

MEAT AND MEAT PRODUCTS

Determination of Total Fat in Meat and Meat Products by a Rapid, Dry Column Method

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A rapid, dry column method is proposed for determining fat in meat and meat products. Unlike AOAC procedures 24.005 and 24.006, this procedure measures total rather than crude fat. A 5 g sample is blended with anhydrous sodium sulfate in a mortar and is then reduced to a fine powder with Celite 545. The fat is eluted on a glass column, using dichloromethane-methanol (9+1). Solvent is removed from the eluate, and the resulting residue is weighed to calculate total fat of the sample. A determination takes 2.5 hr or less. Fat levels ranged from 7 to 90% in 15 meat samples. Quadruplicate determinations by this method and duplicate determinations by 24.005(a) yielded overall means of 29.9 and 29.3% fat, respectively. Repeatability was 0.3% fat. The 0.6% mean difference is significant ($P = 0.05$) and represents a more complete extraction of polar lipids by the proposed method. Results of determinations by this method are compared with results by an accepted but laborious chloroform/methanol procedure for total fat recovery. Overall means and standard deviations of replicate determinations on 4 meats containing 4-30% fat were 12.8 ± 0.1 with this method and 12.7 ± 0.1 with the reference method.

At present there are 2 official AOAC methods for measurement of fat in meat and meat products: 24.005 is a Soxhlet extraction and 24.006 involves use of the Foss-Let analyzer (1). Collaborative studies (2, 3) have shown precision and accuracy of the 2 methods to be similar. The fat recovered by these procedures is reported as crude, or solvent extractable, and is comprised mainly of neutral lipids in the sample. However, in a study in which fat residues from the petroleum ether or ethyl ether Soxhlet extracts of meat were checked for phospholipid content, only partial recovery of these lipids occurred with either solvent (4). It is clear, therefore, that the values obtained for fat content by the Soxhlet methods do not represent a clearly defined portion of the fat in meat and meat products.

Interest has been increasing in methods that measure both neutral and polar lipids, or total fat content, in meat. Investigators have evaluated several methods to determine their suitability for total fat analysis (4-6). Hagan *et al.* (4) compared the effectiveness of the acid hydrolysis-Rohrig, AOAC-Soxhlet, and Bligh-Dyer chloroform/methanol (7) methods for extracting fat from various beef samples. Results showed that the chloroform/methanol method is superior to the other 2 methods for total fat isolation because it extracts more polar lipids than do the other 2 methods. Their conclusions (4) were verified by Prost and Wrebiakowski (5) and Young *et al.* (6), who compared the Soxhlet and the Bligh-Dyer methods. Both groups reported that the Bligh-Dyer technique consistently gave higher values for fat content in meat than did the Soxhlet method, and they concluded that the Bligh-Dyer method effectively isolated total fat.

Based on such studies, recommendations were made (4, 8) that either of the 2 widely used chloroform/methanol extraction methods, the Bligh-Dyer or the Folch, Lees, and Sloane-Stanley (9), be considered when total fat values are required or when the fat residue is needed for further analysis. However, problems may be encountered when these methods are used in routine laboratory applications. Chloroform/methanol extraction techniques must be meticulously carried out, require the use of elaborate apparatus, involve considerable operator time, and use chloroform, a potentially hazardous solvent (8).

We recently developed a dry column method for isolating lipids from adipose and muscle tissue; the method obviates many of the problems encountered in the use of traditional chloroform/methanol extraction techniques (4, 8) or AOAC procedures. The method, as developed for biochemical studies, allows the

lipid to be extracted unaltered, and simultaneously separates it into neutral and polar fractions (10). Pertinent to food analysis, the dry column method is suitable for determining total fat content of meat and meat products. Unlike all currently used methods, the new method is rapid, simple, uses relatively harmless, nonflammable solvents, and requires easily accessible and inexpensive equipment. It is especially suitable for multiple determinations.

This report presents the results of the measurement of total fat in various meats and meat products. Dry column determinations are compared either with those by AOAC-Soxhlet or with those by chloroform/methanol method.

METHOD

Reagents and Apparatus

(a) *Solvents*.—Distilled-in-glass dichloromethane and methanol (Burdick and Jackson Laboratories, Muskegan, MI 49442), or equivalent. Mix (9+1) and store until needed.

(b) *Column packings*.—Granular anhydrous Na_2SO_4 (J. T. Baker Chemical Co., Phillipsburg, NJ 08865); $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$, catalog No. C-123, and Celite 545, not acid-washed, catalog No. C-212 (Fisher Scientific Co., King of Prussia, PA 19406). Mix as follows: In a large mortar, grind to uniform mixture, $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ and Celite 545 (1+9). Store in amber jar with foil-lined cap.

(c) *Porcelain mortar*.—750 mL, Coors No. 17, and pestle (A. H. Thomas, Co., Philadelphia, PA 19105). If sides of porcelain mortar or bulb of pestle become highly polished through extended use, tissue comminution is difficult. Do not use such worn pieces in this determination. Regenerate rough surfaces by grinding a few grams fine mesh (e.g., 60 or 70) abrasive with polished pieces. Grade 60 silicon carbide (Norton Co., Worcester, MA 01606), is adequate.

(d) *Chromatographic column*.—Glass, 35 mm id \times 30 cm long with a drip tip 5 cm \times 8 mm id (prepared by local glassblower); stainless steel spoon; tamping rod, constructed by attaching 32-mm diam. stainless steel disc to a concentric rod (or use a rubber stopper attached to a dowel).

Procedure

Prepare triply ground meat sample for analysis according to sec. 24.001 (1).

Insert glass wool plug into tip of glass column; charge column with 10 g $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ /Celite 545 mixture. Tamp mixture firmly in place with the tamping rod, and set column aside until needed.

Accurately weigh 5 g meat sample (or 3 g if

expected fat content is $>50\%$) and quantitatively transfer to porcelain mortar. Add 20 g anhydrous Na_2SO_4 and grind with pestle until mixture is reduced almost to a powder. Small tissue particles may remain in the mixture after a few minutes grinding with Na_2SO_4 , but are not objectionable at this point. Add 15 g Celite 545 to contents of mortar, and regrind mixture to a completely uniform, free-flowing powder. Quantitatively transfer powder to previously prepared glass column, with a teaspoon or through a glass powder funnel. Moderately tamp powder in place to obtain a uniform bed. A bulk volume of ca 60 mL is adequate. This is equivalent to a 60–70 mm height within a 35 mm id column. Using a disposable pipet and 25 mL solvent mixture, wash pestle, tamping rod, and spoon over the mortar. Swirl rinsings in mortar to extract residual traces of fat, and then rapidly pipet rinsings along walls of column onto column bed. Allow rinsings to percolate into packed bed, always maintaining some solvent above the bed. Continue to add solvent mixture until entire bed is wet and a few drops of eluate have been collected. Then carefully charge column with 150 mL solvent mixture and allow to drip until 150 mL eluate is collected. The eluate may be collected in a tared tall form beaker placed on a hot plate in a fume hood. Thus, eluate can be simultaneously collected and evaporated under a stream of nitrogen. After solvent removal, dry residue in beaker for 30 min at 100°C as specified in sec. 7.056 (1). Determine residue weight and calculate per cent total fat as (residue wt/sample wt) \times 100.

Results and Discussion

The dry column fat determination method was performed on meat samples containing 4–90% fat. Unlike other methods (3, 4, 11), in which standard deviation of replicate analyses tends to increase in proportion to fat content, the dry column method presents no procedural problems regardless of amount of fat in the sample. AOAC method 24.005 requires a lengthy drying step prior to extraction of the sample in the Soxhlet apparatus. This step is unnecessary in the proposed method because contact with anhydrous Na_2SO_4 causes immediate desiccation.

During initial tests of the dry column method, a small amount of nonlipid material was eluted along with the fat. To correct this problem, tests were carried out in which the relative amounts of sample, sodium sulfate,

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and Celite 545 were varied. These experiments showed no reduction of nonlipid carryover. In later experiments, beds of different materials were placed under the sample mixture in attempts to trap nonlipids before they were eluted from the column. We found that pre-packing the column with a mixture of $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ and Celite 545 effectively traps nonlipid contaminants of the fat residue.

The grinding step in the dry column method is rapid, ca 5 min. Grinding the meat sample with anhydrous sodium sulfate results in almost total comminution of tissue, provided mortar and pestle surfaces are not polished. However, at this point it is not necessary to grind the sample until the mixture is entirely homogeneous. Instead, the action of Celite 545 on the mixture of sample and sodium sulfate completes the process of tissue comminution and results in a homogeneous, free flowing, off-white powder, which is easily transferred quantitatively to the glass column.

We performed tests to determine whether the degree of column bed compression influences the amount of fat recovered. No differences were observed in overall recovery between tightly and moderately compressed columns. We did observe, however, that very tightly packed columns sometimes had unacceptably slow flow rates. We therefore recommend that the column bed be compressed moderately to minimize reduction of solvent flow and to permit the entire 150 mL eluate to be collected in less than 1.5 hr. Solvent flow can sometimes be restricted by vapor lock when the laboratory's ambient temperature is above 80°F (27°C). This manifests itself in separation of the column bed. Such flow problems may be obviated by use of water-jacketed columns.

We performed experiments to determine the solvent volume needed for complete extraction of fat from the column bed. Eluate from the columns was collected in 10 mL aliquots and examined for lipids and nonlipids by thin layer chromatography (TLC). Regardless of fat content of the tissue, 150 mL solvent mixture sufficiently eluted all fat. Solvent amounts above 150 mL eluted small amounts of nonlipid material, regardless of whether or not the $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ /Celite 545 trap was at the base of the sample bed.

Table 1. Fat determination (%) by official AOAC^a and dry column methods

Sample ^b	AOAC-Soxhlet ^{a,c}	Dry column ^d
Beef		
1	9.99	10.26 ± 0.25
2	12.11	12.62 ± 0.11
3	7.63	8.39 ± 0.22
4	22.26	23.32 ± 0.11
Mean	13.00	13.65 ± 0.17
Pork		
1	27.60	28.37 ± 0.35
2	18.44	18.92 ± 0.71
3	48.64 ^e	49.48 ± 0.45
4	51.96	52.87 ± 0.24
5	43.98	44.62 ± 0.59
6	90.38	89.78 ± 0.29
Mean	46.83	47.34 ± 0.44
Processed meat		
Bologna	22.50 ^e	22.61 ± 0.25
Frankfurter 1	27.34 ^e	27.95 ± 0.25
Frankfurter 2	17.99	18.67 ± 0.05
Mean	22.61	23.08 ± 0.18
Lamb	22.40	23.24 ± 0.12
Chicken	16.18	17.25 ± 0.12
Mean of all 15 samples	29.29	29.89 ± 0.28

^a Ref. 1, sec. 24.005(a) (petroleum ether).

^b Samples prepared according to ref. 1, sec. 24.001.

^c Duplicate determinations unless otherwise indicated.

^d Mean of 4 subsamples ± std dev.

^e Results from collaborative study (2).

A comparison of the results obtained on various meats and meat products by the AOAC-Soxhlet vs dry column method is presented in Table 1. Most results for Soxhlet determinations are averages of duplicate determinations, but others are taken from collaborative studies run previously (2) on the same samples used in the present study. Results with the dry column method are shown as means and standard deviations of 4 determinations on each sample; i.e., 2 analysts performed duplicate determinations. Each operator performed the grinding operation, transfer of the sample, and elution with slight variations in technique. Nevertheless, good agreement between duplicate pairs was obtained in all cases. Standard deviations of the results (overall mean, 29.9% fat) on the 15 samples indicated repeatability of the method was 0.3% fat. This characteristic compares favorably with the repeatability (0.4% fat) of 24.005(a) reported in a recent collaborative study (2). The overall mean difference, 0.6% fat, between results of the compared methods indicates the amounts of additional phospho-

Table 2. Determinations of total fat (%) by the chloroform/methanol and dry column methods, and phospholipid contents (%) of fat extracts

Sample ^b	Chloroform/methanol ^a		Dry column	
	Total fat, % ^c	Phospholipid, % ^{c,d}	Total fat, %	Phospholipid, % ^d
Beef 5	10.72 ± 0.10	0.61	10.84 ± 0.11 ^e	0.62 ^e
Beef 6	4.29 ± 0.07	0.70	4.34 ± 0.10 ^e	0.70 ^e
Beef 7	29.87 ± 0.22	0.50	29.98 ± 0.06 ^e	0.48 ^e
Pork 7	5.77 ± 0.13	0.67	5.90 ± 0.09 ^e	0.68 ^e
Av. of 4 samples	12.66 ± 0.13	0.62	12.76 ± 0.09	0.62

^a Ref. 12.

^b Samples prepared according to ref. 1, sec. 24.001.

^c Mean of 4 subsamples ± std dev.

^d Phosphorus content determined by method of ref. 13 and expressed as % phospholipid = 25 × % phosphorus.

^e Mean of 6 subsamples ± std. dev.

lipid extracted by the dry column method. This positive mean difference is within the expected range of phospholipid content for such samples, though it already has been pointed out (4) that the Soxhlet extraction does recover small and inconsistent amounts of phospholipid. Additionally, Student's *t*-test ($t = 5.48$) indicates that the difference is significant ($P = 0.05$).

Although no official AOAC method for determining total fat in meat is available to be compared with the dry column method, the traditional chloroform/methanol procedures are known (4-8) to be nearly quantitative in fat recovery. Therefore, we compared the amount of fat recovered by a modified Folch chloroform/methanol procedure (12) with that obtained by the dry column method. One pork and 3 beef samples, containing 4 to 30% fat, each were divided into eight or ten 5 g subsamples. Four subsamples from each meat were extracted by the modified Folch procedure, and 4 or 6 were extracted by the dry column method. The recovered fat residue from each replicate determination was analyzed for phosphorus by an accepted procedure (13), and phospholipid content was calculated as (25 × % phosphorus).

Means and standard deviations of fat determinations and calculated phospholipid contents of the replicate results are shown in Table 2. The degree of agreement of the comparative results demonstrates the accuracy and

precision of the dry column method for the determination of total lipid in meat.

On the basis of these results, the dry column method offers the analyst a convenient, rapid (≤ 2.5 hr, including drying of residue) method for determining total fat in meat and meat products. The method affords consistently higher values than does AOAC method 24.005, and these higher values are due to complete phospholipid extraction. The dry column method, with some modifications to the described procedure, is being evaluated for its applicability to the fat determination of such foods as eggs, fish, dairy products, legumes, grains, and animal feed.

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