Comparative Evaluation of Solvent Extraction Methods for the Determination of Neutral and Polar Lipids in Beef[1]

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ABSTRACT

Samples of lean (< 5% fat), medium (13-15%) and high-fat (> 20%) ground beef were extracted for total lipid by 4 methods of wet extraction employing chloroform/methanol (CM), *n*-hexane/iso-propanol (HIP) and ethyl alcohol/ethyl ether (AE), and by 3 methods of soxhlet extraction of freeze-dried material by petroleum ether (PE) or eithyl ether (EE), CM and methylene chloride/methanol (MM). The purified lipid was fractionated into neutral and polar lipid fractions by silicic acid chromatography and the fractions were analyzed for fatty acid distribution by gas liquid chromatography (GLC). The soxhlet procedure employing either PE or EE extracted less than 75% of total lipid, 89% of triglycerides and 15% of polar lipids from lean beef as compared to other methods, and as the fat content increased from 3 to 20%, extracted amounts of polar lipid which increased to 40% of that extracted by other methods. The fatty acid distribution of the fractionated triglycerides and polar lipids was generally within experimental error for each fraction, irrespective of the method of extraction. The percentages of 16:0 and 18:1 were significantly less in polar lipids than in triglycerides. In addition to significantly higher percentage of 18:2, the polar lipids contained up to 20% of long-chain fatty acids not detected in triglycerides. The soxhlet procedures with CM or MM were as effective as wet extraction procedures in extracting neutral and polar lipids.

INTRODUCTION

Solvent extraction is the most critical step in the analysis of meats for total fat, neutral and polar lipids and fatty acid composition. Several methods have been reported in the literature (1-7) and the most common ones are based on soxhlet extraction of the dried sample or wet extraction with mechanical maceration or reflux with solvents alone or in combination. It is generally recognized that soxhlet extraction with petroleum ether, *n*-hexane or diethyl ether as specified in the official methods of AOAC (6) extracts only the free lipid. Many analysts prefer this method because of its ease and because fewer nonlipid components are extracted. Polar solvent mixtures such as chloroform/methanol (7) can be used for soxhlet extraction where information on total lipid composition is required. The Folch et al. (1) and Bligh and Dyer (2) procedures are extensively used for the analysis of tissue lipids; both employ the solvent mixture chloroform/methanol. Because of the potential health hazard in using chloroform, other solvent mixtures such as hexane/iso-propanol (HIP) (3) and methylene chloride/methanol (MM) (4) have been suggested. Sheppard (5) had earlier employed the Bloor's solvent mixture, ethanol/diethyl ether, for extracting liver lipids. Recent increased interest in the nutritional and physiological properties of the lipid components of foods has made the choice of the analytical method an even more important consideration.

In the work reported here, 7 lipid extraction methods were compared in order to obtain information on the lipid composition of ground beef. The total lipid extracts were fractionated into neutral and polar components and each fraction was analyzed for fatty acid composition.

MATERIALS AND METHODS

Meat Samples

Three composite meat samples containing different levels of fat were prepared from one source of beef round steak. The lean beef sample containing less than 5% fat was prepared by trimming off all visible fatty tissue. The medium-fat beef, with less than 15% fat, was the whole steak, and the high-fat beef was prepared by blending the fatty tissue trimmings from the lean sample. All three samples were ground in a Hobart electrical meat grinder by two passes through a 0.48 cm plate and blended to give a composite sample. Half of each sample was frozen in liquid nitrogen, freeze-dried and then stored in glass jars at −20 C until analysis. The other half was divided into appropriate subsamples and stored wrapped in aluminum foil in plastic bags at −20 C. The samples were analyzed for moisture, nitrogen, ash and phosphorus by the official methods of analysis (6) (AOAC methods 24.002, 24.007, 31.012 and 24.015, respectively).

Extraction Methods

The four wet extraction methods and three soxhlet extraction procedures compared in this study are listed in Table I. The sample size for the wet extraction methods (nos. 1-4) was 5 g, and five determinations were made by each method. Soxhlet extractions (methods 5, 6 and 7) were done on 10 g of the freeze-dried materials, in triplicate, for 2 periods of 8 and 16 hr. The AOAC official method 24.005 for crude fat (6) recommends either petroleum ether or anhydrous ethyl ether. Both solvents were used separately as methods 7a and 7b. Each extract was further purified to remove nonlipid material by biphasic partitioning with chloroform/methanol/water (2:1:0.8) as described earlier (7). The solvents were removed at reduced pressure on a rotary evaporator at 40 C and the lipid extracts were dried to constant weight under a stream of nitrogen at 40 C. BHT was used as an antioxidant in the stored samples.

Fractionation of Lipid Classes

A one-g sample of the purified lipid was fractionated on a 28 g silicic acid column described by Sahasrabudhe et al. (8). Fraction I, eluted with 300 mL benzene containing triglycerides (TG). Fraction II, eluted with 300 mL of diethyl ether, contained the free fatty acids (FFA), mono- and diglycerides and sterols; and fraction III, eluted with 300 mL chloroform/methanol (1:4), contained polar lipids. The fractions were checked for purity by thin layer chromatography (TLC) as described by Sahasrabudhe (7).

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TABLE I

Extraction Methods

Method		Solvents	Reference
1. Folch et al. (1956)	Wet extraction	Chloroform/methanol	1
2. Bligh and Dyer (1959)	Wet extraction	Chloroform/methanol	2
3. Hara and Radin (1978)	Wet extraction	*n*-Hexane/iso-propanol	3
4. Sheppard (1963)	Wet extraction (reflux)	Ethanol/ethyl ether (3:1, v/v)	5
5. Sahasrabudhe (1979)	Soxhlet	Chloroform/methanol (2:1, v/v)	7
6. Present study	Soxhlet	Methylene chloride/methanol (2:1, v/v)	4
7. AOAC	Soxhlet	(a) Petroleum ether (b) Ethyl ether	6

TABLE II

Moisture, Protein, Fat and Ash Contents of Beef (%)[a]

Meat type	Moisture	Protein N X 6.25	Fat	Ash
A. Lean	74.26	21.70	2.87	4.30
B. Medium fat	68.04	17.60	13.55	2.57
C. High fat	60.73	17.09	20.13	2.35

[a]All values except for fat are averages of 2 determinations. Values for fat are means of total lipid values determined by methods 1-6.

Gas Liquid Chromatography (GLC)

Fatty acid methyl esters (7) were analyzed on a Perkin Elmer model 3920 B gas chromatograph equipped with a 30 m SP2330 glass capillary column (Supelco, Bellefonte, PA), a flame ionization detector and a Hewlett Packard model 3390A printer plotter integrator.

Analysis of Variance (ANOVA)

ANOVA was calculated on the data of seven methods with five replicates. The statistical design of the experiment was six orthogonal contrasts as follows: (a) methods 1-5 vs 7a and 7b; (b) methods 1, 2 and 3 vs 4 and 5; (c) methods 1 and 2 vs 3; (d) method 1 vs 2; (e) method 4 vs 5; (f) methods 1 and 2 vs. 5. The contrasts were selected to determine the variations in lipid composition between the wet extraction and soxhlet extraction procedures, and in particular to compare chloroform/methanol as a solvent with petroleum ether and ethyl ether. The variables were as follows: (i) triglycerides (fraction I); (ii) FFA, mono- and diglycerides and sterols (fraction II); (iii) Polar lipids (fraction III); (iv) Neutral lipids (fractions I + II); (v) Total lipids (fractions I + II + III).

RESULTS AND DISCUSSION

Young et al. (9) compared the electronic Anyl-ray, Hobart, Uni-Vex, Honeywell, Bligh and Dyer and AOAC soxhlet procedures for the rapid determination of fat content in retail ground beef containing 15-30% fat. The AOAC and Bligh and Dyer methods correlated highly with each other and consistently yielded significantly higher values in comparison with other methods. In a study on lean beef, Hagan et al. (10) demonstrated that the Bligh and Dyer procedure extracted significantly higher amounts of total lipid than the AOAC procedure. Because of the discrepancy between results obtained for lean and high-fat beef, determinations in this study were made on beef samples containing three levels of fat. Chen et al. (4) demonstrated that substitution of chloroform by methylene chloride in the Folch et al. (1) procedure was equally effective in extracting total fat, fatty acids and sterols from food products. In the present study, methylene chloride/methanol (2:1, v/v) was used as the solvent mixture of soxhlet extraction of the freeze-dried meats. Maxwell et al. (11) and Marmer and Maxwell (12) have recently used the same solvent mixture for the determination of total fat in meat and meat products by a rapid dry column method.

Proximate composition of the three meat samples is shown in Table II. The mean values for the total lipid extracted from lean beef by the various methods and for the neutral and polar fractions obtained are shown in Table III. In comparison with other methods, the AOAC procedure (methods 7a, b) extracted 64.0-74.2% of the total lipid, 82.1-89.2% of TG (fraction I) and 6.0-14.5% of the polar lipids (fraction III). The difference between 8 and 16 hr soxhlet extractions was insignificant, indicating that all extractable lipid was extracted in 8 hr under the conditions used in our laboratory.

Table IV shows the results obtained with the medium-fat beef. The difference between the AOAC and other methods was not as significant for total lipid as it was in the lean beef sample. The AOAC procedure extracted 90% of the total lipid extracted by other methods and extracted all TG. The polar lipids (fraction III) were extracted only to the extent of 25% of that extracted by other methods, but this amount was significantly greater than that extracted from lean beef. The results obtained for high-fat ground beef showed no significant difference between methods with respect to the total lipid extracted (Tables V and VI). However, the polar lipid extracted by the AOAC procedure was still less than 50% of that extracted by other methods from lean meat or the medium-fat meat which indicates a solubilizing effect of excess fat on polar lipids. The

TABLE III

Total, Neutral and Polar Lipids from Lean Beef (g/100 g fresh weight)[a]

Method	Total lipid	Fractions		
		Neutral lipids		Polar lipids
		I Triglycerides	II Other[b]	III
1	2.99 ± 0.116[c]	2.17 ± 0.114	0.23 ± 0.009	0.58 ± 0.023
2	2.77 ± 0.021	2.05 ± 0.016	0.19 ± 0.001	0.53 ± 0.004
3	2.91 ± 0.075	2.18 ± 0.055	0.24 ± 0.006	0.50 ± 0.012
4	3.14 ± 0.049	2.14 ± 0.033	0.33 ± 0.005	0.66 ± 0.007
5	2.71 ± 0.078	2.01 ± 0.027	0.19 ± 0.020	0.48 ± 0.055
6	2.72 ± 0.105	2.04 ± 0.052	0.20 ± 0.025	0.48 ± 0.045
7a	2.01 ± 0.044	1.82 ± 0.040	0.15 ± 0.003	0.04 ± 0.001
7b	2.00 ± 0.041	1.79 ± 0.034	0.14 ± 0.003	0.07 ± 0.001

[a]All values are means of 5 determinations.
[b]Includes FFA, mono- and diglycerides and sterols.
[c]Standard deviation.

TABLE IV

Total, Neutral and Polar Lipids from Medium-Fat Beef (g/100 g fresh weight)[a]

Method	Total lipid	Fractions		
		Neutral lipids		Polar lipids
		I Triglycerides	II Other[b]	III
1	13.70 ± 0.190[c]	11.92 ± 0.166	1.21 ± 0.016	0.56 ± 0.008
2	13.80 ± 0.060	12.64 ± 0.054	0.61 ± 0.002	0.55 ± 0.002
3	13.56 ± 0.176	12.21 ± 0.158	0.81 ± 0.011	0.53 ± 0.006
4	13.16 ± 0.544	11.33 ± 0.460	1.28 ± 0.052	0.56 ± 0.025
5	13.57 ± 0.339	12.48 ± 0.312	0.51 ± 0.012	0.57 ± 0.014
6	13.08 ± 0.205	11.67 ± 0.166	0.58 ± 0.042	0.56 ± 0.002
7a	12.92 ± 0.073	12.43 ± 0.074	0.41 ± 0.002	0.08 ± 0.001
7b	12.19 ± 0.176	11.60 ± 0.132	0.40 ± 0.004	0.19 ± 0.002

[a]All values are means of 5 determinations.
[b]Includes FFA, mono- and diglycerides and sterols.
[c]Standard deviation.

TABLE V

Total, Neutral and Polar Lipids from High-Fat Beef (g/100 g fresh weight)[a]

Method	Total lipid	Fractions		
		Neutral lipids		Polar lipids
		I Triglycerides	II Other[b]	III
1	20.09 ± 0.296[c]	18.30 ± 0.265	1.22 ± 0.005	0.56 ± 0.025
2	20.35 ± 0.542	18.64 ± 0.126	1.10 ± 0.007	0.61 ± 0.004
3	19.97 ± 0.140	18.37 ± 0.129	1.12 ± 0.008	0.48 ± 0.004
4	18.84 ± 0.561	16.74 ± 0.498	1.28 ± 0.038	0.81 ± 0.024
5	21.22 ± 0.324	19.77 ± 0.302	0.81 ± 0.012	0.59 ± 0.009
6	20.32 ± 0.145	18.43 ± 0.132	1.14 ± 0.008	0.54 ± 0.004
7a	20.51 ± 0.385	19.67 ± 0.382	0.70 ± 0.001	0.14 ± 0.001
7b	19.95 ± 0.455	18.96 ± 0.433	0.74 ± 0.014	0.26 ± 0.005

[a]All values are means of 5 determinations with standard deviation.
[b]Includes FFA, mono- and diglycerides and sterols.
[c]Standard deviation.

products of lipolysis eluted in fraction II (Tables III, IV, V), particularly in medium-fat and high-fat samples, were significantly higher in methods of wet extraction (methods 1-4) as compared to those extracted from freeze-dried materials (methods 5, 6, 7a and 7b), which suggests that lipolysis can occur during wet extraction.

ANOVA for selected contrasts for total lipid, neutral lipids (fractions I + II) and polar lipids (fraction III) are shown in Tables VI, VII and VIII, respectively. The total lipid extracted by the official AOAC procedure was significantly different at the 1% level for lean and medium-fat beef but not for high-fat beef. ANOVA for method 6 was calculated separately, as the method was included in the study at a later stage. The polar lipid fraction was essen-

TABLE VI

Analysis of Variance for Total Lipid

Source	DF	Mean squares of meat types A (Lean)	B (Medium fat)	C (High fat)
Methods	6	1.0535	1.5984	2.5141
Contrasts				
Methods 1-5 vs 7a, 7b	1	5.7341[a]	7.1369[a]	0.1597
Methods 1, 2 vs 3	1	0.0025	0.1082	0.2001
Method 1 vs 2	1	0.1221[b]	0.0316	0.1706
Methods 1, 2 vs 5	1	0.0969[b]	0.1002	3.0604[a]
Method 5 vs 6	1	0.0040	0.2482[b]	1.8040[a]
Error	28	0.0045	0.0722	0.0890

[a]Significant at 1%.
[b]Significant at 5%.

TABLE VII

Analysis of Variance for Neutral Lipid (I + II)

Source	DF	Mean squares of meat types A (Lean)	B (Medium fat)	C (High fat)
Methods	6	0.2348	0.8918	3.3890
Contrasts				
Methods 1-5 vs 7a, 7b	1	1.1872[a]	2.4175[a]	2.2338[a]
Methods 1, 2 vs 3	1	0.0310[a]	0.0772	0.0636
Method 1 vs 2	1	0.0719[a]	0.0375	0.1138
Methods 1, 2 vs 5	1	0.0148[b]	0.1155	3.0127[a]
Method 5 vs 6	1	0.0025	0.1172	1.6320[a]
Error	28	0.0027	0.0667	0.0834

[a]Significant at 1%.
[b]Significant at 5%.

TABLE VIII

Analysis of Variance for Polar Lipid (III)

Source	DF	Mean squares of meat types A (Lean)	B (Medium fat)	C (High fat)
Methods	6	0.3072	0.2144	0.2549
Contrasts				
Methods 1-5 vs 7a, 7b	1	1.7031[a]	1.2469[a]	1.1988[a]
Methods 1, 2 vs 3	1	0.1591[a]	0.0026	0.0381[a]
Method 1 vs 2	1	0.0066	0.0002	0.0012
Methods 1, 2 vs 5	1	0.0358[b]	0.0005	0.0002
Method 5 vs 6	1	0.0003	0.0008	0.0013
Error	28	0.0042	0.0013	0.0010

[a]Significant at 1%.
[b]Significant at 5%.

tially the phospholipid (92-98%). The values for polar lipid extracted from all three types of meat by methods 1-6 ranged between 482 and 672 mg per 100 g of fresh sample with a mean of 552 ± 55 mg. Method 4, employing ethanol/diethyl ether, consistenly extracted more polar lipid from high-fat than other methods. The AOAC procedure, employing soxhlet extraction with either diethyl ether or petroleum ether, extracted progressively increasing amounts of polar lipid (from 9 to 40% of that extracted by other methods) as the total fat content increased from 3 to 20%. Methods 5 and 6, employing soxhlet extraction with chloroform/methanol and methylene chloride/methanol, respectively, yielded total, neutral and polar lipids in amounts comparable to the Folch et al. (1) and Bligh and Dyer (2) procedures.

The fatty acid compositions of the lipid fractions are shown in Table IX. Irrespective of the method used or the meat type analyzed, the fatty acid composition of triglycerides and polar lipids was generally within the experimental error. The percentages of palmitic (16:0) and oleic (18:1) acids were significantly less in polar lipids than in triglycerides. In addition to the significantly higher percentages of linoleic (18:2) acid, the polar lipids contained up to 20% of long-chain fatty acids not detected in triglycerides.

TABLE IX

Fatty Acid Composition of Lipid Fractions (%)[a]

Fatty acids[b]	Triglycerides Fraction I	Fraction II	Polar lipids Fraction III
<12:0	2.1 ± 1.2	2.4 ± 0.9	3.3 ± 1.5
14:0	5.1 ± 1.1	3.0 ± 0.5	2.8 ± 1.0
U1[c]	t[d]	+[d]	1.7 ± 1.0
U2	3.2 ± 1.5	+	6.1 ± 1.6
U3	1.7 ± 1.0	+	1.7 ± 1.0
16:0	23.0 ± 1.5	16.9 ± 3.5	13.4 ± 1.9
16:1[e]	9.7 ± 1.7	8.5 ± 2.3	3.8 ± 1.5
U4	2.0 ± 0.9	+	3.8 ± 1.6
U5	ND[d]	+	2.8 ± 0.5[f]
18:0	8.4 ± 1.1	7.7 ± 2.0	10.6 ± 2.0
18:1	37.0 ± 3.5	40.9 ± 10.5	21.8 ± 2.5
18:2	5.5 ± 1.3	7.7 ± 2.8	13.9 ± 2.2[g]
20:0	t	+	2.0 ± 1.0
18:3	1.4 ± 0.4	1.3 ± 0.7	1.7 ± 0.7
U6	ND	+	1.4 ± 1.0
U7	ND	+	2.4 ± 0.2
20:4	t	2.2 ± 1.9	7.6 ± 0.9
U8	ND	+	1.6 ± 0.5
U9	ND	+	1.1 ± 0.3
U10	ND	+	2.6 ± 0.9

[a]All values are means of 20–24 average values obtained for all three types of beef extracted by 7 methods.

[b]Fatty acids are shown in order of retention times.

[c]Exact identities of fatty acids U1–U10 have not been confirmed (see text).

[d]t indicates less than 0.1%; ND indicates not detected; + indicates occurrence up to 1% in some samples.

[e]Includes trace amounts of 2 other isomers, not confirmed.

[f]Not detected in polar lipid extracted by methods 7a and 7b.

[g]Includes methods 1–6 only; values for 18:2 in polar lipids extracted by methods 7a and b ranged between 4.0 and 4.6 in medium- and high-fat beef, and 11.1–11.8 in lean beef.

These observations are in general agreement with the published information on beef lipids (13).

The exact identities of some fatty acids (U1–U10, Table IX) were not determined in the present study but will be published later. One component with a retention time less than that of 16:0 occurred in a range of 0.7–3.0% in the triglyceride fraction extracted by methods 1 and 2 only. A significant difference was noted in the amounts of 18:2 in polar lipids between AOAC procedure (methods 7a, 7b) and other methods. The values for 18:2 in polar lipids extracted by Method 7a and 7b ranged from 4.0 to 4.6% in medium- and high-fat beef and 11.1–11.8% in lean beef, as compared to the range of 12.1–15.9 for methods 1–6 with a mean value of 13.92 ± 2.18 reported in Table IX.

The fatty acid composition of fraction II showed a wide variation between methods in the content of individual fatty acids. For example, 18:1 ranged from 28.7 to 51.2% and 16:0 from 12.0 to 21.8%. This was possibly due to different lipolytic conditions occurring during extraction.

The results of the present study confirm that the official AOAC soxhlet extraction procedure, employing either petroleum ether or ethyl ether, is not satisfactory for the determination of lipid composition in meats and demonstrate that freeze-dried samples of meat can be effectively extracted with chloroform/methanol (2:1, v/v) or methylene chloride/methanol (2:1, v/v) in a soxhlet-type apparatus.

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