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IMPROVED RAPID METHOD FOR DETERMINING TOTAL LIPIDS IN FISH MEAL

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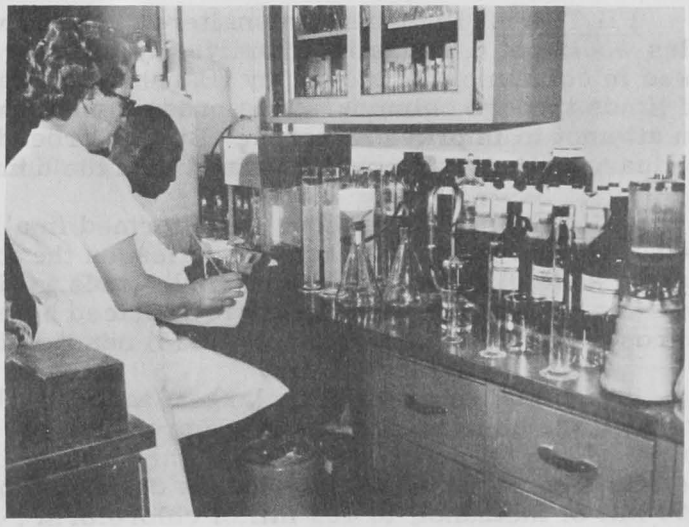
ABSTRACT

In a search for a quicker method for the determination of total lipids in fish meal than the currently used method of the Association of Official Agricultural Chemists, the rapid Bligh and Dyer method for determining the lipid in fish meat was studied. Problems involving filtration and emulsions were encountered, which were largely eliminated, respectively, by using diatomaceous earth and anhydrous sodium sulfate and by increasing the initial ratio of solvent to water in the extraction process. Thus modified, the Bligh and Dyer method gave results in close agreement with those obtained by the more time-consuming AOAC method.

INTRODUCTION

Fish meal is one of several ingredients usually contained in commercial mixed feeds for animals. The manufacturers of those feeds are careful to ensure that the nutritive value of their products remains uniform and consistently high in quality. Hence fish meal and the other ingredients used in the formulations are carefully checked. Ordinarily, fish meal is analyzed for such components as moisture, nitrogen, and lipid content. The nitrogen content is indicative of the amount of protein present, and the lipid content provides an index of the caloric value of the meal.



Determination of lipids in fish meal by the modified Bligh and Dyer method.

Unlike the closely specified procedure used for ascertaining the content of nitrogen, the content of lipid may be determined by any of several methods. The method that has been used quite satisfactorily as an index for caloric value is that of ether extraction (crude fat). Recently, however, there has been a growing demand for information on the fats or lipids in fish meals that cannot be extracted by ether ("total" fat)[1], since there is speculation that the difference between total fat and crude fat may indicate the degree of oxidation of the fat.

The method of the Association of Official Agricultural Chemists for determining total lipids in fish meal provides consistent-

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[1] "Total" fat, for purposes of this paper, will be the value obtained by the method (22.037) of the Association of Official Agricultural Chemists (1960).

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ly reproducible results and gives values among the highest obtained by any method. In the AOAC method, the initial extraction of fish meal is followed by acid hydrolysis and then by second acetone extraction. These conditions presumably free the "bound" lipids and extrac "total" lipids from the fish meal. Because this method requires over 35 hours to complete it is not a rapid analytical tool.

In 1959, Bligh and Dyer developed a rapid extraction procedure to measure the lipids i raw fish. Briefly, their method consists of homogenizing the fish tissue with a mixture of chloroform and methanol in such proportions that a miscible system is formed with the wa in the tissues. Subsequent dilution with chloroform and water then separates the homogena into two phases. After filtration and clarification, the chloroform layer contains the lipids and the methanol-water layer contains the nonlipid material. Unfortunately, though this m od is rapid, it is not entirely satisfactory for use with fish meal.

The purpose of the work reported in this paper therefore was to try to modify the rapi Bligh and Dyer method so that it can be used to determine reproducibly and accurately the total lipids in fish meals. The main topics considered in this report are the following:

1. Modifications needed in the Bligh and Dyer method.
2. Description of the modified method developed.
3. Evaluation of the modified method.

MODIFICATIONS NEEDED

In our study of the modifications needed to adapt the Bligh and Dyer method for use wi such materials as fish meal, five menhaden meals were used. (Menhaden meal constitutes the bulk of the fish meal produced in the United States.) These meal samples differed in a and were from various areas of production. The principal difficulties encountered in the u of the Bligh and Dyer method in the analysis of these meals arose from problems relating filtration and emulsions.

FILTRATION: When the unaltered method was used with the fish meals, two of the sa ples would not filter satisfactorily. Hyflo Super Cel (diatomaceous earth), however, has b used in column chromatography (Hanahan, Dittmer, and Warashina 1957) to enhance the fl of lipids through columns. This product and anhydrous sodium sulfate were therefore use an attempt to improve filtration. By this procedure, all five samples filtered faster and g a clearer filtrate than was obtained with the unmodified Bligh and Dyer method.

EMULSIONS: Emulsions were formed in all five samples tested. The following pro- cedures were tried in an attempt to lessen the formation of emulsions: (1) addition of ne salts--sodium chloride, potassium chloride, and magnesium chloride; (2) use of higher a hols for extraction--methanol was replaced by ethanol and octanol; (3) use of a surfactan Aerosol OT; (4) centrifugation; and (5) changes in the ratio of the organic solvents.

The sample that gave the largest amount of emulsion with the unmodified Bligh and D method was used to evaluate these procedures. Changes in the ratio of the organic solve proved to be the most beneficial of the alterations tried, so this modification was studied detail. The phase-volume ratio was changed from an initial ratio of 50 ml. of chloroform 100 ml. of methanol, to 100 ml. of chloroform : 100 ml. of methanol, and finally to 150 m chloroform : 100 ml. of methanol. Higher values for extractable material were obtained both modifications in ratio of solvents. The last ratio of solvents, however, also resulted both decreased emulsions and in improved filtration and was therefore considered the mo satisfactory.2/ Table 1 presents the results of extraction of lipid by the unmodified Bligh a Dyer method and by the various modifications in the ratio of solvents.

2/In addition, this ratio of solvents resulted in a biphasic system throughout the extraction procedure. Hence, some people may pret this approach as a new application of chloroform:methanol extraction rather than a modification of the Bligh and Dyer m

Table 1 - Fat Extracted from Samples of Fish Meal by Variations of the Bligh and Dyer Method

Extraction Method	Replicate	Fat Content of Fish-Meal Sample: E	6C79	4B120	12C13	G
		(Percent)				
1. Original method 50 $CHCl_3$/100 MeOH	1	11.87	9.44	1/	11.81	1/
	2	12.1	9.38	-	11.93	-
2. Filtering aid 50 $CHCl_3$/100 MeOH	1	11.86	9.19	13.19	11.52	10.23
	2	11.68	9.10	13.44	11.65	10.90
3. Filtering aid 100 $CHCl_3$/100 MeOH	1	12.82	9.90	14.76	12.59	10.36
	2	13.42	9.64	14.78	12.23	10.31
4. Filtering aid 150 $CHCl_3$/100 MeOH		12.80	10.22	14.51	12.51	10.41

1/The samples were not filterable.
Note: Each entry represents a single analysis except Method 4 where each entry is the average of six analyses. Column headings "E," "6C79," etc. identify number of sample.

MODIFIED METHOD

The modified method resulting from the observations reported in the previous section is as follows:

1. Homogenize 10 grams of fish meal in an electric blender for 2 minutes with a mixture of 37 ml. of water, 150 ml. of chloroform (analytical reagent grade), and 100 ml. of absolute methanol (analytical reagent grade).
2. Add 50 ml. of chloroform to the mixture, and blend for an additional 30 seconds.
3. Transfer the mixture to a 600-ml. beaker containing 20 grams of Hyflo Super Cel and 20 grams of anhydrous sodium sulfate.
4. Mix the contents; and filter into a 1,000-ml. filter flask, using a Buchner funnel and Whatman number 1 filter paper.
5. Add 50 ml. of water to the filtrate, and mix thoroughly.
6. Transfer the filtrate to a 500-ml. graduated cylinder.
7. Reblend the residue for 2 minutes with 200 ml. of chloroform, and then refilter.
8. Add the filtrate to the cylinder.
9. Wash the blender jar, beaker, and filtering flask with about 50 ml. of chloroform.
10. Filter the washings and add them to the cylinder.
11. Allow a few minutes for separation and clarification of the filtrate, record the volume of the chloroform, and remove most of the methanol-water layer by aspiration.
12. Mix the contents of the cylinder, and remove the remaining methanol-water layer and also a small volume of the chloroform layer.
13. Take a 25-ml. aliquot of the chloroform layer, and dry it under a stream of nitrogen in a 50° C. water bath.
14. Place the dried sample in a vacuum dessicator over phosphorus pentoxide, and allow to dry overnight.
15. Calculate the weight of the total lipid in the sample as follows:

$$\text{Wt. of total lipid} = \frac{\text{Wt. lipid in aliquot} \times \text{volume of chloroform layer}}{\text{Volume of aliquot}}$$

EVALUATION

In evaluating the modified Bligh and Dyer method, we were concerned with the following four aspects:

1. Purity of the lipid extract obtained by the modified method.
2. Precision of the method.
3. Accuracy of the method.
4. Comparison of the results obtained by the method with those obtained by the AOAC method.

PURITY: The material extracted by organic solvents usually contains some nonlipids. In a satisfactory method for determining lipid, this extract of nonlipid material must, of course, be kept to a minimum. Accordingly, we tested the purity of the extracted material by drying an aliquot of the extracted fat, redissolving it in chloroform, and observing it for undissolved residue. With the improved initial ratio of 150 ml. of chloroform : 100 ml. methanol for extraction, no residue was noted in any of the samples tested. However, in some of the other procedures tested, residue was observed.

PRECISION: To determine the reproducibility of results of the improved procedure, we analyzed each of five menhaden meals six times, and evaluated the results statistically. As is shown in table 2, the maximum deviation from the mean was ±0.23 percent.

Table 2 - Reproducibility of Improved Method of Extraction of Fat

Sample	Mean	Standard Deviation	Standard Error
	(Percent)		
E	12.80	0.14	0.06
G	10.41	0.11	0.04
6C79	10.22	0.23	0.10
4B120	14.51	0.20	0.08
12C13	12.51	0.21	0.09

Note: The mean represents the average of six analyses.

ACCURACY: Test results on samples to which oil has been added should give an indication of the accuracy of the method in terms of the possible errors in the mechanical manipulations, although not in terms of the ability of the system to extract bound fat.

Table 3 - Recovery of Menhaden Oil Added to Fish Meal

Sample	Fat Present			Fat by Analyses	Recovery
	In Sample	In Oil	Total		
			(Percent)		
E	12.71	3.00	15.71	16.05	102.2
		3.04	15.75	15.79	100.3
G	10.56	3.22	13.78	13.93	101.1
		3.16	13.72	13.39	97.6
6C79	10.48	3.04	13.52	13.66	101.0
		3.01	13.49	13.48	99.9
4B120	14.67	3.08	17.75	17.41	98.1
		3.23	17.90	18.20	101.7
12C13	12.72	3.03	15.75	15.51	98.5
		3.20	15.92	15.61	98.1

Recoveries were tested by extracting mixtures of 0.3 grams of menhaden oil with grams each of the same five menhaden meals. Table 3 presents the recovery data, showing that the widest variation in the recovery of menhaden oil was 2.2 percent.

COMPARISON: The AOAC method 22.037 for the determination of fat in fish meal was used for comparison. The data in table 4 show that closely comparable results were obtained by the AOAC method and the modified Bligh and Dyer method. It is interesting to note that values obtained with the modified method were lower for four meals, but that for one meal

ore lipids were found than by the AOAC ethod. In no pair of values, however, was e absolute difference greater than 0.48 per-ent.

Table 4 - Comparison of Fat Extracted by the Improved Method and the AOAC Method

Sample	Fat Extracted	
	Improved Method[1]	AOAC 22.037[2]
	(Percent)	
E	12.80	12.68
G	10.41	10.80
4B120	14.51	14.86
6C79	10.22	10.70
12C13	12.51	12.61

1/Average of six analyses.
2/Average of two analyses.

SUMMARY AND CONCLUSION

In trying to shorten the time of analysis equired by the AOAC method for total lipid fish meal and similar products, we studied possibility of adapting the Bligh and Dyer thod used for analyzing the lipid in fish at. We used five samples of menhaden meal of varying age and lipid content in developing d testing the modified method.

Applying to fish meal the Bligh and Dyer method--which utilizes chloroform, methanol, d water in such proportions that after the initial extraction, a two-phase system is formed--sults in problems involving filtration and emulsions. Filtration was improved by the addi-n of diatomaceous earth and anhydrous sodium sulfate. Emulsions were largely eliminated increasing the initial ratio of solvent to water.

The purity of the extract was tested by drying an aliquot of the extracted fat, redissolving in chloroform, and checking for insoluble material. After the ratio of solvent to water was anged, no residue was noted in any of the five samples tested. The precision of the method as checked by analyzing six replicates of each of the five menhaden meals and evaluating the sults statistically. The maximum deviation from the mean was ±0.23 percent. The accura-y of the improved method was determined by the percent recovery of added menhaden oil to e same five fish meal samples. The widest variation noted in recovery was 2.2 percent. ipid determination by the improved method compared favorably with results from AOAC ethod 22.037.

We concluded that in the analysis of menhaden meal and presumably of other fish meals e modified rapid Bligh and Dyer method could serve satisfactorily as a substitute for the re time-consuming AOAC method.

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