Comparison of Various Methods for the Extraction of Total Lipids, Fatty Acids, Cholesterol, and Other Sterols from Food Products

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ABSTRACT AND SUMMARY

Seven methods were used to compare the efficiency of total lipid extraction from 13 samples of eight different food products. The methyl esters of fatty acids and the butyrate esters of sterols were prepared and analyzed by gas liquid chromatography. On the basis of total lipid recovered and amounts of fatty acids and sterols present, a chloroform: methanol procedure was selected as the most effective method.

INTRODUCTION

In 1973, a Federal regulation on the labeling of foods with regard to the cholesterol, fat, and fatty acid content (1) was promulgated. Suitable methods were required to determine the cholesterol content, as well as the fat and fatty acid content, of a wide variety of readily available foods. This need for analytical methods and procedures to carry out the requirements of the regulation prompted us to undertake methodology studies which are still in progress. Reports on methods based on developments in these areas have been sent out on request by this laboratory as Interim Methodology Instructions Nos. 1 and 2 (2,3).

One of the most critical steps in a method for the analysis of food samples for total lipid, fatty acid, and sterol content is the extraction step. Previously, we compared eight extraction methods by analyzing eight different food samples (4). In that study the analysis of fatty acid methyl esters was the main concern. Two methods proved to be satisfactory: a 4 N HCl digestion followed by ethyl ether extraction and a 2:1 chloroform:methanol extraction. Due to its speed, simplicity, and excellent recoveries the HCl method was the method of choice. Since that time, the HCl method was used prior to the gas liquid chromatographic (GLC) determination of cholesterol and other sterols. The results were disappointing in that recoveries of sterols were low in comparison to accepted values in the literature (5). Recently Punwar (6) reported excellent recoveries with the use of a chloroform:methanol system for extraction. This report describes a second study of the extraction problem, using thirteen samples of eight food products and seven extraction methods or variations of these methods.

EXPERIMENTAL PROCEDURES

Materials

Six different potato chips were obtained from the West Coast area. In addition, the following commercial products were purchased in local supermarkets in the Washington, DC area: medium-size eggs, beef-and-pork frankfurters, mayonnaise, deviled ham, canned beef stew, frozen fish sticks, and frozen chicken pie.

Methods

Sample preparation (A): Samples were passed through a meat grinder (where practical) and thoroughly mixed before sampling. The sample taken for analysis should contain ca. 1 g of fat.

Extraction (B), method 1 (7): The sample is digested on a water bath with 4 N 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; the solvent is removed with a nitrogen bleed and the residue is dried in a vacuum oven.

Method 2 (8): A volume of 2:1 chloroform:methanol ca. 20 times the sample weight is added to the prepared sample. The sample-solvent mixture is homogenized ca. 2 min in a blender and then filtered. The crude extract is washed with 0.2 its volume of water and the chloroform layer is separated. The water layer is washed two more times with the solvent, the solvent layers are combined, and the solvent is removed in a flash evaporator.

Method 3: Same as method 2 but with the HCl predigest

TABLE I

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

Food product[b]	Extraction method[c]						
	1	2	3	4	5	6	7
Potato chip I (1)	29.3	32.3	36.1	35.5	28.0	36.0	38.0
Potato chip II (2)	31.2	37.3	37.1	34.2	30.3	31.9	36.9
Potato chip III (3)	31.1	33.6	34.5	31.4	28.4	32.7	35.8
Potato chip IV (4)	31.1	32.2	37.2	34.6	34.8	38.3	34.3
Potato chip V (5)	34.3	33.8	33.1	34.7	30.5	34.5	34.9
Potato chip VI (6)	36.0	34.1	35.8	37.1	35.7	36.6	35.3
Egg yolks (7)	21.9	29.1	14.0	23.9	29.5	18.5	23.2
Frankfurters (8)	29.3	27.7	29.0	29.9	28.0	28.9	29.9
Mayonnaise (9)	76.7	78.8	53.1	77.3	78.6	78.2	78.0
Deviled ham (10)	26.4	27.0	25.8	26.0	25.7	26.8	26.1
Beef stew (11)	3.2	4.2	2.8	4.4	3.8	3.4	4.2
Fish sticks (12)	11.7	10.1	9.7	12.7	10.3	10.0	7.4
Chicken pot pie (13)	10.0	10.8	8.3	10.7	9.3	10.8	10.8

[a]Each value is a mean of two or more analyses.

[b]The sample number in parentheses is the code number used in Tables II-IV.

[c]See text for description of particular method.

TABLE II

Fatty Acid Methyl Esters as g/100 g of Food Product[a]

Food product[b]	Extraction method[c] 1	2	3	4	5	6	7
1	22.5	26.3	26.4	25.8	22.5	24.8	27.5
2	21.8	26.5	23.4	25.1	20.9	22.0	25.1
3	23.5	24.2	22.6	21.6	20.6	23.7	26.9
4	24.5	24.7	25.3	27.8	26.3	26.5	24.1
5	26.9	25.7	24.8	28.6	23.9	26.0	23.6
6	27.6	24.9	26.2	27.5	24.0	25.6	24.7
7	15.7	20.1	10.0	15.6	19.6	15.2	17.9
8	20.4	19.4	19.9	16.5	17.1	17.5	19.1
9	63.3	62.5	41.9	65.0	65.2	66.5	63.9
10	21.6	20.5	21.0	18.9	20.2	20.4	20.6
11	2.3	2.9	2.2	2.7	2.6	2.3	3.0
12	6.2	6.9	6.9	5.0	7.3	3.7	3.3
13	7.8	7.9	7.0	7.2	6.8	5.4	5.9

[a]Each value is a mean of two or more analyses.
[b]See Table I for description of food products.
[c]See text for description of particular method.

TABLE III

Total Sterols[a] as mg/100 g of Food Product[b]

Food product[c]	Extraction method[d] 1 Chol.	Camp.	Stig.	Sitos.	2 Chol.	Camp.	Stig.	Sitos.	5 Chol.	Camp.	Stig.	Sitos.
1								21				10
2								88				90
3								89				88
4				14				26				24
5				15				28				
6				16				27				
7	791				1012				1019			
8	50				56				54			
9	50	54	43	133	51	51	37	104	56	56	53	150
10	51				56				47			
11	11				14				12			
12	13	6	4	19	29	8	6	27	24	6	2	21
13	15	Trace		6	17	2		7	13	Trace		3

[a]Chol. = cholesterol, Camp. = campesterol, Stig. = stigmasterol, Sitos. = sitosterol.
[b]Each value is a mean of two or more analyses.
[c]See Table I for description of food product.
[d]See text for description of particular method.

outlined under method 1.

Method 4: Same as method 1, but with a further extraction of the aqueous layer with 2:1 chloroform:methanol as outlined under method 2. The extracts are combined and the solvent is removed in a flash evaporator.

Method 5 (9): The sample is homogenized in a blender with a 1:2:0.8 chloroform:methanol:water mixture for 60 sec followed by a 2:2:1.8 chloroform:methanol:water mixture for another 60 sec. (The water ratio includes the amount of water in the sample.) The chloroform layer is separated and the solvent is removed in a flash evaporator.

Method 6: Same as method 5 but with the HCl predigest outlined under method 1.

Method 7: Same as method 1 but with a further extraction of the aqueous layer with the solvent system given in method 5. The extracts are then combined and the solvent is removed in a flash evaporator.

The total extract yield was determined for all extraction methods.

Preparation of Esters

The methyl esters of fatty acids were prepared from the petroleum ether extracts of the fat residues in the manner described previously (4).

The butyrate esters of sterols were prepared as described by Sheppard et al. (3).

Gas Liquid Chromatography

The parameters and column conditions used for determining the methyl (4) and butyrate (3) esters have been discussed in previous publications.

RESULTS AND DISCUSSION

A summary of the total crude lipid extracted, expressed as percent of the original starting material, is presented in Table I for all methods on all foods. Any nonlipid material such as carbohydrate is removed during the several washings of the extracts with water. In addition, since the lipid values obtained are in agreement with the generally accepted values in the USDA Handbook No. 8 (5), it is unlikely that extraneous materials would be present in lipid extracts. It is evident from the data that the extra treatment of HCl predigests and additional extractions performed in methods 3, 4, 6, and 7 did not contribute toward improved results. Since they involved time-consuming additional operations, they were eliminated from serious consideration. Overall, method 2 produced somewhat higher yields of total fat than did method 5, and method 2 was superior to method 1 by a substantial margin for the analysis of egg yolks, the most difficult of the samples to extract.

To determine the reproducibility of results obtained for

total lipid content by method 2, an egg yolk sample was analyzed four times. A mean lipid value of 25.0 ± 1.1 g/100 g of sample with a coefficient of variation of 4.4% was obtained.

The total fatty acid methyl ester(s) as g/100 g of food obtained from GLC measurements after the various extraction methods is summarized in Table II. The individual fatty acid composition of the products studied was reported earlier (4). Many of the conclusions derived from Table I can be supported in Table II. Again the egg yolk (sample 7) results for method 2 are much higher than those for method 1 and slightly higher than those for method 5. The explanation for the lower values in Table II (fatty acid methyl esters) compared to those in Table I (total crude lipid) is that a substantial portion of the crude lipid is made up of lipid material other than fatty acids, as shown previously (4). We are conducting experiments to determine the nature and the amounts present of these additional extractable compounds.

Methods 3, 4, 6, and 7 were eliminated for reasons discussed above and are not shown in Table III. The improved recoveries of sterols obtained by using a chloroform: methanol extraction procedure (method 2) instead of HCl:ethyl ether (method 1) are apparent in Table III, especially for egg yolk (sample 7). Generally higher sitosterol values were reported using method 2 for potato chip samples (samples 1-6). Six different egg yolk samples were analyzed by each method. The coefficients of variation were 10.0, 7.8, and 8.0%, respectively, for methods 1, 2, and 5.

To determine the reproducibility of cholesterol values, a pure all-vegetable oil was spiked with cholesterol palmitate and analyzed by method 2; the butyrate esters were prepared from the fatty acid methyl ester mixture. The mean recovery (six determinations) of cholesterol palmitate was 99.5% ± 0.9% with a coefficient of variation of 0.9%.

The superiority of method 2 is shown in Table IV, where the surviving three methods of the starting seven methods are compared in regard to the amount of sterols extracted. Method 2 produced the best recoveries in 10 of the 13 samples analyzed.

Based on data reported in this paper method 2 is the method of choice. Although the differences in GLC fatty acid analysis between method 1 and method 2 were insignificant, the sterol results after extraction by method 2 improved significantly. Interim Methodology Instructions No. 2 (3) was modified to specify method 2 extraction (chloroform:methanol), based on the superior extraction of cholesterol.

TABLE IV

Ranking of Methods on Basis of Recovered Sterols as mg/100 g of Food Product

Food product[a]	Extraction method[b] 1	2	5
1	3	1	2
2	3	2	1
3	3	1	2
4	3	1	2
5	2	1	3
6	2	1	3
7	3	2	1
8	3	1	2
9	2	3	1
10	2	1	3
11	3	1	2
12	3	1	2
13	2	1	3

[a]See Table I for description of food product.

[b]See text for description of particular method.

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