

## Collaborative Study of Rapid Fat Determination in Meat and Meat Products by Modification of the AOAC Method

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The rapid method for fat determination reported by this laboratory has been evaluated by 10 collaborating laboratories in a comparison with the AOAC method. The method is similar to the official method except that a 3-4 g sample of meat or meat product is dried in an open extraction thimble for 30 min at 125°C and extracted with ethyl ether for 45 min at maximum reflux, and fat content is determined gravimetrically after complete removal of solvent from the extract. Collaborators performed 6 replicate analyses on samples of ground beef, frankfurters, and fresh pork sausage by both methods. A statistical treatment of the results showed that fat contents determined by the modified method agreed with those obtained by the official method. The expected standard deviation,  $s_e$ , averaged for the 3 types of meat product, was  $\pm 0.83\%$  fat which is 1.5 times the value obtained by the official method,  $\pm 0.56\%$  fat.

Fat analysis is important to the meat industry for both quality and regulatory control. Ether extraction as described in the AOAC method (1) is the official procedure for the industry. However, there is a great need for alternative rapid methods. Many rapid methods in use have received only minimal evaluation. With the objective of reducing the time required for the official method, Cohen and Swift (2) varied both drying and extracting times for several types of meat samples and compared the results with those obtained by the AOAC method. Replicate results showed that ground beef required 30 min drying in a 125°C oven and 45 min extraction with ether; frankfurters required either 30 min drying and 30 min extraction, or 45 min drying and 15 min extraction; and pork sausage required either 15 min drying and 30 min extraction or 30 min drying and 15 min extraction. Fat recoveries for these analyses were 99.2-100.5% when compared with results by the AOAC method. Based on these

findings, uniform times of 30 min drying and 45 min extraction were selected for fat analysis of all 3 products. When this analysis time is compared with a minimum of 1.5 hr drying and 4 hr extraction by the official method, time per assay is reduced by 4.25 hr.

In a related study designed to reduce time for fat content analysis, Cohen (3) developed a technique for rapid removal of the last traces of ether from the extracted fat, as follows: After most of the ether is removed from an extract by distillation, a stream of dry, clean nitrogen gas is directed at the surface of the fat extract while the beaker containing the extract is supported on the swing beaker holder and heated by one of the hot plates of the Goldfish extractor. The beaker and contents are heated 3-7 min, and then cooled 1 min in a prechilled (<0°C) aluminum chamber and weighed. As a result, total time for removal of the last traces of solvent was reduced from 60 min to 4-10 min.

Based on these results it was decided to compare this rapid drying and extraction method with the AOAC fat method, 24.005(a), by a collaborative study.

### Collaborative Study

The method of analysis was similar to the official method but drying and extraction times were shortened. The time for each of the steps was selected from the previous evaluation (2) of the method wherein meat and meat products with a fat content range of 9-55% were analyzed. Based on those results, the uniform procedure selected for this collaborative study was that each sample be dried 30 min at 125°C and extracted with ether for 45 min.

Samples of ground beef, frankfurters, and pork sausage were taken from the same lots prepared and described in the preceding report of this series (4). The 10 collaborators were requested to follow all instructions set forth for equipment and procedure for the 2 methods and to perform 6 replicate determinations on each of the 3 samples.

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If unable to comply, they were either to describe the deviation from the procedure or to telephone the Associate Referee for further instruction before proceeding with the analyses.

## METHOD

### Apparatus

(a) *Oven*.—Gravity oven capable of maintaining ca 125°C on single shelf set at fixed height within oven.

(b) *Fat extraction apparatus*.—Goldfisch extractor or Soxhlet extraction unit (Labconco Corp., 8811 S. Prospect Ave., Kansas City, Mo. 64132, or equivalent).

### Method 1

Use 24.005(a) fat method. Weigh 3–4 g sample by difference. Dry 1.5 hr at 125°C. Extract with anhydrous ethyl ether (Baker reagent) for 4 hr at maximum reflux in Goldfisch apparatus, or in Soxhlet unit, using 125–250 ml flask and 22 × 80 mm thimble. Dry extract residue until no ether remains, cool, and weigh as fat. Calculate as per cent fat.

### Method 2

Proceed as in Method 1 except dry sample in open thimble 30 min at 125°C and extract 45 min at maximum reflux.

## Results and Discussion

Analytical results of fat content received from the 10 collaborators for the 3 meat product sam-

ples were treated statistically according to the error analysis techniques described in the preceding report of this series (4).

Tables 1 and 2 show the collaborators' data, means of 6 replicate determinations, ranking, and error statistics for the 2 methods of fat content determination. Collaborators' scores for the 2 methods indicate there was no pronounced systematic error among the laboratories, since all scores were greater than 4 and lower than 29. There was a tendency for some systematic error in the consistently low results by Laboratories 4, 5, and 7 with the official method (Table 1). The numerical values of the standard deviation, the random error, and systematic error obtained for the 3 samples were not proportional to fat level with either of the 2 methods. Dissimilarities in the values of the error components among the 3 samples were more likely due to the fineness of grind of the samples. Frankfurters, the most finely comminuted product, generally yielded the smallest error. The most convenient index for comparing the 2 methods was by means of an average of each error statistic which was obtained for the 3 samples, as shown in Tables 1 and 2.

*Method 1. 24.005(a)*.—The data obtained by this method served as the basis for comparing the trial method. Table 1 shows the reduced data from 180 single determinations. Contrary to the

Table 1. Collaborative fat analysis results by method 24.005(a) for meat product samples, ranked collaborator results, and summary of statistical analysis of the data

Coll.	Fat, %									Coll. score
	Ground beef		Frankfurter		Pork sausage		Ranked results			
	$\bar{x}$	$s_r$	$\bar{x}$	$s_r$	$\bar{x}$	$s_r$	B	F	P	
1(AR)	18.92	0.377	27.48	0.397	44.09	0.413	1	5	2	8
2	18.22	0.601	27.53	0.606	44.03	0.516	4	4	5.5	13.5
3	18.07	0.151	27.47	0.308	44.20	0.268	7	6	1	14
4	17.47	0.208	26.83	0.341	43.12	0.659	10	8	9	27
5	17.48	0.438	26.83	0.187	43.02	0.531	9	9	10	28
6	18.19	0.145	27.57	0.327	44.08	0.469	5	3	3	11
7	17.68	0.266	26.83	0.246	43.76	0.256	8	10	8	26
8	18.14	0.384	27.39	0.288	43.80	0.389	6	7	7	20
9	18.61	0.468	27.87	0.513	44.03	0.303	2	1	5.5	8.5
10	18.40	0.621	27.76	0.177	44.06	0.468	3	2	4	9
Statistic	Beef		Franks		Pork		Average			
Final Results, Per Cent Fat										
Grand mean, $\bar{x}$	18.12		27.36		43.82		—			
Range	-1.34 to 1.28		-1.12 to 1.24		-1.63 to 0.99		—			
$s_d$	0.473		0.390		0.417		0.427			
$s_r$	0.401		0.363		0.444		0.403			
$s_b$	0.444		0.361		0.376		0.394			

**Table 2. Collaborative fat analysis results by modified method 24.005(a) for meat product samples, ranked collaborator results, and summary of statistical analysis of the data**

Coll.	Fat, %									Coll. score
	Ground beef		Frankfurter		Pork sausage		Ranked results			
	$\bar{x}$	$s_r$	$\bar{x}$	$s_r$	$\bar{x}$	$s_r$	B	F	P	
1(AR)	18.00	0.491	28.57	0.397	43.80	0.574	4	1	4	9
2	16.62	0.504	27.23	0.723	35.12	2.179	8	6	10	24
3	18.47	0.151	27.62	0.337	44.25	0.226	2	3	2	7
4	16.33	0.374	27.16	0.310	44.38	0.481	10	7	1	18
5	17.16	0.374	26.58	0.205	43.59	0.343	6	10	5	21
6	16.92	0.251	27.72	0.283	42.63	0.407	7	2	9	18
7	16.53	0.520	27.04	0.252	43.49	0.376	9	8	6	23
8	17.28	0.369	26.87	0.172	44.04	1.020	5	9	3	17
9	18.77	0.734	27.52	0.431	42.87	0.473	1	4	8	13
10	18.25	0.325	27.40	0.619	42.94	1.559	3	5	7	15

  

Statistic	Beef	Franks	Pork	Average
Intermediate Results, Per Cent Fat				
Grand mean, $\bar{x}$	17.43	27.37	42.71	—
Range	-1.88 to 2.42	-1.37 to 1.89	-10.91 to 2.55	—
$s_d$	0.876	0.547	2.733	1.386
$s_r$	0.437	0.409	0.974	0.607
$s_b$	0.858	0.521	2.704	1.361

  

Final Results, <sup>a</sup> Per Cent Fat				
Grand mean, $\bar{x}$	17.52	27.39	43.55	—
Range	-1.97 to 2.33	-1.15 to 1.87	-3.57 to 1.71	—
$s_d$	0.878	0.578	0.629	0.695
$s_r$	0.429	0.358	0.725	0.505
$s_b$	0.860	0.559	0.555	0.659

<sup>a</sup> Data from Collaborator 2 omitted.

usual order of errors, where systematic error (between laboratories) is generally larger than random error (within laboratories), the 2 components of error are almost equal in size and, in fact, are reversed for pork sausage. The value of the expected deviation  $s_e$  is  $\pm 0.565\%$  fat.

**Method 2. Modification of Official Method.**— Table 2 shows the reduced data from 180 single determinations. Summary statistics are presented in 2 parts. Intermediate results were calculated by using all data. Final results were obtained after omitting outlier data. Laboratories 2 and 10 had difficulty in estimating fat content reproducibly in the pork sausage sample by this method, as was evidenced by the high random error values.

A linear regression line was constructed, omitting Laboratory 2, using 27 values (3 meat product samples analyzed by 9 laboratories) to fit the line. The values obtained were: intercept = 0.523; slope = 1.009;  $SS_R = 12.86$  (sum of squares about regression);  $r = 0.997$  (correlation coefficient). To test whether deviation from exact agreement of the 2 methods was significant

rather than due to random error,  $SS_E$  was calculated and found to be 14.9. An  $F$ -ratio of 1.94 was obtained, compared to a tabular value of 3.39. This indicated that there was no significant difference in mean fat level determined by the 2 methods.

Compared with the official method, the average estimates of error  $s_d$ ,  $s_r$ , and  $s_b$  were higher and the resulting expected standard error ( $s_e = \pm 0.829\%$  fat) was about 1.5 times as high.

#### Comments of Collaborators

Collaborator 3 commented that the modified AOAC method could be advantageously substituted for the official procedure. The use, however, of ethyl ether in fat extractions run on a routine basis is not advisable due to its extreme flammability. He questioned whether petroleum ether would have given similar results.

Collaborator 5 used Soxhlet extraction units and Fisher grade anhydrous ether for official

method fat determinations, and Baker petroleum ether for the modified AOAC analyses.

Collaborator 9 set the Goldfish apparatus at maximum temperature. He found that each individual unit dripped at a different rate, ranging from about 3.5 to 6 drops/sec. This could not be remedied and he suggested it may have some effect on both the rapid and the slow extractions.

#### Recommendation

It is recommended that the modified AOAC fat method be adopted as official first action as an alternative method for meats prior to processing, for processed product which is not statutorily borderline, or as a screening procedure for finished product when speed is more important than precision.

#### Acknowledgments

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