Improved Procedure for Extracting Food Fatty Acids

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Abstract

Estimations of the fat content of food are generally based on the weight of the fraction extracted by a solvent. Unfortunately these solvents extract varying amounts of substances which are nonlipids, and they fail to extract all of the fatty acids, especially those present in complex forms. Though current procedures are simple, they are unreliable. Calories contributed by food fats can be calculated accurately only from data on the total fatty acid content of these foods.

An improved method for the complete extraction of food fatty acids is described. This method involves an extraction of food samples with chloroform:methanol (2:1) both before and after treatment with 2 N hydrochloric acid in methanol, removal of the solvent from the combined extracts, and then extraction with chloroform.

This method was compared with the AOAC method in the analysis of 18 foods for fatty acid content. The values obtained by the new method were higher in every case, and significantly higher in most cases, due primarily to a more complete extraction of the bound fatty acids. The usefulness of the new method in the routine analysis of foods was demonstrated in 58 additional food samples.

Introduction

THE USUAL FOOD ANALYST is concerned with the estimation of the total amounts of the various nutrients in foods, and has little interest in their nutritional potential. The analytical values reported in food tables (24) are sometimes too low because of analytical inaccuracies. For instance, the ether extract of a food is commonly assumed to represent its lipid or fat content, in spite of the fact that, first, not all the lipids in foods are extracted by ether, and secondly, ether extracts substances which are not lipids. In a review published 24 years ago Lovern (17) discussed the occurrence of fatty acid derivatives in plant and animal tissues, and remarked that "It is too well known to require detailed reference here that a considerable proportion of the lipoidal matter of most tissues cannot be extracted by simple contact with the usual lipoidal solvents, even although the 'bound' lipoids once obtained free, are quite soluble in such solvents." Recent studies have revealed the presence of many complex lipids (11) which are sufficiently polar to make extraction with petroleum ether difficult.

Even though it has been known for a long time that the simple extraction with a fat solvent will yield unreliable results (2), most of the data on the fat or lipid content of foods were obtained this way. Harris (12) has suggested that it is more correct to express the "fat content" of foods in terms of the total fatty acid content, a value which can be determined with considerable accuracy.

In preparation for a careful investigation of the fatty acid composition of prepared foods, the efficiencies of several published methods were compared: Folch (9,10) Dambergs (7) Bligh (4) Böttcher (5) Insull and Ahrens (13) and the Soxhlet extraction (20). Because none of these methods removed all of the fatty acids from all samples, an improved method was developed using a double extraction procedure in which the solvents are chloroform-methanol (6) and methanolic HCl (1). This method will be described, and then compared with the official AOAC method (20) currently used in food analysis in the United States.

Materials and Methods

Samples

Samples of prepared foods were collected in various parts of the United States, packed immediately in cartons with carbon dioxide ice, shipped frozen to MIT by air express, and stored at −20F until analyzed.

Moisture Content

The moisture content was estimated by placing samples in tared dishes in a vacuum oven under 28 in. vacuum at 65C until constant in weight.

Soxhlet Extraction Method

The dried sample was pulverized by mortar and pestle, placed in a Soxhlet thimble and extracted with petroleum ether (reagent grade, peroxide-free, redistilled) for 17 hr (20).

Cold Extraction Method

The method of Rhodes and Lea (14) was closely followed. All solvents were reagent grade. The sample was weighed, then macerated with a top driven Servall mixer under nitrogen atmosphere.

Double Extraction (MIT) Method

Reagents. 1) Methanolic hydrochloric acid, 2N. Anhydrous HCl was prepared reacting concentrated H_2SO_4 with NH_4Cl, and bubbling the gas through methanol until the required concentration is obtained. 2) Methanol, absolute. 3) Ethanol, absolute. 4) Ethanol—KOH 0.5N. 5) Ether, anhydrous diethyl ether.

Preparation of Sample. A food sample, which had been previously slurried and analyzed for moisture content (21) was weighed into two round bottom flasks. If the sample contained a large amount of carbohydrate, it was desirable to stir in anhydrous sodium sulfate until the mixture was granular in texture. Chloroform-methanol (2:1, v/v) was added to the sample in the proportion 25 volumes per gram, and refluxed on a steam bath for one hour. The insoluble material was filtered on a Buchner funnel, and the residue washed with hot chloroform. The last traces of water from the combined filtrates were removed azeotropically under reduced pressure following the addition of small amounts of ethanol. Chloroform was added to the residue and flushed with nitrogen.

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TABLE I

Total Lipid and Total Fatty Acid Contents of Processed Foods as Estimated by the AOAC Soxhlet Method and by the MIT Double Extraction Method

No.	Description	Moisture, %	AOAC-Soxhlet			MIT-Double extraction		
			Total lipid, %	Total fatty acid, %	Fatty acid in lipid ext., %	Total lipid, %	Total fatty acid, %	Fatty acid in lipid ext., %
	Cereal and farinaceous products							
1.	Cake, hoe	46.9	9.4	.....	.....	14.2	13.1	92.1
2.	Cake, spice	23.7	5.4	4.8	88.9	.....	.....	.....
3.	Cake, spice	23.8	6.2	5.6	90.3	10.1	9.0	91.1
4.	Cupcake, white	23.0	8.2	7.0	85.4	11.0	9.0	81.8
5.	Cupcake, white	23.2	9.4	8.3	87.4	10.8	8.8	81.8
6.	Date-nut, bar	9.4	.....	.....	.....	.....	24.3	.....
7.	Date-nut, bar	9.0	22.6	.....	.....	.....	22.1	.....
8.	Donut	16.9	16.6	.....	.....	17.7	16.3	92.1
9.	Pie, apple	53.7	8.5	.....	.....	9.9	9.0	82.5
10.	Pie crust (N.F.)	1.6	28.6	.....	.....	29.5	27.8	93.0
11.	Pie crust (N.F.)	1.5	.....	.....	.....	29.9	27.4	91.5
12.	Pie crust, hot water	4.5	32.0	27.6	86.3	37.0	35.2	94.0
13.	Pie crust, hot water	5.4	31.9	26.9	84.3	35.0	34.2	97.6
14.	Pie, lemon crust	43.4	8.0	.....	.....	10.0	8.2	81.4
15.	Pizza pie	37.8	10.9	9.5	87.2	14.8	11.9	80.5
16.	Pizza pie	47.9	10.6	8.2	88.1	12.5	10.8	86.2
17.	Rice fried	53.4	10.4	.....	.....	12.1	10.8	89.2
18.	Tortilla (corn cake)	43.0	0.8	.....	.....	4.1	20.4	57.3
	Dairy products							
19.	Ice cream, van.	59.2	.....	.....	.....	11.8	10.9	86.9
	Eggs & poultry							
20.	Chicken fried (leg & thigh, Hydro.)	59.6	12.0	.....	.....	13.4	11.4	84.9
21.	Chicken fried (leg & thigh, corn oil)	60.8	11.0	.....	.....	12.8	10.4	81.8
22.	Chicken oven fried (leg & thigh)	52.8	.....	.....	.....	16.0	13.4	83.9
23.	Chicken oven fried (breast & wing)	51.4	.....	.....	.....	.....	12.5	.....
	Fats & oils							
24.	Grease, fried meat	1.4	4.3	.....	.....	97.7	91.8	94.0
25.	Margarine (brand F)	16.0	.....	.....	.....	81.9	78.0	94.6
26.	Margarine (brand M)	16.2	.....	.....	.....	81.3	75.8	93.3
	Fish & shell fish							
27.	Clams, fried	26.0	26.6	24.1	90.6	29.6	26.8	90.6
28.	Fish cake, fried	60.7	0.8	.....	.....	2.8	2.4	34.3
29.	Haddock, baked Spanish sauce	80.4	.....	.....	.....	2.1	1.4	68.1
30.	Haddock, baked Spanish sauce	79.0	.....	.....	.....	1.8	1.2	69.2
31.	Haddock, fillet raw	78.5	0.1	.....	.....	.....	.....	.....
32.	Haddock fillet fried	71.7	4.3	.....	.....	5.3	4.1	80.8
33.	Haddock fillet fried	71.4	3.6	.....	.....	4.5	3.5	78.6
34.	Haddock, raw	77.3	0.3	0.2	45.4	1.4	0.5	38.7
35.	Haddock, fried	67.9	5.3	4.3	81.1	7.4	5.9	79.8
36.	Sardines	66.2	6.2	5.0	83.3	8.1	6.0	74.3
37.	Sardines	64.6	8.4	6.2	73.8	.....	.....	.....
38.	Tuna fish, canned	55.6	17.9	16.4	91.6	22.2	20.4	91.7
	Food combinations							
39.	Aburage (fried bean curd)	51.9	20.0	.....	.....	20.8	18.5	89.0
40.	Chicken chow mein	87.3	1.7	.....	.....	2.2	1.2	56.5
41.	Chile con carne	77.4	.....	.....	.....	.....	2.9	.....
42.	Chile con carne	76.9	.....	.....	.....	.....	3.2	.....
43.	Chile & cheese fondue	82.0	3.5	.....	.....	8.9	6.8	76.0
44.	Macaroni & cheese	72.4	4.1	3.6	87.8	6.9	5.8	84.8
45.	Shrimp tempera fritters	44.8	7.9	.....	.....	.....	.....	.....
46.	Tuna-macaroni casserole	77.3	2.9	.....	.....	3.8	3.3	88.4
47.	Tuna-noodle casserole	65.5	.....	.....	.....	3.6	2.9	79.9
48.	Tuna-noodle casserole	67.7	.....	.....	.....	.....	3.8	.....
	Meats and Meat Products							
49.	Bacon lean	13.0	37.0	.....	.....	45.6	41.7	91.1
50.	Beef, corned	58.2	18.6	.....	.....	18.9	17.0	90.5
51.	Beef, ground	65.2	13.1	.....	.....	14.4	10.9	75.6
52.	Beef, ground round	53.7	17.0	15.2	89.6	18.4	17.1	93.5
53.	Beef, ground round	55.4	17.4	16.1	92.5	18.1	16.5	91.2
54.	Beef, roast	52.7	17.1	.....	.....	19.2	16.9	88.5
55.	Goat, roast	53.8	13.1	.....	.....	.....	.....	.....
56.	Salt pork, pan fried	18.3	.....	.....	.....	57.5	54.4	94.4
57.	Sausage	44.7	25.1	.....	.....	26.6	23.6	88.6
58.	Veal patty (mock)	66.2	.....	.....	.....	5.9	4.7	79.9
59.	Veal patty (mock)	64.6	.....	.....	.....	6.3	4.8	76.3
60.	Veal steak, Creole	66.5	.....	.....	.....	4.8	3.8	78.4
61.	Veal steak, Creole	61.4	.....	.....	.....	9.8	8.2	84.1
	Nuts & Legumes							
62.	Beans, English pink	68.8	1.5	.....	.....	2.3	1.6	68.0
63.	Bean, navy soup	85.9	1.4	.....	.....	1.7	1.2	71.4
64.	Peanut, butter	0.4	50.8	.....	.....	51.5	46.8	91.0
	Special Dietary Foods							
65.	Bologna, prep.	52.4	26.9	.....	.....	26.9	25.1	92.9
66.	Cheese, prep.	35.3	30.9	.....	.....	31.3	28.6	91.5
67.	Ice cream, van. prep.	63.2	9.1	.....	.....	9.8	9.3	90.4
68.	Liquid diet, low fat	73.7	0.2	.....	.....	1.2	0.6	56.1
69.	Liquid diet, low fat	74.2	0.2	.....	.....	.....	.....	.....
70.	Liquid diet	80.3	2.6	.....	.....	3.2	2.6	82.5
71.	Liver sausage, prep.	47.7	27.8	.....	.....	27.6	23.7	85.7
72.	Milk fluid, prep.	88.0	4.0	.....	.....	6.4	5.9	92.8
	Vegetables (tubers)							
73.	Potatoes, french fried	42.3	13.1	.....	.....	14.0	12.6	90.8
74.	Potatoes, french fried	56.6	8.8	8.1	92.0	11.4	10.6	81.4
75.	Potatoes, escalloped (marg.)	75.3	3.7	.....	.....	4.2	3.7	87.5
76.	Potatoes, escalloped (corn oil)	75.0	4.3	.....	.....	.....	.....	.....

The insoluble residue remaining from the chloroform-methanol extraction was transferred to a round-bottom flask using 2N methanolic HCl, the mixture refluxed on a steam bath for 2 hr, filtered hot on a Buchner funnel, and washed with methanol-ether (1:1, v/v). The filtrate was transferred to a separatory funnel, and ether and water added until two phases formed. The ether layer was removed, and the aqueous layer extracted twice more with ether. The combined ether extracts were washed with water until free from acid and transferred into a round-bottom flask. The solvent was removed by distillation until only a few milliliters remained in the flask. Last traces of water were removed by the addition of ethanol and by further evaporation on a rotary evaporator under a mild vacuum. Chloroform was added to the residue, and the mixture stored under nitrogen.

The two chloroform re-extractions were pooled, and then filtered and washed with chloroform under suction through Celite, which was retained on filter paper supported by a coarse sintered glass funnel. The filtrate and washings were evaporated in a tared flask, the solvent removed on a rotary evaporator and the flask brought to constant weight to obtain the *total lipid* value.

Total Fatty Acid. The total lipid extracts were refluxed for 90 min with ethanolic 0.5N KOH which was added in the proportion of 20 ml per gram of lipid. The saponified mixture was transferred to a separatory funnel by rinsing with water made acid by the addition of 2N H_2SO_4. The aqueous layer was extracted three times with 50, 25, 25 ml portions of ether, respectively, the ether layers pooled and washed twice with water. The fatty acids were isolated from the unsaponifiable matter by extraction into 2% aqueous KOH. To avoid emulsions, the first extractions were carried out by gently rotating the funnel. After the bulk of fatty acids had been extracted, the funnel could be gently shaken with the ether layer without developing undesirable emulsions. Fatty acids were again formed by the addition of 5N H_2SO_4 and extracted into ether as described previously. Combined ether extracts were washed with water until free from mineral acid, the ether evaporated and last traces of water removed by the use of ethanol. The residue was weighed to obtain the *total fatty acid* value.

In the analysis of milk and milk products, it was preferable to dry the final ether extract over anhydrous Na_2SO_4, filter to remove the last traces of solvent under reduced pressure at room temperature, and weigh.

Results and Discussion

The total lipid and the fatty acid contents of the samples, expressed as percentage of the wet sample weight and percentage of the total lipid, are presented in Table I. Data on several of the food samples extracted with petroleum ether by the AOAC Soxhlet method are also presented in this table. All values in Table I are reported on a wet basis. The moisture values have been included to permit calculation to a dry basis. Each value represents the mean of at least duplicate estimations. When significant differences were noted, the analyses were usually repeated in triplicate to confirm. Where two results are given for one foodstuff, these represent data from the analysis of two samples taken from the same batch of material.

The data in Table I show that the values for both total lipid and total fatty acid content as determined by the double extraction (MIT) method are higher than those obtained by the Soxhlet method. The two series of results differ by 0.0% to 93.3%. The differences were apparently due to peculiarities in lipid composition and the degree of lipid binding, rather than the total amount of lipid present. However, it is difficult to compare the results obtained by these two methods of analysis in terms of the composition of the food lipids since the physical and chemical characteristics of the natural food lipids were undoubtedly altered by the processing.

Data on the total lipid and fatty acid contents of four different foods prepared in different ways (fried, boiled, baked) and analyzed by three different methods are presented in Table II. All four samples were extracted least efficiently by the Soxhlet procedure and best by the double-extraction (MIT) method.

The first extraction with chloroform-methanol yielded approximately 95% of the total lipid (Table III). Since no additional lipid was obtained by additional extractions, it may be assumed that the extraction was complete. Treatment with methanolic-HCl in the second stage of extraction converts fatty acid ester complexes to the corresponding methyl esters. It is admitted that water-soluble fragments resulting from the methanolysis of the residual complex lipids will affect the estimation of total lipid. However, this error is small since the second extraction removes less than 5% of the total lipids in any foodstuff.

The data in Table I indicate that the two methods yield divergent results when the food samples contained significant amounts of bound fatty acids. Foods like beef pattie and corned beef in which triglyceride is the predominant lipid, and which consequently show high fatty acid to total lipid ratios, show small differences between total lipid and fatty acid values as determined by the two methods. Triglycerides occur in animals and plants mostly in the free form (18). Because of this and because they are soluble in nonpolar solvents, such as petroleum ether, they are readily extracted. Agreement was noticed with prepared foods such as bologna and

TABLE II
Comparison of Soxhlet Extraction (20), Cold Extraction (14), and Double Extraction MIT Procedure in the Lipid Analysis of Different Foods

Description	Soxhlet method				Cold extract ($CHCl_3$:MeOH 2:1)				Present method (Double extract)			
	Total % wet	Lipid % dry	% FA of Sample	% FA of Fat	Total % wet	Lipid % dry	% FA of Sample	% FA of Fat	Total % wet	Lipid % dry	% FA of Sample	% FA of Fat
Chicken, leg & thigh fried in hydrog. veg short.	12.0	29.0	10.5	87.0	12.4	29.8	10.1	81.3	13.4	32.3	11.4	84.9
Chicken, leg & thigh fried in cornoil	11.0	28.0	9.8	89.7	12.4	31.5	10.6	85.8	12.8	32.6	10.4	81.8
Soup, Navy bean, boiled	1.4	9.71	1.2	82.9	1.6	11.3	1.3	81.4	1.7	11.7	1.2	71.4
Pie, lemon crust, baked	8.0	14.0	7.3	93.0	9.4	16.6	8.0	84.6	10.0	17.7	8.2	81.4

TABLE III
Total Lipid and Fatty Acid Content of the Fractions Obtained in the Double Extraction MIT Procedure Used in This Study

No.	Description	Wt. of original sample (gm)	Extraction fraction	Wt. of total lipid (gm)	Lipid in fraction (% of sample)	Fatty acids in fraction (% of sample)	Total lipid (% of wet sample)	Total fatty acid (% of wet sample)
NJ226A	Cookies, oatmeal	3.4489	1	1.0608	30.8	28.8	32.5	29.6
			2	0.0590	1.7	0.8		
NJ226B	Cookies, oatmeal	3.4372	1	1.0770	31.3	....	33.5	....
			2	0.0772	2.2	....		
NJ229A	Pie crust	2.3830	1	0.6620	27.8	26.1	29.5	27.8
			2	0.0396	1.7	1.7		
NJ229B	Pie crust	2.3533	1	0.6665	28.3	25.8	29.9	27.4
			2	0.0384	1.6	1.6		
NJ243	Steak veal, Creole	3.0001	1	0.2818	9.4	7.9	9.8	8.2
			2	0.0130	0.4	0.3		

liver sausage because these were especially prepared for dietetic purposes and lipids, mostly triglycerides high in polyunsaturated fatty acids, were added during the manufacturing process. These added lipids were not bound, and were easily extracted.

In contrast to this group, there are foods such as raw haddock which contain proportionately large amounts of bound fatty acids (19). These fatty acids are difficult to extract by simple solvent treatment, and as a result the data obtained by the two extraction methods are in serious disagreement. This difference was evident also in the fried fish (haddock) which had been cooked in vegetable shortening.

The lipids of some of the farinaceous-products (i.e., pie crust, cakes and cupcakes) were incompletely removed by simple extraction with petroleum ether, possibly because significant amounts of the fatty acids in wheat flour are bound, probably in lipoproteins during "wetting" and "doughing" (22). However, the presence of monoglycerides in shortening and of small amounts of galactosyl glycerides in wheat flour and other cereal products (6) may also have contributed to the difference in the total fatty acid values of baked products, since this type of polar lipid is readily extracted by chloroform-methanol, but not by petroleum ether.

Wide differences were noted between the petroleum ether extraction and the double extraction (MIT) values of a third group of foodstuffs (i.e., fried meat grease, fried fish cake). Bound lipids are formed during the storage of foods and food products (15), and when mixtures of protein and lipid are heated in air (26). These complexes must be cleaved (such as by treatment with mineral acid) before they can be extracted with solvents. Almquist (2) has shown that the ether extract of fish meals decreases considerably (from 6.8% to 2.8% during storage for 331 days), and most of this decrease occurs during the first two months of storage. In fried foods this is masked by the absorption of large quantities of oil which is easily extractable. Aburage, a fried bean curd, for example, gave a 20.0% value by the Soxhlet method and 20.8% by the double extraction (MIT) procedure. Practically the same amount was extracted by the two methods because most of the lipid was contributed by the triglycerides in the frying oil.

It should be pointed out that the percentage of fatty acid in the total lipid was lower in the double extraction (MIT) method than in the Soxhlet method. This may be due in part to the fact that in the double extraction (MIT) method nonlipid material could be extracted either as residues esterified to fatty acids or as contaminants of the lipid extract. Another possible explanation is that complex lipids which contain a smaller proportion of fatty acids than triglycerides are more readily extracted by the chloroform-methanol than by petroleum ether. Rectification of the chloroform-methanol extract with chloroform is insufficient to prevent the inclusion of nonlipid material in the total lipid extract. It has long been recognized that the solubilities of lipid and nonlipid compounds present in plant and animal tissues vary depending on the nature of the solvent.

The results obtained in this study clearly indicate that the continuous extraction of desiccated food with petroleum ether does not remove all the lipid contained in these foods. The unextracted fatty acids are probably more unsaturated than those easily extracted, and may be of greater nutritional significance. Presumably these bound lipids are released during digestion and are nutritionally available to human beings.

Though there is dearth of information on the extent to which complex lipids are digested and absorbed, it is apparent that hydrolysis of phosphatidyl ethanolamine, phosphatidyl choline (16) and monophosphoinositide (8) can take place by the action of pancreatic enzymes (23). It is however, noteworthy that phosphatidyl choline may be absorbed to some extent without hydrolysis (3).

The nutrition scientist is more interested in specific fatty acids than in total lipids. In order to calculate accurately the caloric value of a food it is necessary to know the total content of fatty acids, and this value cannot be obtained by simple extraction with petroleum ether. Often it is important to know the exact amounts of saturated, monounsaturated and polyunsaturated (linoleic and arachidonic) fatty acids, and the proportions that are in *cis*- and *trans*-isomeric forms. The ultimate method of lipid analysis of foods will, therefore, involve the hydrolysis of the lipid fraction, the extraction of the released fatty acids, and the quantitative estimation of each fatty acid.

Calculation of the calorie value of a food from data on the "fat content" as determined by simple extraction with solvents is not entirely reliable since significant amounts of bound lipids may not be extracted and since significant amounts of the extracted material are not fatty acids and, therefore, have lower caloric value.

More valid data can be obtained by extracting all the fatty acids from the food sample, isolating these fatty acids and quantitating each of them.

It is encouraging to note that data of this type are beginning to appear in food tables (25). Hopefully these data will be expanded until statistically valid figures are available for all types of food and food products.

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