FAT IN FISH MEAL

By M. E. Stansby*

Tests carried on in laboratories of the Fish and Wildlife Service during the past ten years have shown that when the fat content of fish meal is determined by conventional methods, that is, by extraction with ethyl ether, the apparent fat content drops during even short storage periods. After six months to a year, values obtained are as low as 30 percent of the original. It has also been shown that when acetone is substituted for ethyl ether, much higher recoveries of the initial fat are obtained, although a small drop in the apparent fat content does take place after extended storage periods.

More recent studies have shown that somewhat higher results are obtained with certain mixed solvents than with acetone, but other difficulties in technique have thus far prevented the use of such mixed solvents on a routine basis. Inasmuch as such extremely low values are obtained when using conventional methods, it seemed advisable to test the acetone-extraction method on a collaborative basis even though it has not been perfected. Accordingly, during the past year such a collaborative analysis has been conducted.

Before starting the collaborative analysis, it was necessary to standardize on a procedure. In preliminary experiments carried out during the past years, it was customary to first extract the meal with acetone, then to evaporate the acetone and redissolve the fat in ethyl ether. This solution was then evaporated and the ether-soluble material weighed as fat. This purification step was carried out to be certain that the acetone was not dissolving extraneous material and the final reported value represented true fat. Extensive experience with this procedure, however, showed that the gross, unpurified acetone extract obtained from old meals was never higher than the purified ether extract of the same meal in the fresh condition nor was it higher than the initial fat content as determined by standard AOAC procedure. Thus, once having shown that the acetone extract contained no significant amount of extraneous materials, it was felt that, for routine analyses, it would be unnecessary to include the so-called "purification" step. By merely weighing the crude acetone extract, a minimum value would be obtained which in no case would be higher than the true fat content of the meal. Accordingly, no purification with ethyl ether was included in the final procedure as adopted for the collaborative analysis.

Another decision which had to be made was whether a second step, namely hydrolysis of the extracted meal with acid, followed by a second extraction, should be included in the standardized method. Previous tests had shown that a considerable amount of additional fat could be extracted from meals in this way, especially from meals which had been stored for a considerable length of time. Such a hydrolysis and extraction procedure on fresh meals usually yielded about 1 percent additional fat by this method. For very old meals, a considerably larger proportion of the fat was not extracted by the initial solvent but could be obtained by this hydrolysis procedure. Accordingly, in order to more nearly determine the true fat content in older meals, it was decided to include the acid hydrolysis in the standard procedure. By so doing, the value obtained for the initial fat content of fresh meals is increased by about 1 percent over values which would have been obtained had this process not been included.

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Following is the procedure exactly as sent to collaborative laboratories for this analysis:

FAT DETERMINATION IN FISH MEALS

I. Acetone method.

Weigh four to five grams of the meal to the nearest 1/100 gm. into an alundum or paper extraction thimble, cover with a light layer of cotton, and extract in a Bailey-Walker or Underwriters type continuous extraction apparatus for 16 hours, using acetone as the solvent. If this type extraction equipment is not available, Soxhlet or other types may be used, but a notation describing the type used should be made with the report on results obtained.

At the end of the extraction period, distill off acetone until volume in flask is 10-15 ml. Transfer this oil solution to a 100 ml. tared beaker, washing flask free of all oil with fresh acetone, and evaporate with a current of warm air. A convenient method is to place flask on a grill (e.g. over a steam radiator) and place in front of a small electric fan.

When no moisture or acetone can be observed, place beaker in a vacuum oven at 80° C. and apply a vacuum of 24 to 25 inches for 1 hour. Transfer to desiccator, cool, and weigh.

Next, transfer the extracted meal residue from the thimble to a 150 ml. beaker. Remove any remaining solvent by heating on a warm grid and then add 60 ml. 4N HCl. Digest for one hour at or near the boiling point, occasionally stirring with a glass rod. Heat on a hot plate for which the temperature can be controlled and add water as necessary to maintain volume in the beaker. (Note: Thorough removal of all acetone is necessary preliminary to this digestion for otherwise vaporization of solvent will carry meal particles over side of vessel on to hot plate.) Filter through a 12.5 cm. fluted filter. Wash residue on filter until free of acid, using methyl red indicator on portions of filtrate to follow progress of washing. Place filter and meal in a 150 ml. beaker and dry in air oven at 80-90° C. for one hour. Transfer filter paper and contents to a thimble and extract 16 hours with acetone. Finish removal of solvent and weighing of extract as with initial extraction.

II. AOAC method.

Determine oil content by the official AOAC method for grain and stock feeds as described on page 408 of the sixth edition of "Methods of Analysis AOAC", under section 27.24 and 27.25, first drying the meal as described under section 27.3. It should be noted that in drying to constant weight, ordinarily, a truly constant weight will never be attained with fish meal because both loss in weight due to evaporation of moisture and gain of weight due to oxidation of fish oils (even in vacuum oven) occur simultaneously. Drying is therefore discontinued after the sample ceases to lose weight.

Average results obtained in the collaborative analysis are shown in Table 1 while individual determinations in triplicate reported by the different laboratories are given in Table 2. The meal used in this collaborative assay was an experimental pilchard meal[1/] prepared in the pilot plant of the Seattle Fishery Technological Laboratory of the Fish and Wildlife Service on September 25, 1946. The meal was stored in a paper bag at room temperature under conditions similar to

1/ The meal was prepared by a method somewhat similar to commercial wet process rendering, but on a small scale. This involved cooking the pilchard with steam, pressing out oil in a hydraulic press, and drying the meal. The latter step differed from commercial practice in that a tunnel drier was employed using a blast of air at 150° F.

those prevailing in the storage of commercial meals. A large sample was withdrawn during the latter part of April 1947, ground, and mixed thoroughly to insure homogeneity. Samples were placed in glass-stoppered paraffined bottles and mailed to collaborative laboratories with instructions that all tests should be run during the 10-day period of May 10 to May 20. As shown in Table 1, the average fat content of the meal had fallen from the original 14.75 percent in the freshly prepared meal (when determined by the ether extract method) to an apparent value of 14.66 percent when determined by the acetone method. A value of only 5.33 percent was obtained by the ether extract method (AOAC).

Table 1 - Average Values for Fat in Fish Meal as Reported by Collaborative Laboratories

Laboratory	Fat Content of Pilchard Meal Stored 7½ Months			
	Acetone Extraction Method			AOAC Ethyl Ether Method Extract
	Initial Extract	Acid Hydrolysis Extract	Total Extract	
	Percent	Percent	Percent	Percent
A	11.15	2.57	13.72	5.25
B	13.32	2.57	15.67	4.92
C	11.9	3.7	15.6	5.8
D	11.0	2.1	13.1	4.57
E[1]	12.31	2.05	14.36	6.12
F	13.5	2.5	16.0	5.7
G	11.38	2.80	14.18	4.97
Average	12.08	2.58	14.66	5.33
	Initial fat content of freshly prepared meal			
	17.96	0.93	18.89	14.75[2]

1/ Soxhlet extraction equipment was used by laboratory E.
2/ With hydrochloric acid digest extraction included this value was 16.96 percent.

There was no outstanding difference in precision between the AOAC method and the acetone method either in regard to reproducibility between the triplicate samples run within the same laboratory or with respect to differences between individual laboratories. As a rule, individual laboratories were able to obtain checks between the triplicate determinations better than they checked each other and this was true with both the acetone extraction method and the AOAC method.

Table 2 - Precision Attained by Individual Laboratories on Acetone and Ethyl Ether Extraction of Fish Meal

Laboratory	Fat Content of Meal			
	Initial Extract	Acid Hydrolysis Extract	Total Extract	AOAC Ethyl Ether Method Extract
	Percent	Percent	Percent	Percent
A	11.21; 11.27; 10.98	2.75; 2.32; 2.65	13.96; 13.59; 13.63	5.17; 5.27; 5.33
B	13.10; 13.18; 13.68	2.05; 2.77; 2.24	15.15; 15.95; 15.92	4.93; 4.90; 4.92
C	10.8; 12.0; 11.9	4.0; 3.5; 3.7	14.8; 15.5; 15.6	6.0; 5.7; 5.8
D	11.2; 10.6; 11.0	2.1; 2.1; 2.1	13.3; 12.7; 13.1	4.55; 4.70; 4.60
E	12.24; 12.17; 12.51	1.99; 2.08; 2.09	14.23; 14.25; 14.60	6.00; 6.20; 6.07
G	11.14; 11.48; 11.51	3.00; 2.72; 2.67	14.14; 14.20; 14.18	4.93; 4.98; 5.00

NOTE: Laboratory F results not shown because they were not reported in triplicate.

While absolute differences were greater in the case of the acetone extraction method, relative percent differences were about the same. Values obtained for the acetone extraction fat determinations were about three times as high as those for the AOAC method, and differences between duplicate samples and individual laboratories were likewise about three times as high. Thus, the relative difference was of the same order of magnitude for the two different methods. On

the whole, the precision was not nearly as good as could be desired, and future studies should be carried out to determine the cause for such lack of precision and, if possible, to improve upon the procedure in order to obtain better agreement.

In spite of the fact that the acetone extraction method does not give ideal and complete extraction of fat from old meals and that the degree of precision is not all that could be expected, the results by this method are so far superior to those obtained by ethyl ether extraction that it is felt the acetone method should be adopted tentatively pending improvements which may require a considerable number of years to be attained. Accordingly, the following recommendations are made:

1. It is recommended that the acetone extraction procedure as outlined above be made tentative.

2. That study be continued to improve the precision of the above outlined acetone extraction procedure.

3. That studies be continued on the use of other solvents or mixtures of solvents which might eventually lead to a better procedure than is possible by the use of acetone.

LIST OF COLLABORATIVE LABORATORIES

Commonwealth of Virginia
Department of Agriculture and Immigration
Division of Chemistry
Richmond, Virginia

Maine Agricultural Experiment Station
Orono, Maine

State of California Department of Agriculture
Sacramento, California

State of Michigan Department of Agriculture
Lansing, Michigan

U. S. Department of the Interior
Fish and Wildlife Service
Fishery Technological Laboratory
Seattle, Washington

Wirthmore Research Laboratory
Malden, Massachusetts

THE OYSTER AND THE OYSTER INDUSTRY IN THE UNITED STATES

The oyster industry in the United States is one of the very valuable fishery industries, yielding annually (1938-1942) from 75 to about 90 million pounds of oyster meat and giving to our fishermen an annual income varying from 7.5 to 11.8 million dollars. Three species are of commercial value: Ostrea virginica, the eastern oyster; O. lurida, so-called Olympia oyster of the Pacific Coast; and O. gigas, a Japanese oyster introduced into the United States.

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