Evaluation of Eight Extraction Methods and Their Effects upon Total Fat and Gas Liquid Chromatographic Fatty Acid Composition Analyses of Food Products[1]

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ABSTRACT

Samples of corn beef hash, frozen turkey pie, frozen beef pie, and beef stew were extracted by eight methods. Methyl esters of the fatty acids contained in the extracted fat residue were prepared with BF_3-methanol reagent and measured quantitatively by gas liquid chromatography. A 4N HCl digest followed by ethyl ether extraction was the most effective extraction method. Total lipid extracted, fatty acid distribution, and triglyceride recovery were the primary evaluation criteria. Recovery studies were carried out on eight different foods ranging from high meat content to pure vegetable shortening.

INTRODUCTION

A series of proposals relating to labeling of foods with information on fat composition has been published in the *Federal Register* (1-2) in recognition of the desire and need of many consumers for such nutritional information. This information might help persons to make changes in their diet which could have potential health benefits.

In view of the forthcoming requirements for product labeling (3), laboratories will require methods for determining the fatty acid content of a wide variety of readily available foods. Over the years, a number of methods has been published, each of which deals with the fatty acid analysis of a particular type of food or food product. Generally, the method performs quite well when applied to that product.

This need for analytical methods and procedures to carry out the anticipated analytical requirements of the new regulation prompted us to undertake the current study. This study is the first of a series to develop methodology that will fill the need of industrial and governmental laboratories to either comply with or enforce the recently published final regulation (3).

The initial samples used in this study were selected for the anticipated difficulty of successfully extracting them. If an extraction method proved successful with these samples, it then was expected that the method probably would have wide applicability. The successful method then was subjected to additional testing on four other foods possessing widely different physical and chemical characteristics. For the purposes of the regulation, the method of choice should be widely applicable, simple, direct, reproducible, and capable of handling a large number of samples quickly. Eight methods, some of which are used widely, were selected for this evaluation study.

PROCEDURES

Materials

The following commercial products were purchased off the shelf in local supermarkets: Libby's corned beef hash; Manor House frozen beef pie; Manor House frozen turkey pie; Dinty Moore canned stew; Skippy peanut butter; Mrs. Filbert's Golden Quarters margarine; and Heinz mayonnaise. A vegetable shortening that is distributed by the USDA as "A Section 416 Commodity for Distribution to Eligible Outlets" also was used. The first four products were utilized in the first experiment to screen out the least desirable methods and the latter four were used for further studies of the best method.

Methods

Sample preparation (A): The first four products listed above were passed through a meat grinder and thoroughly mixed before sampling.

Dry matter (B): Dry matter (DM) determinations were made in duplicate at 60 C and 26 in. vacuum.

Extraction (C), method 1 (4): The Association of Analytical Chemists' (AOAC) Continuous Extraction-Crude Ether Extract Method involves extracting the dried sample 4 hr with anhydrous ethyl ether, using a thimble with porosity permitting rapid passage of ether. The extract then is dried, cooled, and weighed.

Method 2 (5): This method originally was applied to liver samples. The sample is ground in 95% ethanol, diethyl ether is added to make Bloor's reagent, and the mixture is brought to a rolling boil on a magnetic stirring hot plate. The extract is filtered and the process repeated twice more. The combined solvents are removed on the steam bath.

Method 2A: Same as Method 2 but with HCl predigest as outlined under Method 3. Methanol was used in place of ethanol.

Method 3 (6): This method was applied originally to sausage meat. The sample is digested with 4N HCl for 30 min at 60 C and then on a 90 C water bath for 30 min. The fat is extracted three times with ethyl ether; the combined extracts are washed with water; and the solvent is removed.

Method 4 (7): The method involves digestion of the sample in concentrated HCl by boiling 1 hr. Methanol and CCl_4 are added, and the fat is extracted into the CCl_4 layer by shaking. The CCl_4 layer is removed by distillation.

Method 5 (8): In this method, the sample first is warmed in dilute NH_4OH; then HCl is added; and the mixture is boiled. After cooling, the mixture is transferred to a Mojonnier flask where it is extracted first with ethyl ether and then with petroleum ether. The ether layer is drawn off and the solvent evaporated.

Method 6 (9): The sample is homogenized with a 2:1 chloroform-methanol mixture and filtered. The crude extract is washed with water; the chloroform layer is separated; and the solvent is removed.

Method 6A: Same as Method 6 but with HCl predigest, as outlined under Method 3.

The total lipid extract yield was determined for all extraction methods.

Preparation of Methyl Esters

The fat residues from the various methods were dissolved in petroleum ether; the solutions were transferred to 100 ml volumetric flasks and diluted to mark with petroleum ether. Fatty acid methyl esters were prepared by the boron trifluoride method as outlined by the AOAC (10). Aliquots containing 350 mg lipid content were used. Dupli-

[1] Presented at the AOCS Meeting, New Orleans, May 1973.

TABLE I

Total Fat in Foods as Percent of Starting Material as Received[a]

Extraction method[b]	Hash	Turkey pie	Beef pie	Stew
1	7.9	9.3	10.3	3.4
2	15.8	11.2	9.6	4.7
3	12.3	11.1	11.8	4.4
4	13.9	12.2	13.3	3.5
5	14.2	12.2	10.1	3.2
6	11.2	10.7	12.1	3.7
2A	15.2	9.3	11.2	3.6
6A	14.4	10.6	9.2	2.6

[a]Mean of duplicates.

[b]See text for description of particular method.

TABLE II

Fatty Acid Methyl Esters (g)/100 g Stew[a]

Methyl esters	Extraction method[b] 1	2	3	4	5	6	2A	6A
14:0	0.10	0.08	0.12	0.08	0.10	0.10	0.10	0.08
14:1	0.04	0.02	0.04	0.04	0.04	0.04	0.04	0.02
16:0	0.60	0.45	0.64	0.54	0.58	0.61	0.60	0.45
16:1	0.09	0.07	0.10	0.07	0.09	0.10	0.09	0.06
18:0	0.46	0.36	0.50	0.41	0.46	0.47	0.47	0.36
18:1	0.74	0.56	0.80	0.59	0.71	0.78	0.70	0.51
18:2	0.21	0.28	0.30	0.23	0.28	0.30	0.31	0.13
18:3	0.06	0.08	0.14	0.08	0.11	0.12	0.09	0.05
20:0	Tr[c]	Tr	Tr	Tr	Tr	Tr	None[d]	Tr

[a]Mean of duplicates.

[b]See text for description of particular method.

[c]Trace = less than 2% full scale deflection on gas liquid chromatographic chart.

[d]Not detected.

cates were used throughout the study, and, when solvents needed to be removed, a stream of nitrogen was used. The fatty acid methyl ester solutions were filtered into 10 ml volumetric flasks and diluted to the mark with n-hexane. Gas liquid chromatographic (GLC) analyses were performed directly on these solutions. Quantitative recoveries were a major problem with the AOAC method. It was found that 350 mg or larger samples gave ca. 100% yields (11).

GLC

For GLC analyses, the following were used: a Barber-Colman 5000 instrument equipped with H_2 flame detector; 6 ft x 4 mm Pyrex column packed with 15% ethylene glycol succinate; column, 182 C; detector, 252 C; injector, 244 C; argon carrier gas, 14 psi; H_2 pressure, 20 psi; air pressure, 40 psi. These operating parameters fall within the general GLC conditions outlined by the AOAC (12). The calibration procedure used is not that prescribed by the AOAC, since the regulation calls for actual fatty acid wt, rather than percentage distribution. The gas chromatograph was calibrated by injecting four levels of a mixture of equal wt of 6 pure fatty acid methyl esters into the GLC and preparing standard curves in which μg of methyl esters served as the abscissa and response in mm^2 as the ordinate. The extracts then were analyzed by GLC; the response was calculated (mm^2); and the wt of the particular fatty acid ester was obtained directly from the individual fatty acid methyl ester calibration plots by reading the wt corresponding to peak area (response) from the graph. The amount of the individual fatty acid methyl esters then was calculated back to the total ether extract and finally to 100 g product. The volume of sample injected ranged from 1.9-3.0 μliter. Appropriate changes in attenuation were made to ensure good measurable peaks. Peak areas were calculated by the method of ht x wt at half the peak ht.

TABLE III

Fatty Acid Methyl Esters (g)/100 g Beef Pie[a]

Methyl esters[b]	Extraction method[c] 1	2	3	4	5	6	2A	6A
14:0	0.17	0.15	0.17	0.10	0.17	0.19	0.17	0.13
16:0	1.68	1.43	1.79	0.95	1.64	1.83	1.48	1.31
16:1	0.18	0.13	0.18	0.10	0.18	0.19	0.15	0.12
18:0	1.19	0.94	1.20	0.71	1.12	1.25	1.02	0.97
18:1	2.43	1.88	2.43	1.25	2.32	2.55	1.86	1.66
18:2	1.26	1.00	1.25	0.64	1.15	1.21	0.93	0.89
20:0	Tr[d]	Tr	Tr	Tr	Tr	Tr	Tr	Tr

[a]Mean of duplicates.

[b]No 14:1 or 18:3 was found in any of the samples.

[c]See text for description of particular method.

[d]Trace = less than 2% full scale deflection on gas liquid chromatographic chart.

TABLE IV

Fatty Acid Methyl Esters (g)/100 g Turkey Pie[a]

Methyl esters[b]	Extraction method[c] 1	2	3	4	5	6	2A	6A
14:0	0.08	0.15	0.13	0.08	0.12	0.15	0.10	0.13
16:0	1.21	1.86	1.71	1.09	1.49	1.85	1.19	1.65
16:1	0.13	0.18	0.22	0.12	0.18	0.15	0.16	0.16
18:0	0.90	1.32	1.11	0.81	0.97	1.22	0.79	1.09
18:1	1.66	2.00	2.44	1.47	2.10	1.92	1.97	1.90
18:2	1.05	0.66	1.31	0.95	1.18	0.11	0.78	0.57
18:3	0.11	0.26	0.53	0.09	0.45	0.11	0.17	0.20
20:0	Tr[d]	0.02	Tr	Tr	Tr	Tr	Tr	Tr

[a]Mean of duplicates.

[b]No 14:1 in any of the samples.

[c]See text for description of particular method.

[d]Trace = less than 2% full scale deflection on gas liquid chromatographic chart.

TABLE V

Fatty Acid Methyl Esters (g)/100 g Hash[a]

Methyl esters[b]	Extraction method[c] 1	2	3	4	5	6	2A	6A
14:0	0.31	0.33	0.50	0.21	0.40	0.42	0.54	0.40
14:1	0.08	0.13	0.17	0.08	0.15	0.15	0.21	0.13
16:0	1.88	1.86	2.73	1.15	2.19	2.32	2.89	2.19
16:1	0.22	0.36	0.51	0.22	0.40	0.43	0.54	0.40
18:0	1.49	1.32	1.75	0.79	1.52	1.57	1.81	1.50
18:1	2.06	2.60	3.94	1.68	3.10	3.31	4.21	3.12
18:2	0.05	0.77	0.95	0.34	0.66	0.85	1.10	0.89
18:3	0.08	0.32	0.71	0.21	0.57	0.39	0.48	0.44

[a]Mean of duplicates.

[b]No 20:0 detected in any samples.

[c]See text for description of particular method.

Lipoxidase

The Canadian Food and Drug Directorate FA-59 method (13) was used to measure any change in the double bond configuration, i.e. *trans-* vs *cis*; during studies designed specifically to determine the extraction effects, if any, of the extraction method of choice.

RESULTS AND DISCUSSION

The results for DM were as follows: hash 31.9%, turkey pie 17.3%, beef pie 39.0%, and stew 17.3%.

A summary of the total fat extracted expressed as percent of the original starting material is presented in Table I for all methods on all foods. Method 1 yielded the lowest amount of total lipids of all methods for two foods (hash and turkey pie) and the third lowest for beef pie and stew.

TABLE VI

Ranking of Methods: Fatty Acid Methyl Esters/100 g of Product

Food product	Extraction method[a]							
	1	2	3	4	5	6	2A	6A
Stew	5	7	1[b]	6	4	2	3	8[c]
Turkey pie	7	2	1[b]	8[c]	3	4	6	5
Hash	7[c]	6	2	8[c]	4	3	1[b]	5
Beef pie	3	6	2	8[c]	4	1[b]	5	7

[a]See text for description of particular method.

[b]Significantly high at $P<0.05$.

[c]Too low to be considered as part of the group, $P<0.05$.

Methods 2, 3, and 4 gave the highest overall yields of total fat.

The g fatty acid methyl ester(s)/100 g food, as related to the various extraction methods, is summarized in Tables II-V. The results in these tables are a culmination of the various effects previously examined individually, i.e. total fat extracted and the fatty acid composition of the fat extract as affected by the extraction. It can be readily seen that, of the eight extraction methods, Method 3 consistently gave the second highest values in the amounts of fatty acids found for beef pie, turkey pie, and hash extracts and the highest values for stew extracts. Method 3 gave the best overall performance in the essential fatty acid group, indicating that the extraction was quantitatively superior and less damaging to the 18:3 double bonds than the other seven extraction systems.

Table VI presents the ranking of the various extraction methods as related to the respective food products. The methods are ranked with the method showing the highest recovery of fatty acids as number 1, etc. For the purposes of this phase of the study, the assumption that the highest fatty acid values are the most desirable was adopted. The ranking and determination of outliers were performed by the method of Youden (14). Method 3 gave significantly higher fatty acid values for stew and turkey than any other method. Method 3 was ranked number 2 for the remaining foods. No other method exhibited the overall sustained ranking values of Method 3.

Based upon the results discussed above, Method 3 appeared to be the method of choice. To further evaluate the method, two recovery studies using known wt of tripalmitin and trilinolein were conducted. Known amounts of each triglyceride were added to weighed samples of the above individual foods plus four additional foods and extracted by Method 3. Controls were individual food samples of the same size with no added triglycerides. The recoveries were determined gravimetrically. The results of the two recovery studies are summarized in Table VII. The recoveries were excellent both for the unsaturated and polyunsaturated triglycerides, further supporting the previous indication that Method 3 was the method of choice.

One final experiment was conducted to determine whether Method 3 was causing any destruction and damage to the polyunsaturated fatty acids. Samples of pure (99% plus) methyl linoleate were subjected to the rigors of the Method 3 extraction systems with no protection, such as the use of antioxidants. Three such experiments were performed before and after treatment on the methyl linoleate.

TABLE VII

Recovery of Added Tripalmitin and Trilinolein (Single Recoveries)

Food product	Tripalmitin %	Trilinolein %
Hash	99.1	99.5
Turkey pot pie	97.4	99.8
Beef pot pie	97.0	102.3
Stew	97.6	97.8
Mayonnaise	102.2	99.9
Peanut butter	97.9	100.9
Vegetable shortening	99.5	99.6
Margarine	99.5	99.8
Mean	98.8	100.0

Both methods of analysis showed that no destruction or bond shifting occurred during the Method 3 extraction procedure. The GLC and lipoxidase analyses yielded $98.4 \pm 0.4\%$ and $98.9 \pm 0.4\%$, respectively, for the treated samples.

One final consideration was the ease of performing the various methods in the laboratory. Method 3 utilizes standard laboratory glassware and is more easily applied because of its simplicity, than most of the other seven methods tested.

Finally, Method 3 is the method of choice based upon ease of application, fatty acid analysis, recovery studies, and lack of fatty acid alteration during the extraction process.

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[Received May 24, 1974]