	3.06	0.17	—	0.72	0.06	—	11.77	0.96	12.59	0.07	2.13	0.07	—
Cheese crackers	2.73	0.56	—	0.26	—	—	10.13	4.86	7.71	—	0.50	—	—
Cereal with raisins	3.56	—	—	0.07	—	—	12.49	6.84	10.28	0.11	0.43	0.12	—
Oat cereal	5.01	—	—	0.12	—	—	23.88	—	24.19	—	0.89	0.50	—
Graham crackers, cinnamon	7.81	—	—	—	—	—	28.14	28.61	7.21	1.14	0.53	—	—
Potted meat product	6.73	0.17	—	4.68	0.71	0.54	42.38	2.29	10.08	0.17	0.85	0.77	0.95
Breaded fish fillets	9.31	—	—	0.14	—	—	29.27	9.97	32.41	0.30	2.63	0.25	—
Taco dinner	9.69	—	—	0.08	—	—	38.47	30.52	13.12	0.07	0.38	—	—
Chili without beans	7.05	1.02	—	4.87	0.59	1.64	47.23	5.78	4.39	—	0.45	0.22	0.12
Chocolate chip cookies(1)	8.42	0.09	—	0.24	—	—	43.07	20.06	10.04	1.63	0.91	—	—
SRM 1548, Total Diet	6.42	0.35	—	2.38	0.55	0.54	37.89	6.98	2.41	—	0.46	0.31	—
Infant formula powder (1)	9.38	—	—	0.22	—	—	51.38	0.48	31.77	0.13	2.58	0.30	—
Crackers	10.39	0.20	—	0.09	—	—	39.58	26.95	16.05	0.81	1.82	0.35	0.04
Blue cheese dressing	18.09	—	—	—	—	—	50.57	8.00	97.68	—	14.62	—	—
Peanut butter/grape crack	12.01	0.89	—	1.86	0.72	0.39	62.89	2.07	41.19	0.09	2.00	0.16	0.20
Biscotti	13.78	0.64	—	1.88	0.42	—	70.43	4.30	43.36	0.31	7.14	—	0.21
Chocolate chip cookies(2)	8.57	0.78	—	1.35	1.63	0.67	55.58	4.54	8.30	0.44	0.86	0.13	0.30
SRM 1846, Infant Formula	13.17	—	—	0.13	—	—	46.07	36.37	30.97	0.47	0.86	—	—
Infant formula powder(2)	15.28	0.61	—	2.72	—	1.05	102.51	3.55	31.74	—	3.09	—	—
Peanut butter crack. sand.	23.11	0.21	—	0.40	—	—	115.67	39.46	44.85	0.28	0.84	1.68	—
Chicken—flavored crack.	28.77	—	—	—	—	—	100.30	105.85	12.63	3.38	—	0.77	—
Margarine, low—fat	27.78	—	—	0.29	—	—	173.01	11.11	59.32	0.61	24.12	6.53	0.63
Chocolate bar/almonds	19.30	0.39	—	1.75	—	—	149.27	0.84	31.09	—	0.79	—	—
Peanut butter/reduced fat	29.68	—	—	—	—	—	180.61	—	106.44	—	5.93	—	—
Margarine, light	37.46	—	—	—	—	—	93.20	44.60	208.62	—	6.96	—	—
Vegetable oil spread	54.90	—	—	0.29	—	—	138.84	97.34	275.95	—	6.56	—	—
Mayonnaise	67.18	—	—	0.86	—	—	170.60	—	417.28	—	76.35	—	—
Margarine	66.02	—	—	0.47	—	—	189.29	92.34	286.12	—	53.20	—	—
Margarine blend w butter	66.27	—	—	0.90	—	—	198.61	132.99	225.08	2.64	39.48	—	—
Canola oil	89.40	—	—	2.07	—	—	558.49	0.97	205.11	—	98.35	14.53	6.71

[a]Values (% unsaturated fat by weight as fatty acids, or mg fatty acid/g product) are means of 2 determinations. Trans fatty acids t-16:1, t-18:1, and t,t-18:2 were identified by comparisons of their retention times with those of known standards and quantitated. The sums of individual unsaturated fatty acids may differ from the value found in the 'unsat. fat, %' column because of the presence in some products of unknown cis, trans; cis, cis; cis, trans; trans, trans fatty acids for which standards are not available. The designations (1) and (2) for fat-free margarines, chocolate chip cookies, and infant formula powder are used to distinguish products from different manufacturers.

Cis fatty acids

Fatty acid C15:1 was not found in any of the food products examined (Table 3). Fatty acids C14:1 and C17:1 were found in the foods at levels of 0.07–1.02 and 0.39–1.64 mg/g, respectively.

Both *cis* and *trans* isomers of C16:1 were found in this investigation, with the *cis* isomer found in 77% of products and the *trans* isomers found in 19% of the products. These isomers were present at levels of 0.07 to 4.87 mg/g and 0.06 to 1.63 mg/g for *c*-16:1 and *t*-16:1, respectively. The meat products (potted meat product and chili without beans) had the highest levels of *c*-16:1 (4.68-4.87 mg/g). Chocolate chip cookies product (2) had the highest level of *t*-16:1 (1.63 mg/g). Fatty acid *t*-16:1 was less common in food products than the *t*-18:1 isomer and the *t,t*-18:2 isomer.

Fatty acids *c*-18:1, *c*-18:2, and *c*-18:3 were found in the majority of foods analyzed. The ranges for monenoic (*c*-18:1), dienoic (*c*-18:2), and trienoic (*c*-18:3) acids were 1.02 to 558.49, 0.14 to 417.28, and 0.22 to 98.35 mg/g, respectively.

Canola oil contained the highest levels of fatty acids C22:1 and C20:1. Concentrations found were 6.71 and 14.53 mg/g for C22:1 and C20:1, respectively.

Trans fatty acids

Trans fatty acid content of the foods varied widely. Three margarine products whose ingredient lists included partially hydrogenated vegetable oils (soybean, corn and/or canola) contained *trans* fatty acid *t*-18:1 at levels of 44.06–132.99 mg/g (Table 3). The principal *trans* fatty acids found in this investigation were *t*-18:1 fatty acids present at levels of 0.40 to 132.99 mg/g in 70% of the products. *Trans* fatty acid C18:2Δ9*t*,12*t* was found at levels of 0.06–2.64 mg/g in 44% of the products. Among the 18:2 *trans* isomers, only isomer *t*,*t*-18:2 was quantitated in this study because of the availability of the appropriate standard (i.e., C18:2Δ9*t*,12*t*). The 18:2*c*,*t* and 18:2*t*,*c* isomers, for which standards are not readily available, are eluted from the column after 18:2*t*,*t* (Ratnayake, 1995). The elution positions of these isomers are shown in Fig. 2b and Fig. 3b but, because of the lack of standards, we could not quantitate them.

Standard reference materials

The results of analysis of SRM 1846 are reported in Table 4 with packed column-GC data (Rader *et al.*, 1995) shown for comparison. The value for total fat derived by quantitation of FAMEs (24.7%) agreed well with the Certificate of Analysis value (27.7%) and with the value obtained by the AOAC direct gravimetric analysis (26.9%). Values for unsaturated fatty acids 18:1 and 18:2 were lower in samples analyzed by the acid hydrolysis-capillary column GC method than in samples analyzed by the acid hydrolysis-packed column-GC methodology.

For SRM 1548 total diet, total fat determined by the AOAC direct method (19.9 and 20.8%, determined in two studies) agreed closely with the NIST certified value of 20.6 ± 2%. Calculation of total fat after acid hydrolysis and capillary column GC separation of FAMEs

Table 4. Determination of total fat and distribution of fatty acids in SRMs 1846 and 1548

Composition	SRM 1846[a] Infant Formula			SRM 1548[b] Total Diet	
	Cert mg/g	Study 1	Study 2	Study 1	Study 2
		GC column type		GC column type	
		Packed mg/g	Capil. mg/g	Packed mg/g	Capil mg/g
Saturated fat					
6:0	0.00	—	—	—	—
8:0	6.81	—	17.88	—	4.23
10:0	5.40	2.33	4.87	0.16	1.45
12:0	41.27	30.98	36.97	1.31	1.63
14:0	17.51	14.38	15.67	5.16	6.23
15:0	—	—	—	—	0.80
16:0	32.96	27.88	28.17	35.06	42.67
17:0	—	—	0.22	—	1.15
18:0	32.13	26.38	27.03	17.78	22.32
20:0	0.86	0	0.76	0	0.52
22:0	—	0.63	0.24	4.56	0.37
24:0	—	—	0.20	0	0.28
Unsaturated fat					
14:1	0.00	0.09	0.00	1.05	0.35
16:1	0.44	0.47	0.13	3.42	2.93[c]
17:1	—	—	0.00	—	0.54
18:1	96.67	97.71	82.44[c]	47.53	44.87[c]
18:2	40.72	35.51	31.44[c]	3.81	2.41
18:3	0.97	0.85	0.86	0.39	0.46
20:1	0.00	0.40	—	0.31	0.31
22:1	—	0.05	—	0	0
SUM mg/g	275.75	237.66	246.88	120.54	133.52
Fat, %	27.6	23.8	24.69	12.1	13.35
AOAC direct, Fat, %	No value	27.0	26.9	20.8	19.94

[a]Fatty acid composition of SRM 1846 was determined by Analytical Systems Research Corp., Indianapolis, IN. A value for total fat determined by a gravimetric method was not reported. The value of 27.6% fat represents the sum of the fatty acids found.
[b]Portions of the Infant Formula and Total Diet reference materials were analyzed by AOAC direct gravimetric method 922.06, the acid hydrolysis-packed column GC method reported previously (Study 1; Rader *et al.*, 1995), and by the acid hydrolysis-capillary column GC method (Study 2). Values represent the means of two (Study 2) or 4 (Study 1) independent analyses of each material. Three gravimetric methods were used to obtain the NIST Certificate of Analysis value of 20.6 ± 2.0% fat for SRM 1548.
[c]Values represent the sums of cis and trans isomers listed in Table 3.

gave values that were significantly lower than the gravimetrically determined value, but in good agreement with corresponding calculations performed after packed column GC separation of FAMEs. National Institute of Standards and Technology (NIST) scientists have speculated that reduction in the levels of fatty acids in SRM 1548 may have resulted from the ^{60}cobalt radiation sterilization used to prevent bacterial growth in this SRM (Rader *et al.*, 1995). Irradiation may have altered some fatty acid components in SRM 1548 in such a way that the resultant material remained quantifiable by gravimetric methods but not by methods dependent upon the release and methylation of fatty acids. These uncertainties limit the use of SRM 1548 when quantifying fatty acids.

SUMMARY AND CONCLUSIONS

The literature analyzing the fatty acid composition of foods in the general food supply is limited (Ratnayake *et al.*, 1991, 1993). This study was conducted to extend our knowledge of the distribution of saturated and unsaturated fatty acids in commonly consumed foods. More complete data on amounts of *trans*-unsaturated fatty acids and *cis*, *cis*-methylene-interrupted diene fatty acids in foods are needed because of the known or suspected nutritional effects of these fatty acids.

The foods included in this study were non-dairy products and contained fatty acids of 10–24 carbon chain lengths. Dairy products, which contain short chain volatile fatty acids that require preparation and analysis of butyl esters because of volatility and solubility problems, were not studied here. The acid hydrolysis methodology followed by SP-2560 capillary column-gas chromatography of methyl esters described above allows extraction and quantitation of many fatty acids. AOAC method 990.27 Butyric acid in fats containing butter fat-GC chromatographic method (Official Methods of Analysis, 1995) is a validated method for the quantitative determination of butyric acid and could be used for products containing the lower chain length fatty acids.

Numerous fatty acid isomers are present in hydrogenated vegetable oils which are ingredients in the majority of products tested. Suitable standards for all of these isomers are not readily available. In addition, satisfactory separation of all *trans* isomers from *cis* isomers in the 18:1 region is not possible on the SP-2560 column. Because of overlap in elution of *cis* and *trans* isomers in the 18:1 region, the direct capillary column-GC method underestimates total *t*-18:1 in favor of the *cis* isomers. Ratnayake (1995) recently published the results of a collaborative study of an infrared spectrophotometric method and a combined GC chromatographic/infrared spectrophotometric method that addresses this problem.

The fatty acids *t*-16:1, *t*-18:1, and *t,t*-18:2 were quantified in this study. Of the 43 food products analyzed, 70% contained measurable levels of *t*-18:1. *Trans* fatty acid content (e.g. the sum of *t*-16:1, *t*-18:1, and *t,t*-18:2) as percent of total fat content was calculated and ranged from 1.97% for a cookie (biscotti) to 30.56% for cinnamon Graham crackers. An intermediate value of 16.48% *trans* fat was found for a margarine product.

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