Simplified Gravimetric Determination of Total Fat in Food Composites After Chloroform–Methanol Extraction

Katherine M. Phillips*, Maria Teresa Tarragó-Trani, Tina M. Grove, Ingolf Grün, Rita Lugogo, Robert F. Harris, and Kent K. Stewart

Department of Biochemistry, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061-0308

ABSTRACT: A modification of Association of Official Analytical Chemists (AOAC) method 983.23 for the quantitative determination of total lipid in food composites was evaluated for the measurement of total fat. The procedure is based on the Bligh and Dyer chloroform/methanol total lipid extraction. Relative to AOAC 983.23, the proposed method is less labor-intensive and is applicable to batch analysis of a larger number of samples, thus reducing the cost of analysis and increasing sample throughput. Total lipid values from the proposed method are comparable to those from AOAC 983.23 and slightly higher than total fat determined by acid hydrolysis (AOAC 954.02, 945.44, or 922.06). Recoveries of standard additions of different food-grade oils from a mixed food composite were essentially quantitative, ranging from 96 to 101%. Total lipid measured in Total Diet Standard Reference Material 1548 (SRM 1548, National Institute of Standards and Technology) was 101% of the certified mean total fat content and within the certified range. The method is to be suitable for analysis of food composites with between 0.15 and 1.5 g total fat (3 to 30% by weight). More than 600 samples of a variety of total diet composites were collected and assayed as diet quality control samples for two National Heart, Lung and Blood Institute-sponsored multicenter clinical feeding trials: DELTA (Dietary Effects on Lipoproteins and Thrombogenic Activity) and DASH (Dietary Approaches to Stop Hypertension). The mean coefficient of variation was 1.2% for duplicate assays of these samples over the course of two years and multiple analysts. In addition, total lipid values for more than 200 samples of a diet composite quality control material, used in this laboratory over a two-year period, had a 3.99% coefficient of variation. Although the accuracy of all gravimetric total fat methods with respect to the U.S. Food and Drug Administration's Nutritional Labeling and Education Act (NLEA) definition of total fat as the sum of triglycerides remains to be determined, the reported modification of AOAC 983.23 yields a total fat content of acceptable accuracy relative to other gravimetric methods, and with proper quality control the method has excellent precision.
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KEY WORDS: Diet composition, fat, food composition, total lipid extraction.

*To whom correspondence should be addressed at Department of Biochemistry, Engel Hall, Virginia Tech, Blacksburg, VA 24061-0308.

Chloroform/methanol extraction of total lipid from mixed food composites has been used for quantitative determination of total fat content and as a preliminary step in the analysis of fatty acids, cholesterol, and other lipids. Gravimetric methods are used routinely for determination of total fat. One such method applicable to a variety of food matrices is Association of Official Analytical Chemists (AOAC) method 983.23 (1), which is Daugherty and Lento's modification (2) of the Bligh and Dyer total lipid extraction (3). AOAC 983.23 involves incubating a minced food sample with 1% Clarase® 40,000 (an enzyme preparation that contains proteases and α-amylase) in 0.5 M sodium acetate at 45–50°C for one hour; transferring the solution to a blending assembly; homogenizing with multiple additions of chloroform and methanol; transferring the mixture to a centrifuge bottle; centrifuging; then pipetting, drying, and weighing an aliquot of the chloroform layer. In spite of the reported accuracy and applicability of this method to a variety of foods (3), it is labor-intensive and requires availability of Clarase®. Typical sample throughput in this system is about 8 per day per analyst if one individual blending cup (*ca.* $540.00 each) is procured for each sample. In addition, transferring the volatile solvent mixture can result in variable solvent loss, which would decrease the routine precision and accuracy of the gravimetric measurement.

In this paper we report a modification of AOAC 983.23 that was validated for the quantitation of total fat in mixed food composites. The accuracy, precision, and analytical concentration range of the method were assessed by analysis of the Total Diet Standard Reference Material 1548 [SRM 1548, National Institute of Standards and Technology (NIST)], recovery of standard additions of food-grade fats and oils, and routine use over the course of approximately two years for repeated assays of a quality control composite and for duplicate assays of over 600 food composites, ranging from 2.4 to 8.7% by weight total lipid and 56 to 80% moisture. Results from the proposed procedure were also compared with those from acid hydrolysis, an alternative widely used gravimetric total-fat method.

MATERIALS AND METHODS

Food composites. Each food composite consisted of a combination of the following foods: fruits, fruit juices, breads, cereals, vegetables, pasta, dairy products (milk, cheese, yogurt, butter), eggs, meats (poultry, beef, pork), seafood, sweets, and cooking fats (oils, margarine, etc.) in proportions that represented a range of total diet consumption patterns and contained approximately 2 to 9% by weight total fat and 56 to 80% moisture. The majority of the samples were collected as part of diet quality control for two National Heart, Lung and Blood Institute (NHLBI)-sponsored multicenter feeding trials: DELTA (Dietary Effects on Lipoproteins and Thrombogenic Activity) and DASH (Dietary Approaches to Stop Hypertension). Composites were prepared in such a way that a homogeneous mixture (i.e., aliquots derived from a specific composite were representative of the entire composite) was ensured. Briefly, the frozen mixture of foods (−20°C) was partially thawed, then homogenized in a stainless steel industrial food processor (Model R-6, Robot Coupe USA, Inc., Jackson, MS), with periodic scraping of any food adhering to the lid back into the processor bowl. The temperature of the mixture was maintained below 25°C throughout the procedure. The composite was immediately aliquotted, with stirring, into 30-mL wide-mouth glass jars with Teflon®-lined screw caps. The samples were stored in tightly sealed jars at −60°C. The frozen samples were brought to room temperature, then thoroughly stirred for approximately 30 s prior to aliquotting for assay. Complete mixing at this stage is critical to obtain uniform representative subsamples because fat separation can occur when composites are frozen and/or allowed to settle.

Standard reference material. Standard Reference Material 1548 (SRM 1548), Total Diet, National Institute of Standards and Technology (NIST, Gaithersburg, MD).

Chemicals and standards. Solvents were ACS-certified chloroform and HPLC-certified methanol (Fisher Scientific, Fair Lawn, NJ). Sodium acetate (Sigma Chemical Co., St. Louis, MO) was prepared as a 0.5 M solution in distilled deionized water. Dimethyldichlorosilane (Sigma Chemical Co.), 5% in hexane, was used for siliconization of bottles. Fats used were coconut oil, which contained cholesterol, retinyl acetate, ergocalciferol, and dl-α-tocopherol acetate (Standard Reference Material 1563, NIST, Gaithersburg, MD), and menhaden fish oil (Southeast Fisheries Center, National Marine Fisheries Service, Charleston, SC). Clarase® 40,000 was obtained from Miles Laboratories, Inc., Elkhart, IN. The DELTA study fat blend was supplied by Kraft Foods Technology Center (Glenview, IL), and contained the following fatty acids (weight percentage, as triglycerides): $C_{12:0}$ (0.9%), $C_{14:0}$ (0.6%), $C_{16:0}$ (14.7%), $C_{18:0}$ (6.8%), $C_{18:1}$ (60.4%), $C_{18:2}$, (18.6%).

Moisture determination. Moisture in each composite was determined prior to total lipid extraction with a microwave moisture–solids analyzer (LabWave 9000, CEM Corporation, Matthews, NC). Approximately 2.0 to 2.5 g of food composite were used to determine moisture in the moisture–solids analyzer, at microwave power of 377–442 watts (65% of maximum power) for 5 min. Moisture in the Total Diet Standard Reference Material (NIST SRM 1548), which is lyophilized, was measured by vacuum drying at 25°C for 24 h. Moisture contents were used to calculate the amount of sodium acetate required for the extraction of each composite as described below.

Total lipid extraction by proposed method. Five grams (±0.1 g) of thoroughly mixed food composite or 1 g of Total Diet Standard Reference Material 1548 was weighed (to nearest 0.0001 g) into a 500-mL polypropylene centrifuge bottle that was previously siliconized with dimethyldichlorosilane. Based on the measured moisture content of the sample, enough 0.5 M sodium acetate was added so that the total volume of water in the sample plus sodium acetate solution was 32 mL. Next, 80 mL methanol and 40 mL chloroform were precisely measured and added to the sample with repipet dispensers (Brinkmann Dispensette®, 10–50 mL with quick release connector, #5010050-2; Brinkmann Instruments, Inc., Westbury, NY). The resulting proportion of chloroform:methanol:water was 1:2:0.8 (vol/vol/vol). The centrifuge bottles were capped and shaken on an orbital platform shaker (New Brunswick Scientific Co., Inc., Edison, NJ) for 2 h at 325 rpm. Next, precisely 40 mL chloroform was added to each sample, and capped bottles were shaken for 30 min at 300 rpm. Then, 40 mL water was added to each sample, and the capped bottles were shaken for 30 min at 275 rpm. The final ratio of chloroform:methanol:water in the samples was 2:2:1.8 (vol/vol/vol). The capped bottles were centrifuged at 2300 rpm at a temperature between 4 and 22°C for 10 min to clarify the chloroform (bottom) layer. To avoid disturbance of the chloroform, methanol/water, and interphase food layers, the centrifuge was set with brake at half speed. Bottles were placed in a 25°C water bath and allowed to equilibrate for 15 min before dispensing.

Gravimetric determination of total lipid in extracts by proposed method. Corex® centrifuge tubes (30 mL; Cat. No. 05-566-55, Fisher Scientific, Pittsburgh, PA), one per sample, were labeled with sample numbers, placed in a metal test tube rack, and heated in a drying oven at 101 ± 2°C for 30 min. The tubes were cooled in a desiccator for at least 30 min and then weighed on a microbalance to the nearest 0.0001 g. When manipulating Corex® tubes, clean powder-free gloves were used at all times to prevent transferring any lipids or other materials (e.g., fingerprints) from the hands of the analyst to the tubes and hence altering the total lipid weight measurement. A semiautomated pipetter/diluter (Hamilton MicroLab® 910, Hamilton Company, Reno, NV) was utilized to pipet aliquots accurately and precisely from the chloroform (bottom) layer. A 25-mL gas-tight syringe, macrovalve, and 12-gauge fill and dispense tubing were used in the operation of the pipetter, which was calibrated and primed first with 3 × 25 mL distilled deionized water, 3 × 25 mL methanol, and 3 × 25 mL chloroform. To prevent entry of food sediment and/or aqueous methanol into the dispenser tubing, a polypropylene tubing

plug for 1/16″ ID tubing (Part #PIP210-6; Value Plastics, Inc., Fort Collins, CO) was inserted into the tip end of a clean 2-mL glass pipet or polypropylene macropipet tip (Rainin, #RC-10; Rainin Instrument Co., Inc., Woburn, MA), with a separate pipet/plug assembly per sample. The assembly was introduced carefully to the bottom of the centrifuge bottle. A piece of wire (1-mm diameter, 37–50 mm length) was threaded through the pipet to dislodge the plug while care was taken to ensure that the pipet tip remained in the chloroform layer. The pipetter tubing was inserted through the pipet to the tip. The tubing lines of the Microlab® pipetter/diluter were then primed by aliquotting and discarding 5 mL of the sample extract twice before 20 mL of the chloroform layer was aliquotted and dispensed into the tared Corex® tube. Between samples, the pipetter tubing lines were flushed first with 20 mL chloroform and then with 5 mL chloroform.

The aliquotted extracts in the tubes were evaporated to dryness in a 60°C water bath under a stream of nitrogen (N-Evap Analytical Evaporator; Organomation Associates, Inc., Berlin, MA). The tubes were then placed in a metal test tube rack and heated in a drying oven at 101 ± 2°C for 30 min, cooled in a desiccator for at least 30 min (usually overnight), then weighed on a microbalance to the nearest 0.0001 g. Total lipid in the food sample was calculated from the formula:

$$\text{Total lipid (g/100 g wet weight)} = (W_2 - W_1) \times V_C \times 100/(V_A \times \text{SW})$$

where W_2 is the weight of glass tube + dried extract (g), W_1 is the weight of empty dried glass tube (g), V_C is the total volume of chloroform (80 mL), V_A is the volume of extract dried (mL), and SW is the weight of food sample assayed (g).

Total lipid extraction by AOAC 983.23. Food composite samples were extracted as specified by AOAC 983.23 (1). Briefly, 5 g of well-homogenized food composite was weighed into a 50-mL Pyrex® screw-cap tube, and enough 1% Clarase 40,000® in 0.5 M Na acetate was added so that the total water content, including moisture in the sample, was 32 mL. The tube was shaken gently until the sample was well mixed, and the tube was then incubated in a water bath at 45–50°C for 1 h. The sample was mixed thoroughly and then transferred to a semimicro stainless-steel blending assembly (Omni Mixer-Homogenizer, Omni International, Waterbury, CT). The sample tube was rinsed first with 80 mL methanol, then with 40 mL chloroform, and the rinses were added to the blending assembly. The blending assembly was covered, and the contents were blended at high speed for 2 min. Then, 40 mL chloroform was added, covered, and the contents were blended for 30 s; 40 mL water was added, and the contents were blended for an additional 30 s. The extract was transferred to a 500-mL siliconized polypropylene bottle, capped and centrifuged for 10 min at 3000 rpm to clarify the chloroform layer (bottom layer). The bottle was placed in a 25°C water bath and allowed to equilibrate for 15 min before dispensing. Gravimetric determination of total lipid in the extract was carried out as explained above. Statistical analyses were accomplished with Stat View 4.01, FPU version software and manual (4) for Macintosh computers.

RESULTS

Comparison of total lipid content determined by proposed method and AOAC 983.23. Total lipids in three different diet composites assayed by the proposed method and AOAC 983.23 were compared; the results are shown in Table 1. For each of the three composites, the mean total lipid content, determined by the two procedures, did not differ significantly ($P > 0.05$ for the 95% confidence intervals).

Total fat content of total diet standard reference material (NIST SRM 1548). The accuracy of the proposed method for total fat analysis was evaluated via results obtained for NIST Standard Reference Material (SRM) 1548. SRM 1548 is a freeze-dried homogeneous mixture of foods representative of the typical U.S. adult diet (5), and it is the only available mixed-food standard reference material certified for total fat content. The certificate of analysis reports a total fat level of 20.6 ± 2 g/100 g dry weight (5). The mean total fat content, determined by the proposed method, was 20.9 ± 0.52 g/100 g dry wt. [$n = 5$, CV was 2%, and the statistical uncertainty for a 95% confidence interval (= 2.78 × standard deviation/$\sqrt{n}$) was 0.52 (6)]. This value is 101% of the reported mean and well within the certified range for total fat content.

Recovery of fats spiked into food composites. Fat recovery for the proposed method was assessed by analyzing aliquots of a typical food composite (3.2% by weight total lipid), spiked with either coconut oil, menhaden oil, or fat blend. An accurately weighed amount of oil (ranging from 0.1 to 1.0 g) was added to an accurately weighed aliquot (*ca.* 5 g) of the composite. Total lipid was measured in the spiked and unspiked samples by the proposed method. Recovery was calculated as the weight of lipid recovered divided by the sum of the weight of lipid in the composite plus weight of fat added, expressed as a percentage.

Results show that recovery ranged from 96 to 101% (Table 2), indicating that loss of lipid during the assay was minimal and that recovery was essentially quantitative for all oils. The efficiency of the total lipid extraction may be attrib-

TABLE 1
Comparison of Total Lipid Content of Mixed Food Composites by AOAC 983.23 and Proposed Method

				Total lipid (g/100 g wet)		
Method	Composite	*n*	Moisture (g/100 g)	Mean[a]	Standard deviation	CV[b] (%)
Proposed	A	8	78.2	3.05[a]	0.14	4.6
AOAC	A	6	78.2	2.94[a]	0.06	2.2
Proposed	B	8	78.1	3.21[b]	0.03	1.1
AOAC	B	8	78.1	3.16[b]	0.14	4.3
Proposed	C	8	78.0	3.39[c]	0.13	3.8
AOAC	C	6	78.0	3.31[c]	0.04	1.2

[a]Means superscripted with the same letter were not significantly different for 95% confidence interval ([a]$P = 0.11$, [b]$P = 0.34$, [c]$P = 0.20$).
[b]Abbreviation: CV, coefficient of variance.

TABLE 2
Recovery of Fats Spiked into Mixed Food Composite (Composite A)

Fat spiked	Wt. of spike (g)	Wt. of fat in composite (g)	Total wt. of fat recovered (g)	Recovery[a] (%)
Coconut oil	0.1656	0.160	0.3295	101
Coconut oil	0.1582	0.157	0.3154	100
Menhaden oil oil	0.1716	0.161	0.3198	96
Menhaden oil oil	0.2024	0.159	0.3582	99
Fat blend	0.1017	0.159	0.2556	98
Fat blend	0.1019	0.157	0.2584	100
Fat blend	0.2074	0.158	0.3604	100
Fat blend	0.2081	0.158	0.3624	99
Fat blend	0.3018	0.158	0.4540	99
Fat blend	0.2995	0.158	0.4504	98
Fat blend	0.6087	0.158	0.7576	99
Fat blend	0.6058	0.158	0.7532	99
Fat blend	0.9981	0.158	1.1448	99
Fat blend	1.0283	0.159	1.1632	98

[a]Recovery = (Total wt. of fat recovered)/(wt. of fat in composite + wt. spike) × 100.

uted to the fact that food samples have been composited and homogenized prior to solvent extraction. Also, it appears that the solvent-to-sample ratio employed is suitable for complete removal of lipids from the composite at the levels tested.

Analytical concentration range. To determine the analytical limits of the method, recovery of a range of weights (0.01 to 6.0 g) of the pure fat blend was evaluated. The results are shown in Figure 1. Below 0.15 g and above 1.5 g of total fat, variability was high, ranging from 99 to 169%. At the low end (<0.15 g), the variance is likely due to background noise and defines the limit of detection. At the high end (>1.5 g fat), variance was probably caused by incomplete evaporation of the chloroform solvent prior to weighing. Thus, for optimal accuracy and precision, the total weight of fat in the food aliquot assayed should be between 0.15 and 1.5 g, which is 3 to 30% by weight of a 5-g aliquot.

In separate assays, a number of blanks (i.e., no sample) were analyzed by the same procedure as samples to evaluate the magnitude and variability of any background "contamination." The "blank" residue weight ranged from 0.0005 to 0.0014 g ($n = 6$), which represents 0.03 to 0.9% of the residue weight for samples in the analytical range (0.15 to 1.5 g total fat). Total fat values were not corrected because of sample-to-sample variability in the "blank" value; thus any residual weight is part of the overall assay variance. This variance can be minimized by taking the precautions described in Table 3.

Precision in repeated assays. In this laboratory, composite A (Tables 1 and 2) is used as a quality control material for total lipid assays, and therefore, more than 200 values have been obtained for this sample over a span of *ca.* two years by several analysts. These data are illustrated in Figure 2. The coefficient of variance (CV, expressed as a percentage) of these measurements was 3.99% ($n = 232$). Although no experiments were performed to determine the respective contributions of sample variance (i.e., composite heterogeneity and/or aliquotting) and assay variance to the overall assay precision, a CV of 3.99% is acceptable for most quantitative lipid assays of food composites in the concentration range typically encountered in food analysis. Less precise measurements would be expected for poorly homogenized samples and/or assay of smaller aliquots, or inadequate analytical technique.

Precision in routine duplicate assays of mixed food composites. The total lipid contents of a variety of mixed food composites ($n > 600$) were assayed in duplicate with the present method over the course of about two years. The moisture content of these composites ranged from 56 to 80%, and mean total lipid from 2.4 to 8.7 g/100 g wet weight. The within-sample CV was 0 to 9% (mean 1.2%, median 0.9%, $n = 608$) and was unrelated to total lipid or moisture content in the range of samples analyzed.

Comparison of total fat content determined by proposed method and by an acid hydrolysis method. Six samples each of two different diet composites were sent to outside laboratories for total fat analysis by acid hydrolysis (AOAC 954.02,

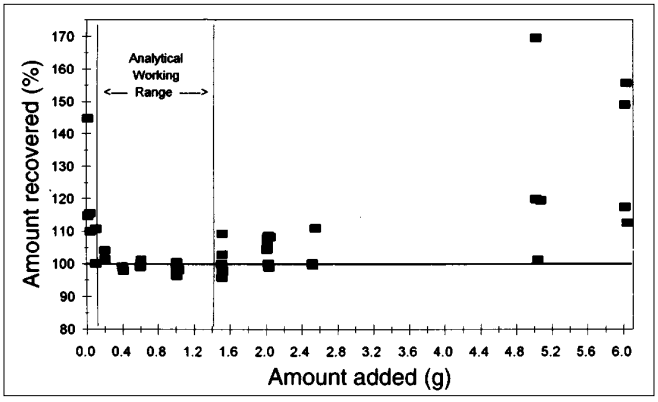

FIG. 1. Recovery of pure fat blend.

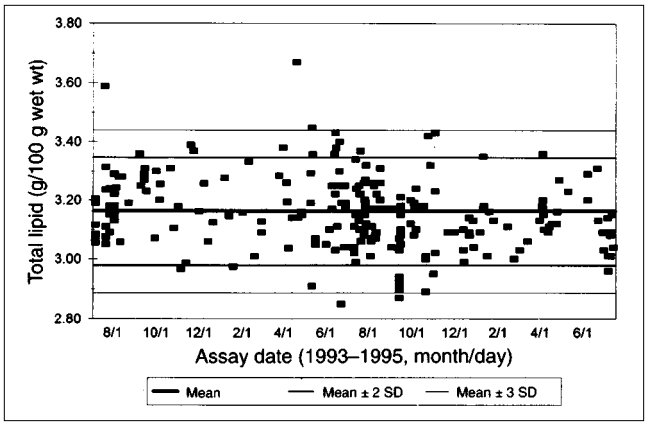

FIG. 2. Control chart for total fat in mixed-food quality control composite ("Composite A"), assayed over two years with proposed method. Mean, standard deviation (S.D.), and % coefficient of variance (CV) were calculated from data obtained between July 7, 1993 and July 15, 1995. For a total of 232 samples assayed, the mean, S.D., and CV (%) were 3.15 g/100 g wet weight, 0.13 and 3.99%, respectively.

TABLE 3
Critical Factors That Affect Accuracy and Precision of Proposed Method

Factor	Control measures
Accurate and precise measurement of volume of chloroform extract aliquotted for gravimetric determination	Calibrate measuring device; set performance criteria for accuracy and precision of measuring device; avoid loss of volatile solvent while measuring and aliquotting; ensure absence of bubbles while aliquotting; equilibrate extracts to constant room temperature before aliquotting
Accurate and precise weighing of tubes	Avoid contamination of tubes (fingerprints, dust/dirt, etc.)—handle with clean, lint-free gloves or tongs; use fresh deionized water in clean water bath; calibrate balance; label empty tubes with permanent marker prior to drying and taring; thoroughly clean all lipid residues from test tubes before use
Homogeneity of sample	Bring frozen samples to room temperature; include thorough mixing of homogenized sample while aliquotting for assay
Sample loss while drying extract	Use gentle stream of nitrogen to avoid splashing sample; keep gas line from contact with sample
Thorough contact of sample with solvent during extraction	Weigh sample into bottom of bottle; use siliconized bottles to prevent adherence of sample to bottle during extraction
Sample contamination	Thoroughly clean all lipid residues from centrifuge bottles before use; avoid contamination of sample with extraneous material (dust/dirt, etc.); use clean nitrogen gas lines for evaporation; use fresh pipet and thoroughly prime measuring device to avoid cross-contamination of extracts during aliquotting
Quality control monitoring	Include control sample with each assay batch to monitor assay performance

945.44, or 922.06) (1). In this procedure, samples are acid-digested prior to extraction with organic solvents. A summary of the results is given in Table 4.

Total fat via acid hydrolysis was lower than that measured by the proposed method (4% lower for Composite A, 11% lower for Composite Z, Table 3). These results suggest a bias between the two methods. It is generally accepted that chloroform/methanol quantitatively extracts all lipid components relative to other solvent extractions. Triglycerides and phospholipids extracted from acid-hydrolyzed samples are recovered as free fatty acids whereas in the chloroform/methanol extraction method, triglycerides and phospholipids are recovered intact. It is possible that some of the more polar fatty acids (shorter-chain fatty acids) are not completely extracted by the acid hydrolysis method and therefore yield lower recoveries of total fat.

TABLE 4
Total Fat Content of Mixed Food Composites A by Proposed Method and an Acid Hydrolysis Method

Method	Composite	*n*	Mean total fat[a] (g/100 g wet)	Standard deviation	CV[b] (%)
Acid hydrolysis	A	6	3.03[b]	0.14	4.5
Proposed method	A	24	3.15[c]	0.07	2.3
Acid hydrolysis	Z	6	3.68[d]	0.23	6.2
Proposed method	Z	6	4.15[e]	0.19	4.6

[a]Means for same composite superscripted with different letters are significantly different ($P < 0.05$ for 95% confidence interval).
[b]See Table 1 for abbreviation. [b,c]$P = 0.006$; [d,e]$P = 0.004$.

DISCUSSION

Based on the validation data presented, the modification of AOAC 983.23 is suitable for routine analyses of total fat in homogenized food composites, especially when high sample throughput is required. The entire extraction is carried out in a single 500-mL centrifuge bottle and does not require the individual stainless-steel blending apparatus per AOAC 983.23. The blending step was eliminated with the rationale that it is unnecessary if foods are already thoroughly homogenized prior to the assay. Additionally, homogenization of foods before aliquotting for assay ensures that subsamples analyzed are representative of the whole food mixture. The modified procedure requires less analyst hands-on time, is applicable to batch processing of samples, does not involve transferring the volatile solvent mixture, and does not require reagent (Clarase®) of uncertain procurability. Sample throughput is increased to *ca*. 22 per day per analyst, and the procedure is less labor-intensive than AOAC 983.23. The chloroform extract has also been used regularly for subsequent chromatographic quantitation of specific lipids, such as fatty acids and cholesterol. Although it is desirable to reduce the use of halogenated solvents in developing new methods (7,8), no suitable substitute for chloroform extraction of lipids has yet been evaluated. The proposed method is reported as a simplification of an established standard procedure.

Although total fat has recently been defined by the FDA Nutrition Labeling and Education Act (NLEA) as the sum of

fatty acids from C_4 to C_{24}, expressed as triglycerides (9), the accuracy of gravimetric total-fat methods with respect to the NLEA definition of total fat remains to be determined. Currently, no standard methodology exists for the determination of total fat by the NLEA definition. The total fat contents for selected diet composites by the proposed method were 3–11% higher than values obtained by acid hydrolysis, another common gravimetric procedure for quantitation of total fat, but the significance of this difference with respect to accuracy of the measured total fat concentration is unknown. On the one hand, it is expected that chloroform/methanol extraction might overestimate total fat because lipids other than fatty acids are quantitated, but on the other hand, low-molecular weight triglycerides, monoglycerides, diglycerides, and free fatty acids might not be extracted and quantitated. Acid hydrolysis also extracts nonfat materials, especially from low-fat or high-carbohydrate samples (7). The only mixed food standard reference material certified for total fat content, NIST SRM 1548, has an acceptable range of ±10% of the certified mean, which is too imprecise to detect significant bias between methods. Total fat in SRM 1548, measured by the proposed method, was 101% of the certified mean and well within the certified acceptable range; however, the reference value is based on gravimetric measurements.

The accuracy and precision, obtained with any gravimetric method, are integrally dependent on the accuracy and precision of weight and volume measurements. The potential bias and variance in these measurements, which cannot be managed with internal standards, coupled with the uncertainty about the chemical entities actually quantitated, are reasons for disfavor of gravimetric assays in general. For quality control to optimize results, it is useful to identify and control critical steps; these points are summarized for the proposed method in Table 4. In addition, a control sample should be analyzed with each assay batch to monitor assay performance.

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