Comparison of Methylene Chloride and Chloroform for the Extraction of Fats from Food Products[1]

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ABSTRACT

Ten food products with a wide range of total fat, fatty acid and sterol content were obtained from a supermarket in the Washington, DC area. These food products were extracted by a new method, using methylene chloride/methanol as the extraction solvent. The results were compared to the Folch et al. procedure, in which chloroform/methanol is the extraction solvent. Total fat was determined, and fatty acid methyl esters and sterol butyrates were prepared and measured by gas liquid chromatography. An analysis of variance indicated that methylene chloride is as effective as chloroform, a suspected carcinogen, in the Folch et al. extraction of total fat, fatty acids and sterols from foods.

INTRODUCTION

Extraction is one of the most critical steps in the analysis of foods for total fat, fatty acids and sterols. Previous studies (1,2) have shown that the Folch et al. method (3), which uses chloroform/methanol (2:1,v/v), is the most effective and satisfactory extraction procedure for subsequent determination of total fat, fatty acids and sterols in various food products. However, there is some evidence indicating that chloroform may be a carcinogen and that long-term exposure may cause liver enlargement and kidney damage in man (4,5). There is considerable evidence that chloroform induces malignant tumors in the liver, kidney and other organs in mice, rats and dogs (4,5). Therefore, it is desirable to minimize the use of chloroform wherever feasible.

Methylene chloride (dichloromethane, methylene dichloride) is similar to chloroform in its physical and chemical properties but is much less hazardous (4). The American Conference of Governmental Industrial Hygienists has recommended and the Occupational Safety and Health Administration has adopted a safety standard in which the permitted maximum level of exposure to methylene chloride is 10 times higher than the safety standard for chloroform (50 ppm vs 500 ppm) (6,7).

Methylene chloride is a logical replacement for chloroform since reducing the risk of carcinogenicity is desirable. This study was conducted to evaluate the effectiveness and suitability of methylene chloride as a replacement for chloroform in the Folch et al. lipid extraction method by analyzing 10 different food products containing various amounts of lipids.

EXPERIMENTAL PROCEDURES

Materials

Ten food samples, beef stew, chicken pot pie, corned beef, deviled ham, mayonnaise, potato chips, salad dressing, canned meat, sausage and small hot dogs, were purchased from a local supermarket.

Methods

Sample preparation. The foods were thoroughly homogenized in a Waring blender before subsampling. Each subsample taken for analysis contained ca. 1 g fat.

Extraction. Method 1: chloroform/methanol extraction method (8).

Method 2: same as method 1 except that methylene chloride was substituted for chloroform.

Preparation of esters. The methyl esters of the fatty acids (FAME) were prepared as described by Sheppard et al. (1). The butyrate derivatives of the sterols were prepared by reacting an aliquot of the FAME solution with butyric anhydride/pyridine solution (2:1, v/v). The details of this procedure have been described by Sheppard et al. (9).

Gas liquid chromatography (GLC). The parameters and column conditions used for determining the methyl and butyrate esters have been published (1,9).

Recovery Study

Cholesterol palmitate (0.1 g/g food lipid) was added to samples of the 10 foods and the spiked samples were homogenized. The homogenates were then extracted using either method 1 or 2. The amount of cholesterol palmitate recovered was determined gravimetrically; the difference between the total fat obtained from the spiked sample and a nonspiked duplicate sample was attributed to cholesterol palmitate.

Statistical Method

The data were analyzed using the type IV sum of squares generated in an analysis of variance by the SAS computer program (10).

RESULTS AND DISCUSSION

The amount of total fat extracted using chloroform/methanol (method 1) and methylene chloride/methanol (method 2) is presented in Table I. Both methods gave essentially the same total fat values for each product. The analysis of variance showed no overall significant difference ($p > 0.05$) between lipid values obtained due to solvent

TABLE I

Total Fat Content (g/100 g Product) of Foods[a]

Food product	Extraction method	
	Chloroform/methanol	Methylene chloride/methanol
Mayonnaise	81.27	80.96
Salad dressing	50.10	49.09
Potato chips	34.01	33.67
Sausage	32.08	31.34
Canned meat	30.19	31.92
Deviled ham	25.83	25.67
Small hot dog	23.24	22.82
Corned beef	12.27	12.12
Chicken pot pie	10.30	10.23
Beef stew	4.18	4.00

[a]Each value is the mean of 2 or more analyses.

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system used. As expected, the total fat content varied among food products analyzed, ranging from 4 g/100 g beef stew to more than 80 g/100 g mayonnaise. These values agree with the generally accepted values in the USDA's Handbook No. 8 (11) and those reported by other workers (1,2,8,12).

Data for the FAME of foods are summarized in Table II. Values for nine FAME, which are the fatty acids most commonly found in foods, were obtained by GLC. An analysis of variance was calculated for each fatty acid to assess the effect of solvent and solvent-product interaction. The results revealed no statistically significant differences in FAME caused by extraction with the two different solvents. The FAME pattern obtained for each product was not affected by the extraction methods. The interaction of product and solvent was also not significantly different ($p > 0.05$) for any of the nine fatty acids, i.e., the solvent did not affect the mean amount of each FAME for all products. The FAME pattern differed from product to product because of the nature of the various lipid sources used in the food products. The results for mayonnaise, potato chips, deviled ham, chicken pot pie and beef stew are consistent with previously reported values (1,8,12,13).

The sterol content of foods, expressed as mg/100 g product, is presented in Table III. Individual sterol components, such as butyrate esters, were quantitatively determined by GLC. There was no significant difference ($p > 0.05$) between solvents on the sterols extracted, nor could a significant interaction of solvent and product be demonstrated. Thus, the choice of solvent did not affect the mean amount of any sterol measured. Furthermore, this was true for all products since in no case did the interactions of product and solvent reach a significant difference ($p > 0.05$). The amount and type of sterols varied among different food products (Table III). Campesterol was found only in mayonnaise and salad dressing. Cholesterol was present in all samples except potato chips. Stigmasterol was found in beef stew, corned beef, mayonnaise and salad dressing. Sitosterol was found in beef stew, chicken pot pie, mayonnaise, potato chips and salad dressing.

The data obtained from the recovery of added cholesterol palmitate are summarized in Table IV. Both extracting solvent systems produced excellent cholesterol palmitate recoveries. The mean recovery value of method 1 was 98.90%, with a standard deviation of 1.99%. The mean recovery value for method 2 was 99.11%, with a standard deviation of 2.03%. There was no statistical difference ($p > 0.05$) between the two solvent systems tested.

Based on the data obtained in this study, methylene chloride is a suitable replacement for chloroform in the Folch et al. method for extracting total fat, fatty acids and sterols from foods, regardless of the food source or amount of lipid present.

A hexane/isopropanol extraction method that also avoids the chloroform toxicity of the Folch et al. method has been recently reported by Hara and Radin (14). Their method was satisfactory for extracting lipids from rat and mouse brains; however, further studies are needed to evaluate the suitability of the Hara and Radin method for the extraction of lipids from food products.

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TABLE II

Fatty Acid Content (g/100 g Product) of Foods[a,b]

Food product	Chloroform/methanol extraction								Methylene chloride/methanol extraction							
	14:0	14:1	16:0	16:1	18:0	18:1	18:2	18:3	20:0	14:0	14:1	16:0	16:1	18:0	18:1	18:2	18:3	20:0
Mayonnaise	0.54	ND[c]	12.22	ND	4.55	26.49	31.02	2.95	ND	0.54	ND	11.69	0.08	4.80	26.17	31.28	3.25	ND
Salad dressing	0.14	ND	5.93	0.12	2.55	13.78	22.31	3.12	ND	0.09	ND	5.93	0.15	2.57	13.94	23.46	3.28	ND
Potato chips	0.43	ND	9.94	0.36	1.32	7.88	15.43	0.13	ND	0.39	ND	8.27	0.36	1.16	7.27	16.62	0.15	ND
Sausage	0.96	0.43	8.31	1.55	5.03	15.02	0.95	0.41	0.63	1.07	0.46	7.95	1.76	4.94	14.64	0.67	0.43	0.69
Canned meat	0.52	ND	7.84	1.06	3.57	13.99	2.10	ND	ND	0.56	ND	8.12	1.12	3.60	14.10	1.98	ND	ND
Deviled ham	0.46	ND	6.20	0.98	2.79	12.11	2.44	ND	ND	0.50	ND	6.42	0.95	2.89	12.13	1.84	ND	ND
Small hot dog	0.43	0.18	5.37	1.02	3.04	9.74	0.69	0.23	0.08	0.42	0.17	5.31	1.02	3.01	9.74	0.60	0.32	0.07
Corned beef	0.37	0.15	2.92	0.53	2.20	4.68	0.16	0.21	ND	0.35	0.14	2.86	0.53	2.16	4.64	0.19	0.17	ND
Chicken pot pie	0.14	0.01	2.12	0.44	1.52	4.47	1.83	0.15	0.02	0.15	0.01	2.28	0.40	1.42	4.40	1.48	0.11	0.02
Beef stew	0.14	0.07	0.94	0.33	0.73	1.73	0.16	0.07	ND	0.13	0.07	0.92	0.29	0.66	1.74	0.17	0.06	ND

[a]Each value is the mean of 2 or more analyses.
[b]Fatty acids were determined as their methyl esters by gas liquid chromatography.
[c]ND = none detected.

TABLE III

Sterol Content (mg/100 g Product) of Foods[a]

Food product	Chloroform/methanol extraction				Methylene chloride/methanol extraction			
	Cholesterol	Campesterol	Stigmasterol	Sitosterol	Cholesterol	Campesterol	Stigmasterol	Sitosterol
Mayonnaise	19.00	16.68	27.11	123.84	19.93	14.85	22.98	127.38
Salad dressing	32.49	14.78	17.38	75.22	36.43	15.50	17.88	75.46
Potato chips				136.15				130.98
Sausage	82.95				83.35			
Canned meat	64.02				63.85			
Deviled ham	57.82				57.04			
Small hot dog	34.84				35.85			
Corned beef	71.30		8.78		70.55		7.71	
Chicken pot pie	22.69			7.13	24.55			5.00
Beef stew	14.50		1.81	1.21	14.71		2.51	1.75

[a]Each value is the mean of 2 or more analyses.

TABLE IV

Recovery (%) of Added Cholesterol Palmitate from Foods[a]

Food product	Extraction method	
	Chloroform/methanol	Methylene chloride/methanol
Mayonnaise	100.31	100.32
Salad dressing	98.16	99.37
Potato chips	95.10	94.15
Sausage	99.08	99.60
Canned meat	100.10	97.11
Deviled ham	99.59	100.37
Small hot dog	96.46	99.41
Corned beef	102.03	101.06
Chicken pot pie	99.72	100.00
Beef stew	98.43	99.70
Average ± SD	98.90 ± 1.99	99.11 ± 2.03

[a]Values are the result of single or duplicate determinations.

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