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Determination of total fat and saturated fat in foods by packed column gas–liquid chromatography after acid hydrolysis

Jeanne I. Rader*, Gerald Angyal, Roger G. O'Dell, Carol M. Weaver, Alan J. Sheppard & Martin P. Bueno

Food and Drug Administration, Office of Food Labeling, Center for Food Safety and Applied Nutrition, 200 C Street, S.W., Washington DC 20204, USA

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The new definition of total fat in FDA regulations implementing the Nutrition Labeling and Education Act of 1990 necessitates the quantitation of all lipid fatty acids and the summation of their triglyceride equivalents. A gas–liquid chromatographic (GLC) method using a packed column has been developed for quantitative measurement of total fat and saturated fat in foods. Fatty acids are released from food matrices by acid hydrolysis, and then extracted, esterified to their methyl esters and determined by GLC. Total fat and saturated fat are calculated in accordance with the new definitions of these components. Fat content determined by the acid hydrolysis–GLC methodology was compared with fat content determined by a direct AOAC gravimetric method for 23 food products containing between about 1 and 75% fat by weight. For all food products studied, the relationship between the results obtained by the two methods was best described by a straight line that had a correlation coefficient of 0.94. Results of repeated extractions and analysis of a milk-based infant formula (SRM 1846) suggest that this material may be useful as a quality control standard.

INTRODUCTION

The Nutrition Labeling and Education Act of 1990 (NLEA) mandated that nearly all processed foods be labeled with information about their nutrient content. The US Food and Drug Administration (FDA), in responding to the NLEA, defined 'total fat' for labeling purposes as the sum of fatty acids (i.e. total lipid fatty acids) expressed as triglyceride; 'saturated fat' as the sum of all fatty acids containing no double bonds expressed as fatty acids; 'polyunsaturated fat' as *cis*, *cis*-methylene-interrupted polyunsaturated fatty acids, expressed as fatty acids; and 'monounsaturated fat' as *cis*-monounsaturated fatty acids expressed as fatty acids (Code of Federal Regulations, 1994). The new food labels are required to list fat and saturated fat content, whereas labeling for polyunsaturated fat and monounsaturated fat is voluntary. At this time, *trans* fatty acids are included in the definition for total fat but are not included in the definition for monounsaturated or polyunsaturated fat. The requirement that total fat be calculated as the sum of lipid fatty acids from all sources, expressed as triglyceride, points to the need for appropriate methods which will ensure that individual fatty acids will be quantitatively extracted and recovered, and accurately measured.

A number of methods are available for gravimetric analysis of fat or crude fat in a variety of food matrices. In addition, a number of techniques exist for extraction of fatty acids from foods. For example, foods may be subjected to acid or alkaline hydrolysis before ether extraction. After extraction, the fatty acids may be converted to their methyl esters (FAMEs), which are well resolved by gas–liquid chromatography (GLC) either on packed columns (Official Methods of Analysis, 1990*b*) or capillary columns (House *et al.*, 1994). Carpenter *et al.* (1993) have provided an extensive discussion of fat methodology that includes consideration of extraction methods, acid and alkaline hydrolysis methods, and methods specific for fatty acids.

The fat or crude fat content of food products has traditionally been determined by methodologies that involve extraction with organic solvents (e.g. ether, hexane, etc.), drying of the extract and gravimetric determination of the fat (Carpenter *et al.*, 1993). Existing databases for the fat content of many foods are based on results obtained by use of such methodologies. In addition, before the new definition of fat, there was

*To whom correspondence should be addressed.

no requirement to quantitate fatty acids in foods for labeling purposes. Hence, values for fat on food labels were also primarily determined by gravimetric measurement of ether extracts. Therefore, data are needed that compare results based on the new definition of fat with results based on fat content obtained by traditional methodologies.

In this report we describe an acid hydrolysis method for extraction of fatty acids from foods followed by packed column GLC that addresses the new definitions of total fat and saturated fat in the NLEA. We also report a comparison for all foods analyzed of the results for fat obtained by the acid hydrolysis–packed column GLC methodology with results obtained by an AOAC direct gravimetric method.

The ability of the acid hydrolysis–packed column GLC combination to obtain values for total fat also was determined with Standard Reference Material (SRM) 1548 (Total Diet) and SRM 1846, an infant formula reference material. The use of the infant formula reference material as a quality control standard for determination of saturated fat also is reported.

MATERIALS AND METHODS

Preparation of foods

Twenty-three food products with fat contents (calculated from label declarations) between about 1 and 75% (w/w) were purchased locally. Products included breakfast foods (e.g. dry cereals, powdered breakfast drink, waffles), meat-containing products (e.g. ravioli, fish fillets, chicken), meal-type products (e.g. taco dinner, turkey dinner), snack foods (e.g. cookies, pretzels, sandwich crackers, snack crackers, chocolate bar), pudding, a powdered infant formula, and mayonnaise. Foods were blended (composited, homogenized) in a dual-speed food processor (Osterizer) and stored in tightly sealed glass containers. Composites were stored frozen (meal-type products, meat-containing products, mayonnaise, pudding, chocolate bar) or at room temperature (dry foods) until analyzed.

Determination of dry matter

Test portions of composited food products were weighed (2 g, or 4 g of low-or no-fat product) on an analytical balance into a tared weighing vessel. The test portions were dried overnight to a constant weight in a vacuum oven (60° C, 26 in. Hg). The difference between initial weight and final weight was estimated to be the water in the products and the final weight was estimated to be the dry matter.

Fatty acid standards

Fatty acid methyl ester (FAME) standards were prepared by dissolving individual fatty acid methyl esters (99% pure; Nu Chek Prep., Inc., Elysian, MN; Matreya, Inc., Pleasant Gap, PA) in *n*-hexane. The following fatty acid methyl ester standards were used: $C_{10:0}$, Me decanoate; $C_{12:0}$, Me dodecanoate; $C_{14:0}$, Me tetradecanoate; $C_{16:0}$, Me hexadecanoate; $C_{18:0}$, Me octadecanoate; $C_{20:0}$, Me eicosanoate; $C_{22:0}$, Me docosanoate; $C_{10:1}$, Me-decenoate; $C_{12:1}$, 11-dodecenoate; $C_{14:1}$, Me 9-tetradecenoate; $C_{16:1}$, Me 9-hexadecenoate; $C_{18:1}$, Me 9-octadecenoate; $C_{20:1}$, Me 11-eicosenoate; $C_{22:1}$, Me 13-docosenoate; $C_{18:2}$, Me 9,12-octadecadienoic; and $C_{18:3}$, Me 9,12,15-octadecatrienoic.

Individual stock solutions containing 10 mg/ml of the respective FAMEs were diluted with *n*-hexane to prepare working solutions containing 1 or 2 mg of each FAME/ml. A mixture containing equal weights of the methyl esters (1 or 2 mg/ml) was prepared.

Standard reference materials (SRMs)

A limited number of reference materials are available for use in validating methodologies for fat determination. Among standard reference materials available from the National Institute of Standards and Technology (NIST), only SRM 1548 (Total Diet) has a certified value for weight of total fat. NIST's value for total fat in SRM 1548 does not correspond to FDA's 'total fat', which is defined as total lipid fatty acids expressed as triglycerides (Code of Federal Regulations, 1994). Informational values for weight of total fat are available for several US/Canada collaborative reference materials, and several reference materials available from the Community Bureau of Reference (BCR, Brussels, Belgium) have certified values for weight of total fat (Wolf, 1993). Certified values for saturated fat are not available for any of the reference materials.

Two SRMs were used in this study. SRM 1548 (Total Diet) was purchased from NIST (Gaithersburg, MD). Three gravimetric methods (i.e. AOAC direct, Folch-CH_3Cl/CH_3OH, and Weibull-Soxhlet, petroleum ether) were used to obtain the fat value for this material. The Certificate of Analysis does not include values for saturated or unsaturated fatty acids. SRM 1846, a milk-based infant formula reference material, was produced in large quantity as a liquid, spray-dried and packaged for FDA in 1991. The resultant dry material was analyzed for fat, protein, ash, moisture, vitamins and minerals by Analytical Systems Research Corp., Indianapolis, IN. The certificate of analysis provided by Analytical Systems Research Corp. included values for total fat as well as saturated and unsaturated fatty acids.

Ether extract

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

Fatty acid determination

Acid hydrolysis

Test portions (2 or 4 g) of each food composite or reference material were quantitatively transferred to a tared digestion flask with the aid of absolute ethanol. The digestion flask was swirled to moisten all particles of the material to prevent lumping when the hydrolyzing reagent was added. Then 10 ml (20 ml for a 4 g-test portion) of 6*N* HCl and 2 ml (or 4 ml for a 4-g test portion) of ethanol per gram of dry matter were added to the digestion flask. Digestion was carried out for 30 min in a 70–80°C water bath with frequent shaking (e.g. about 60 cycles/min). The digestate was cooled and quantitatively transferred to a separatory funnel. The digestion flask was rinsed sequentially with diethyl ether and petroleum ether (boiling range, 30–60°C, Baxter, Muskegon, MI) 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 (boiling range, 30–60°C) mixture. The ether extract was filtered through a glass wool pledget covered with anhydrous sodium sulfate (approximately 25 g) into a pre-weighed 250-ml beaker. The ether was removed under a stream of nitrogen on a steam bath. After all traces of the ethers were removed from the composite extract, the extract was dried in a vacuum oven (60°C; 26 in. Hg) for 2–3 h to constant weight, transferred to a nitrogen-flushed desiccator and allowed to equilibrate to room temperature. The dried extract was weighed and dissolved in redistilled petroleum ether (boiling range, 30–60°C).

Preparation of fatty acid methyl esters (FAMEs)

An aliquot of the petroleum ether extract representing > 350 mg of the total petroleum ether extract whenever possible was quantitatively transferred to a tared flask. FAMEs were prepared as described in AOAC Official Methods of Analysis (1990*a*). After preparation of the methyl esters, the solvent was removed under a stream of nitrogen on a steam bath. The FAMEs were dissolved in Phillips 66 *n*-hexane (pure grade), quantitatively transferred to a 25-ml volumetric flask and diluted to volume with *n*-hexane.

Gas chromatography of FAMEs

Gas chromatography was performed in a Shimadzu GC-14A chromatograph equipped with a hydrogen flame detector and a Shimadzu CR501 Chromatopac integrator (Shimadzu Scientific Instruments, Inc., Columbia, MD). Typical operating parameters are as follows: Glass column, 180 cm × 2.6 mm i.d., with 10% Silar 10C mobile phase and 100/120 mesh GCQ inert support (Alltech Assoc., Inc., Deerfield, IL); temperatures, 200°C column, 250°C injector, 250°C detector; hydrogen flame detector; nitrogen carrier gas, 60 ml/min adjusted with $C_{18:0}$ eluting at 5–7 min; 5×10^{-11} A electrometer output; 1 cm/min chart speed.

The gas chromatography column and operating parameters used are generally within the range of conditions given in Official Methods of Analysis (1990*b*).

Calibration

The calibration procedure used in this method differs from that specified in the AOAC method (Official Methods of Analysis, 1990*b*). The AOAC method uses

1. Prepare a calibration plot relating GLC response (e.g., area, computer output units) to concentration of methyl stearate. Obtain corresponding response data for each peak being quantitated.

2. Using $C_{16:0}$ as an example:

a. Calculate response correction factor (RCF) for $C_{16:0}$

$$RCF\ C_{16:0} = \frac{\text{Response to 1 } \mu\text{g methyl stearate}}{\text{Response to 1 } \mu\text{g } C_{16:0}}$$

Calculate corrected response for $C_{16:0}$ = (Response $C_{16:0}$) x (RCF $C_{16:0}$)

b. Using corrected response value obtained above, calculate $C_{16:0}$ from methyl stearate standard curve by simple proportionality or by linear interpolation.

c. Correct for dilutions and express results per g product:

$$C_{16:0} = \frac{(\text{mg } C_{16:0}/\text{ml})(\text{volume FAMEs})}{(\text{weight of product for FAMEs})} = \text{g } C_{16:0} \text{ per g product}$$

d. Convert $C_{16:0}$ value to its triglyceride equivalent using factors in the Lipid Manual or Methods of Analysis for Nutrition Labeling:

$C_{16:0}$ = (Weight $C_{16:0}$)(Me-->TRIG conversion factor) = g triglyceride equivalent/g product

3. Perform steps 2.a.-d., above, for each FAME found in the product. Calculate the sum of the triglyceride equivalents. This is the "total fat" content of the product as per the NLEA definition. Total saturated fat content is obtained by summing $C_{10:0}$-$C_{22:0}$.

Fig. 1. Summary of calculations of response correction factors and conversion of fatty acid methyl esters to triglyceride equivalents.

the method of normalization which assumes that all components of test materials are represented on the chromatograms (i.e. total elution), so that the sum of the areas under all of the peaks represents 100% of the constituents. Using the AOAC method, results are obtained as a percentage distribution of each component, expressed as methyl esters. Such results are not consistent with the new labeling regulations, which require that weight values for individual fatty acids be determined and converted to their triglyceride equivalents. While area percentage values represent a close approximation of weight percents, appropriate response factors must be employed to convert values to true weight percents (Ackman, 1992).

The calibration procedure used in the method described in this report uses a correction response factor for each FAME based on elution of an external standard, methyl octadecanoate (methyl stearate, $C_{18:0}$). Thus, results are expressed by weight of each component corrected for the response factor obtained as described below and summarized in Fig. 1 (Sheppard, 1992).

A curve showing concentration versus response for methyl stearate was obtained by injecting three or four different quantities (e.g. 0.5, 1, 2, 4 μg) onto the column and plotting the results in peak area units versus μg of the $C_{18:0}$ methyl ester. The calibration plot was constructed to bracket the concentration of methyl stearate estimated to be present in the foods.

Response correction factors (RCFs) for equivalent weights of all other fatty acids of interest with respect to methyl stearate were obtained by analyzing the equal weight mixture of fatty acid methyl esters (see **Fatty acid standards**, above). The RCFs were calculated by dividing the area response of methyl stearate by the area response of an equal weight of individual FAMEs. The RCFs are used to correct the responses of the specific FAMEs to that of methyl stearate. The corrected response for each FAME was thus expressed in terms of the response to methyl stearate.

Calculations

After GLC analysis of each food product, the RCFs determined above were used to correct the responses of individual FAMEs to that of methyl stearate. Concentrations of individual FAMEs were then calculated from the methyl stearate standard curve, corrected for dilutions, and expressed as milligrams or grams of FAME per gram of test material. Values for individual FAMEs were converted to their triglyceride equivalents by using factors for conversion of fatty acid methyl esters and butyl esters published in the *Lipid Manual* (Sheppard, 1992). The AOAC has adopted these conversion factors (Carpenter *et al.*, 1993).

Total fat was calculated by obtaining the sum of the individually calculated triglyceride weights. Total saturated fat was calculated by obtaining the sum of individually calculated weights for all saturated fatty acids found. Calculation steps are summarized in Fig. 1.

RESULTS AND DISCUSSION

Representative chromatograms of FAMEs prepared from two foods are shown in Fig. 2. The upper panel shows the profile of FAMEs prepared from a ravioli product containing meat sauce. The total fat content of the product was approximately 3% as determined by the methodology involving acid hydrolysis and packed column GLC. The lower panel shows the profile of FAMEs prepared from a grilled snack food containing about 28% total fat. With the packed column GLC methodology, each saturated FAME elutes as an individual peak and each FAME of a given chain length and degree of unsaturation elutes as a single peak regardless of position or geometric configuration of the double bonds. The conversion of FAMEs to their triglyceride equivalents (Fig. 1) for purposes of calculating total fat content and saturated fat content according to

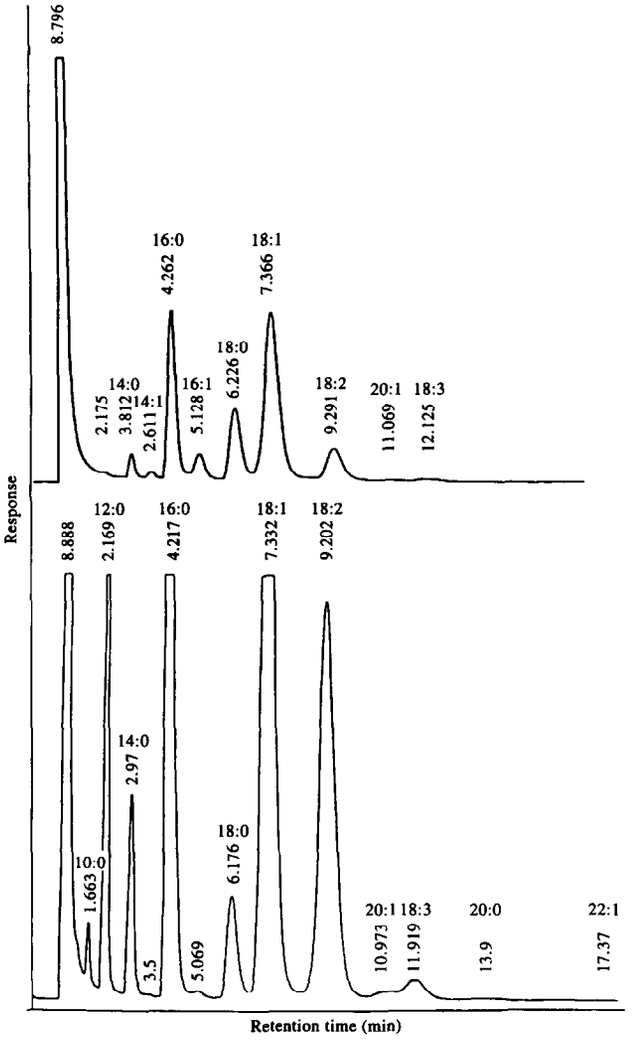

Fig. 2. Gas chromatograms of FAMEs prepared from test portions of ravioli containing meat sauce (upper panel) and grilled snack chips (lower panel) after acid hydrolysis of sample composites. Mean total fat contents of the products determined by the acid hydrolysis–packed column-GLC methodology were 3% (ravioli) and 28% (grilled snack chips). Representative operating conditions are listed under **Gas chromatography of FAMEs**, above.

Table 1. Determination of total fat by AOAC gravimetric methodology and by acid hydrolysis–packed column-GLC methodology

Product	AOAC direct gravimetric fat(%)	Acid hydrolysis –GLC method fat(%)	CV(%)	Difference (AOAC - GLC) fat(%)	Relative difference (%)
Tapioca pudding, fat-free	0.84	0.16 ± 0.03	21.2	0.68	–81
Honey mustard chicken, low-fat	1.50	0.88 ± 0.19	21.7	0.62	–41
Waffles, fat-free	1.73	0.56 ± 0.05	8.2	1.17	–68
Chicken chow mein, low-fat	1.91	1.57 ± 0.31	19.6	0.34	–18
Crackers, fat-free	2.55	0.94 ± 0.19	20.5	1.61	–63
Hard pretzels, no-fat	2.88	0.92 ± 0.25	27.1	1.96	–68
Rice cakes, fat-free	3.14	1.58 ± 0.24	15.2	1.56	–50
Cookies, fruit-center, fat-free	3.48	1.31 ± 0.16	12.3	2.17	–62
Instant brkfst powder, low-fat	3.57	1.44 ± 0.19	12.9	2.13	–60
Turkey gravy dressing meal	3.94	2.93 ± 0.19	6.5	1.01	–26
Beef ravioli in meat sauce	4.06	3.14 ± 0.29	9.0	0.92	–22
Muesli cereal, low-fat	6.61	3.57 ± 0.70	19.6	3.04	–46
Cereal with raisins, low-fat	8.77	5.28 ± 0.71	13.5	3.49	–40
Breaded fish fillets	9.84	8.22 ± 0.32	3.9	1.62	–16
Taco dinner without meat	11.7	10.7 ± 0.4	3.7	1.0	–9
Infant formula powder	20.0	17.4 ± 0.7	4.1	2.6	–13
Chocolate chip cookies	26.2	20.8 ± 0.2	1.2	5.4	–20
Chicken-flavored snack crackers	26.8	24.8 ± 0.6	2.4	2.0	–8
SRM1846 Powdered Infant Formula	26.8	23.8 ± 0.6	2.6	3.0	–11
Grilled snack chips	29.3	27.8 ± 1.0	3.6	1.5	–3
Cheese cracker sandwiches	30.0	27.4 ± 0.8	3.0	2.6	–9
Corn chips	36.5	34.4 ± 1.4	4.0	2.1	–6
Chocolate bar with almonds	36.9	29.7 ± 1.3	4.5	7.2	–20
Mayonnaise	72.6	75.9 ± 0.9	1.1	–3.3	+5

Values for % fat by acid hydrolysis-packed column–GLC methodology are means ± SD of four or five independent replications. Coefficient of variation (CV, %) = [(SD/Mean) × (100)]. Relative difference, % = [(GLC)–(AOAC direct)]/[(AOAC direct) × (100)].

the new NLEA definition is straightforward, since each FAME peak contains all fatty acids of the same molecular weight.

Total fat in foods determined by the AOAC direct method and the packed column–GLC method

Values for crude fat in foods obtained by the AOAC direct gravimetric method and for total fat determined by the GLC method are shown in Table 1. Products are listed in order of increasing crude fat content as determined by the AOAC gravimetric method. We did not compare values obtained by either methodology with label declarations for fat for the products analyzed since such comparisons were outside the purpose of the study.

With the exception of the mayonnaise sample, total fat content based on GLC fatty acid analysis was lower than the crude fat content of the same food sample determined by the AOAC gravimetric method. Relative differences between the two methods declined markedly with increasing fat content, and for many foods containing >4% fat (w/w) the relative differences were less than 20%. The coefficients of variation (CV, %) for replicate determinations by the GLC method were <5% for most food products of >3% total fat content.

Replicate analyses by the GLC method for two fruit-containing dry breakfast cereals gave CVs of 14 and 20%, respectively, values which were higher than those observed for other products of lower fat content (Table 1). These large variations may be due to inhomogeneity in the dry cereal composites.

The food samples were blended (homogenized, composited) extensively. High-temperature drying and freeze-drying were avoided, however, because of their potentially adverse effects on fat components (Ackman, 1992). However, unrecognized inhomogeneity may have existed in samples of some products. With respect to the dry cereal products, the high variances may also be related to the higher content of plant material in these products or to effects of processing on the fat. In addition, increased variances with the low-fat products in general might also be expected because of the apparent inverse relationship between component level and variability (Horwitz *et al.*, 1980; Holden *et al.*, 1994).

Although the relative differences between the two methods were very large for products of low fat content (i.e. 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 to determine the relationship between the data pairs. A correlation coefficient (r) of 0.941 was found for the straight line that best described all of the data (Fig. 3). Fig. 3 is cropped at 40%. One point (i.e. mayonnaise) outside of the axis range of 40% is not shown but was included in the regression analysis.

After reviewing the results of the regression analysis including all food products, we considered whether a correlation coefficient greater than 0.94 would be found if, for example dry cereals or meat-containing products

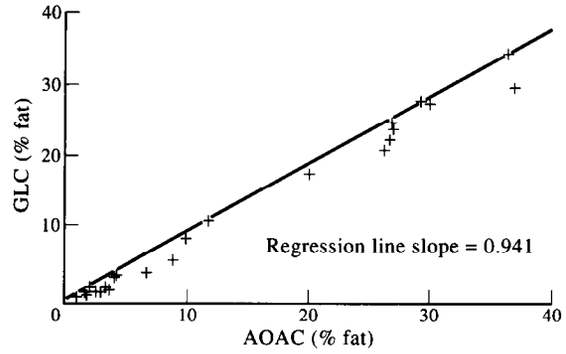

Fig. 3. Values for total fat determined by the acid hydrolysis–packed column-GLC methodology were regressed on values for crude fat obtained by the AOAC gravimetric method. The resulting line, forced through the origin, had a slope of 0.941. Each value represents the mean of four or five independent determinations. The point for mayonnaise is outside of the axis range and is not shown. The data were included in the regression analysis.

were excluded from the analysis. Baily *et al.* (1994) recently noted that depending upon the food matrix and the method used, traditional methodology can either overestimate or underestimate fat according to the new definition. We were interested, therefore, in determining whether results with specific types of food were biasing the results of the regression analysis. However, we found that correlation coefficients calculated from data excluding specific types of products (e.g. dry cereals, meat-containing products) were consistently weaker (i.e. in the range of 0.54 – 0.79) than those obtained when all data sets were included. Fig. 3 therefore shows the regression analysis using all data pairs.

When the crude fat contents of specific foods include components in addition to lipid fatty acids, the NLEA-defined fat content of the foods will fall below a regression line such as that shown in Fig. 3.

House *et al.* (1994) recently described a hydrolytic extraction of fat from foods followed by quantitative measurement of FAMEs by capillary GLC. These authors reported total fat contents determined by several methods for an oat-based cereal, a yogurt, and a cake. For each of these products, values for crude fat determined gravimetrically from the weight of the hydrolysis extraction residue (7.27, 0.93 and 15.5%, respectively) were higher than the values for total fat determined chromatographically from fatty acid quantitation (6.61, 0.82 and 12.9%, respectively). The higher gravimetric crude fat values were attributed to the weight of material other than fat in the extraction residues. The results reported here (Table 1) are consistent with these observations. Differences between the gravimetric method and the chromatographic method found in this study were larger than those reported by House *et al.* (1994).

Distribution of fatty acids

The distributions of fatty acids in six representative foods are shown in Table 2. Total fat content of these products ranged from about 3 to 28%. Coefficients of variation were high for many of the fatty acid components in the two meat-containing products. These products contained about 3% total fat by weight. Coefficients of variation were 5–12% for the major fatty acids present in these products ($C_{16:0}$, $C_{18:0}$, $C_{18:1}$, and $C_{18:2}$) and above 20% for all others fatty acids.

In contrast, coefficients of variation were 3–4% for the major fatty acid components in the two products of 8 and 11% total fat content. Higher coefficients of variation (10–15%) were observed for fatty acids estimated to be present in these products at levels of $<0.05\%$ (w/w). For products of 17 and 28% total fat content, coefficients of variation for essentially all component fatty acids were 3–5%.

Table 2. Distribution of fatty acids in several food products

FAME	Turkey gravy dressing meal		Beef ravioli in meat sauce		Breaded fish fillets		Taco dinner without meat		Infant formula powder		Grilled snack chips	
	mg/g	CV(%)	mg/g	CV(%)	mg/g	CV(%)	mg/g	CV(%)	mg/g	CV(%)	mg/g	CV(%)
10:0	0	-	0	-	0	-	0	-	1.01	13.7	0	-
12:0	0	-	0	-	0	-	0	-	14.19	5.8	0.06	11.3
14:0	0.19	14.2	0.82	10.1	0.12	22.9	0.09	3.2	7.03	4.5	2.26	4.0
14:1	0.06	43.0	0.32	12.7	0	-	0	-	0.05	5.4	0	-
16:0	5.59	9.5	7.68	9.3	9.53	3.6	12.20	3.2	42.34	3.9	67.03	3.5
16:1	0.88	12.6	1.53	9.4	0.22	15.1	0.22	3.9	0.51	5.2	1.93	3.3
18:0	2.48	5.4	4.28	9.8	3.48	2.8	7.06	3.9	6.45	4.0	7.14	3.2
18:1	12.78	9.2	13.42	9.6	37.81	3.3	69.93	3.4	58.68	3.9	45.69	3.5
18:2	6.71	5.3	2.89	11.7	28.81	4.8	16.23	4.3	39.24	3.9	151.66	3.6
20:0	0	-	0	-	0	-	0	-	0	-	0	-
20:1	0.08	22.9	0.10	25.0	0.52	10.12	0.30	14.5	1.14	5.8	0.41	4.8
18:3	0.38	18.9	0.41	21.5	2.22	9.9	0.41	13.5	3.10	3.1	1.57	1.8
22:0	0.10	53.6	0	-	0.49	4.1	0.54	24.0	0.40	2.9	0.61	8.6
22:1	0	-	0	-	0	-	0	-	0.02	-	0	-
Sum, mg/g	29.25		31.44		82.49		106.97		174.17		278.25	
Fat, %	2.93		3.14		8.25		10.7		17.4		27.8	

Values (mg/g) are means of four or five independent replicates. Coefficient of variation [CV(%)]) = [(SD/Mean) × (100)]. The value for $C_{20:1}$ in grilled snack chips (0.41 mg/g). represents the mean of three independent determinations. A value of 0.00 mg/g was obtained for a fourth determination. Mean ± SD of all four determinations = 0.31 ± 0.20 mg/g; CV = 66.9%.

Table 3. Saturated fat content of food samples

Product	Saturated fat (%)	Coefficient of variation CV(%)
Honey mstd chicken, low-fat	0.23	7.2
Chicken chow mein, low-fat	0.35	23.2
Inst brkfst powder, low-fat	0.71	15.1
Turkey gravy dressing meal	0.84	8.5
Beef ravioli in meat sauce	1.29	8.9
Breaded fish fillets	1.36	3.5
Taco dinner without meat	1.99	3.8
Chicken-flavr snack crackers	4.28	2.5
Corn chips	4.57	4.4
Cheese cracker sandwiches	6.36	3.2
Grilled snack chips	7.71	3.4
SRM 1846 infant formula	10.3	2.4
Mayonnaise	10.7	1.2
Chocolate chip cookies	12.4	1.0
Chocolate bar with almonds	13.6	1.0

Values for % saturated fat are means of four or five independent replicates; CV(%) = [(SD/Mean) × (100)]. The saturated fat content of SRM 1846 was also calculated from the certificate of analysis as the sum of $C_{10:0}$ -$C_{22:0}$. The value obtained was 13.0%.

Total saturated fat content of nine foods was determined by summing $C_{10:0}$ through $C_{22:0}$ (Table 3). Coefficients of variation were <4% for this determination with two exceptions. Higher coefficients of variation (8–9%) were found for two meat-containing products whose total fat contents were <4%.

Standard reference materials

Analysis of crude fat in SRM 1548 by the direct AOAC method gave excellent agreement with the certified value (Table 4). However, calculation of total fat after acid hydrolysis and GLC separation of FAMEs gave values

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

FAME	SRM 1846[a] Infant Formula, mg/g	SRM 1846[b] Infant Formula, mg/g	CV(%)	SRM 1548[b] Total Diet, mg/g	CV(%)
4:0	0.00	–	–	–	–
6:0	0.00	–	–	–	–
8:0	6.81	–	–	–	–
10:0	5.40	2.33	15.5	0.16	29.3
10:1	0.00	0	–	0	–
12:0	41.27	30.98	3.6	1.31	6.8
12:1	0.00	0	–	0	–
14:0	17.51	14.38	2.2	5.16	3.1
14:1	0.00	0.09	7.4	1.05	3.7
16:0	32.96	27.88	2.4	35.06	3.3
16:1	0.44	0.47	10.5	3.42	8.8
18:0	32.13	26.38	3.0	17.78	3.7
18:1	96.67	97.71	3.0	47.53	10.0
18:2	40.72	35.51	2.8	3.81	12.1
20:0	0.86	0	–	0	–
20:1	0.00	0.40	10.3	0.31	10.1
18:3	0.97	0.85	7.6	0.39	25.2
22:0	–	0.63	4.8	4.56	9.1
22:1	–	0.05	70.4	0	–
Sum, mg/g	275.75	237.66		120.54	
Fat, %	27.6	23.8		12.1	
AOAC direct Fat, %	–	27.0		20.8	

[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% represents the sum of the fatty acids. [b] Portions of the Infant Formula and Total Diet reference materials were analyzed by the AOAC direct gravimetric methodology and by the acid hydrolysis–packed column-GLC methodology. Values represent the means of four independent replicates 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. Coefficient of variation (CV%) = [(S.D./Mean) × (100)].

that were significantly lower (by about 40%) than anticipated. This finding was unexpected.

We discussed our observations with NIST scientists, who noted that SRM 1548 was subjected to ^{60}Co radiation sterilization at a dose of 2.5–5.0 mrad to prevent bacterial growth (National Institute of Standards and Technology, 1991).

NIST scientists noted that lipids are known to degrade with exposure to ionizing radiation. NIST scientists had found that the original level of cholesterol in SRM 1548 fell by approximately 50%, probably because of the radiation treatment. Cholesterol undergoes various oxidations during irradiation, and the reaction products would be expected to react differently than cholesterol during analysis. The principal end products of irradiation of fatty acids are carbon dioxide, carbon monoxide, hydrogen, hydrocarbons, mainly alkanes, and aldehydes, while hydrocarbons, aldehydes, ketones, polymerized fats, and free fatty acids are formed from irradiated triglycerides (Josephson & Peterson, 1983; Nawar, 1983; Urbain, 1986). Triglycerides undergo a series of reactions similar to those of fatty acids, with a large number of radiolytic products postulated (Urbain, 1986).

NIST scientists speculate that reductions in the levels of fatty acids may have occurred as a result of the radiation sterilization (personal communication, Dr Michael J. Welch, NIST). Thus, irradiation may have altered some fatty acid components in SRM 1548 in such a way that the resultant material remained quantifiable by the AOAC gravimetric method but not by a method dependent upon the release and methylation of fatty acids. Because of these uncertainties, we do not use SRM 1548 as a reference material when quantifying fatty acids.

NIST scientists further noted that SRM 1548 was not intended as a reference material for fatty acids. NIST is currently developing a freeze-dried composite food SRM that will be certified for cholesterol and several fatty acids (personal communication, Dr Michael J. Welch, NIST).

SRM 1846, developed from a milk-based infant formula, has proved to be a much more useful reference material. Crude fat content determined by the AOAC gravimetric method (27.0%) was 97.3% of the certificate of analysis value of 27.6% found by Analytical Systems Research Corp. (Table 4). The value for total fat derived by quantitation of FAMEs (23.8%) compared well with the value of 27.6% calculated from the sum of individual fatty acid values determined by Analytical Systems Research Corp. Coefficients of variation of 2–4% were found for the major fatty acid components of this material ($C_{10:0}$, $C_{14:0}$, $C_{16:0}$, $C_{18:0}$, $C_{18:1}$, and $C_{18:2}$), and CVs of 5–11% were found for fatty acids $C_{14:1}$, $C_{16:1}$, and $C_{20:1}$. These reproducibility results are very consistent with the reproducibility of our analyses of other foods, and suggest that this reference material is appropriate for the type of studies reported here.

Effects of method modifications

In the methodology described above, fatty acids contained in lipid components of foods are released by acid hydrolysis. Fatty acids are quantitatively extracted from the acid digestate with diethyl and petroleum ethers, converted to their methyl esters and quantitated by GLC on a packed column. Several aspects of the methodology were modified to determine whether they affected the results.

No significant differences in results were found when 8*N* HCl was used in place of 6*N* HCl in the extraction phase. Omission of the prescribed water wash during the saponification and methylation steps did not result in significant differences in the values obtained. Increasing the methylation time with BF_3 from 2 min (as prescribed) to 5 min did not result in any significant differences.

No additional peaks were obtained for the foods when butyl esters (used to measure short chain fatty acids) were prepared and analyzed. Two materials, SRM 1846 and the chocolate bar with almonds, exhibited C:10 peaks for both methyl ester and butyl ester preparations.

The diethyl ether extraction was repeated for some foods because a residue appeared to remain in the bottom of the extraction flask. The extraction vessel was also reweighed. No additional fatty acid peaks were obtained for the residue after this procedure.

In conclusion, none of the modifications above had an impact on the results of the analyses, and the method appears adequate as described.

Other columns

The 10% Silar 10C packed column used in this study elutes saturated FAMEs as individual peaks and all FAMEs of a given chain length and degree of unsaturation as single peaks regardless of double bond position or geometric configuration. Other standard column packings such as a 12–15% ethylene glycol succinate column or a 10% ECNN-S column may also be used (Sheppard, 1992; Official Methods of Analysis, 1990*b*). These columns provide excellent FAME separations based on chain length and degree of unsaturation independent of double bond position or geometric configuration. However, the resolution times are somewhat longer with these columns than with the 10% Silar 10C column described above (Sheppard, 1992).

In addition to the analyses on the packed column described above, we performed capillary gas chromatography following acid hydrolysis on replicate samples of four food products. Gas chromatography was performed in the Shimadzu instrument described above. A standard mixture of fatty acid methyl esters in heptane was purchased from Matreya, Inc. (catalog #4210) and used as received. Typical operating parameters were as follows: SP-2330 fused silica capillary column, 60 m, 0.25 mm i.d., 0.20 μm film thickness (Supelco, Bellefonte, PA); temperatures, 175°C column, 225°C injector,

225°C detector; helium carrier gas adjusted to allow runs to end in about 50 min. An internal standard, methyl tridecanoate ($C_{13:0}$), was added to the samples prior to acid hydrolysis. Resultant values for total fat, %, mean ± SD, in four products were as follows for the two types of columns: whole grain cereal, 7.55 ± 0.30 (packed), 7.28 ± 0.23 (capillary); NIST SRM 1548, 15.6 ± 0.8 (packed), 16.3 ± 0.0 (capillary); chocolate cookies, 23.7 ± 0.1 (packed), 20.9 ± 0.8 (capillary); and mayonnaise, 75.4 ± 4.6 (packed), 69.4 ± 6.7 (capillary). Based on these observations, comparable results can be obtained with the packed column or the SP-2330 capillary column.

CONCLUSIONS

Most foods contain fatty acids of 10–20 carbon chain lengths. Some foods, particularly if they contain some marine material, may have fatty acids of chain lengths up to 24. Dairy products contain short chain volatile fatty acids that require use of butyl fatty acid esters because of volatility and solubility problems. The results of this study indicate that the acid hydrolysis–packed column GLC methodology is applicable to determination of total fat and saturated fat in a wide range of food products.

Many laboratories currently use capillary columns rather than packed columns for routine GLC determination of fatty acids. Such columns are capable, to varying degrees, of separating *cis* and *trans* isomers of unsaturated fatty acids, which is beyond the capability of the packed column used in the present study.

At the present time, total fat and saturated fat content are required for labeling purposes. The NLEA definition of total fat is based on conversion of total fatty acid content to the triglyceride equivalent. Therefore, there is no analytical requirement to resolve positional and/or geometric isomers but rather a requirement to determine the total amount of each fatty acid of a given chain length and degree of unsaturation, regardless of double bond position and/or geometric configuration. The acid hydrolysis–packed column GLC methodology described satisfies the labeling requirements for determination of total fat and saturated fat for many foods. Comparable results have been obtained using an SP-2330 fused silica capillary column.

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