STUDIES ON METHODS OF EXTRACTING VITAMIN A AND OIL FROM FISHERY PRODUCTS[1/]

PART III - EXPERIMENTS ON THE EXTRACTION OF LOW-OIL-CONTEN LIVERS WITH PETROLEUM ETHER BY THE SHAKING METH

By F Bruce Sanford and Neva L. Karrick*

ABSTRACT

This report deals with experiments that were directed toward improving the laboratory method of analyzing for oil in low-oil-content fish livers. The particular procedure studied was the shaking method. The study was limited to three variables: (1) the weight of the liver sample (5 and 18 grams in one series of experiments, and 1 and 5 grams in another series); (2) the type of dispersing agent (powdered pumice and powdered anhydrous sodium sulfate); and (3) the volume of dispersing agent (25, 50, and 100 milliliters). A better dispersion of the ground liver was obtained with the pumice than with the anhydrous sodium sulfate. Fifty milliliters of dispersing agent appeared to be about the optimum volume to use with 50 milliliters of petroleum ether and a shaking bottle of 180-milliliters capacity. The liver samples weighing 5 grams were more thoroughly extracted than those weighing 18 grams; and the samples weighing 1 gram were more thoroughly extracted than those weighing 5 grams. The clumping of the liver material appeared to decrease the amount of oil extracted.

INTRODUCTION

Because of the decrease in the abundance of soupfin and certain other shar the livers of food fish are becoming increasingly important as sources of natur vitamin A. In contrast to the livers of most sharks, the livers of food fish us ally have a low content. The rapid method of analysis (Anonymous 1947) for oil and vitamin A that was developed for shark livers is not suitable for livers th contain only a small amount of oil. Therefore, a rapid method of assay is need for the low-oil-content livers.

The purpose of these experiments was to obtain data that may ultimately re sult in the development of the needed method. The observations reported here a part of a series of studies being conducted to investigate the factors involved in the solvent extraction of oil and vitamin A from low-oil-content livers for analytical purposes. This particular group of experiments applies to the sh

* Chemists, Fishery Technological Laboratory, Branch of Commercial Fisheries, U. S. Fish and Wildlife Service, Seattle, Washington.

1/ Part I - "Vitamin A Potencies of Oil from Grayfish Livers Obtained by Extraction with Petroleum Ether and by Cooking with Water," by D. Miyauchi and F. B. Sanford, appeared in Commercial Fisheries Review, September 1947, Vol. 9, No. 9, and also as Separate No. 186. Part II - Experiments on the Solvent Extraction of Low-Fat Livers, appeared in same Review, February 1949, Vol. 11, No. 2, and also as Separate No. 224.

ing method (Anonymous 1947).[2/] The following variables were tested: (1) the weight of the sample (5 and 18 grams in one series of experiments, and 1 and 5 grams in another series), (2) the type of dispersing agent (powdered pumice and powdered anhydrous sodium sulfate), and (3) the volume of dispersing agent (25 milliliters, 50 milliliters, and 100 milliliters). In order to simplify the immediate problem, no analyses were made for vitamin A.

PROCEDURE

To maintain continuity and facilitate comparison with previous results, the liver samples were from the same material employed in the work reported earlier (Sanford and Manalo 1949). A quantity of rockfish (Sebastodes sp.) livers had been ground, stirred until uniform, sealed under atmospheric pressure in half-pound cans, and stored at -18° C. (0° F.). Because the cans had not all been opened, a number of them were available for the present study. After these had been used, a series of sole livers was prepared in a similar manner.

The concentration of oil in the liver material was determined as follows:

> A can of the frozen liver was placed overnight in a refrigerator maintained at a temperature slightly above freezing. On the following morning, the material was removed from the can and blended for 3 minutes in a Waring Blendor. A portion (approximately 1, 5, or 18 grams, depending upon the experiment) of the material was transferred to a tared, square, 180-milliliter bottle, and the bottle and its contents were accurately weighed. Exactly 50 milliliters of petroleum ether (bp 35° - 60° C.) and the desired volume (25, 50, or 100 milliliters) of dispersing agent (powdered pumice or powdered anhydrous sodium sulfate) were added to the bottle. It was corked, machine-shaken for one hour (144 one-inch strokes a minute), and centrifuged. A 10-milliliter aliquot portion was then pipetted into a tared beaker and the solvent was evaporated by placing the beaker on a wire screen suspended above an electric hot plate. Three minutes after the solvent had disappeared, the beaker was removed, allowed to cool, and weighed. The oil content of the sample was then calculated.

The choice of 5 and 18 grams as the sample weights to be studied in the first series of experiments, although somewhat arbitrary, was based upon the following considerations:

> The sample should be large enough that the extra precautions required in micro techniques need not be employed. On the other hand, the dimensions of the shaking bottle imposed an obvious upper limit on the size of the sample. Another consideration was that the samples must be sufficiently different in weight so that, if sample size was a significant variable, this fact would become apparent from the data. Previous work with the liver material indicated that 5 and 18 grams would be satisfactory weights. The choice of 1 and 5 grams in the second series

2/ The shaking method employs the following procedure: A weighed sample of liver is shaken in a bottle with a measured volume of solvent and a desiccant or a dispersing agent. The bottle and its contents are centrifuged and an aliquot portion of the supernatant solution is taken by means of a pipet. The weight of oil obtained, after the solvent in the aliquot portion is evaporated, gives the remaining datum necessary for the calculation of the liver oil concentration. A second aliquot portion, suitably diluted with isopropanol, is taken for a determination of optical density by means of a spectrophotometer. The vitamin A potency of the oil can then be calculated from the resulting data.

of experiments was based upon similar reasoning. It was realized, however, that the results with the 1-gram samples would be more variable than those with the 5-gram samples. To compensate somewhat for the decrease in precision, the number of replicate samples in the second series was increased to 10.

Because the blended liver material was fluid, the sample was transferred from the blender jar to the shaking bottle, using a pipet constructed from glass tubing one centimeter in diameter. Markings on the pipet indicated the volume of liver material needed to obtain samples fairly reproducible in weight.

DISCUSSION

If anhydrous sodium sulfate is placed in the shaking bottle before the petroleum ether is added to it, the liver material tends to form clumps or balls when the bottle is shaken. This results in less efficient extraction of oil from the liver. The order of addition of the pumice and solvent, however, appears to make no difference because there is little tendency for the pumice to cause balling of the liver particles. If the dispersing agent can be placed in the bottle before the solvent, the manipulative procedure can be slightly improved because the possibility of losing the solvent by evaporation is decreased. Therefore, when powdered pumice was used as the dispersing agent, it was added before the petroleum ether; when anhydrous sodium sulfate was used, it was added after the petroleum ether.

The amount of a powder is usually measured by weight. In experiments reported here, however, the amounts of powder were measured more conveniently by volume.

The data in Table I indicate that, under the conditions of the experiments, oil was more completely extracted from the 5-gram samples of liver than from the 18-gram samples. The effect of changing the sample weight from 5 grams to 18 grams was somewhat more noticeable if powdered anhydrous sodium sulfate was used as the dispersing agent than if powdered pumice was used.

The pumice appeared to be a more effective dispersing agent than the sodium sulfate. Increase in sample size accentuated the difference in the apparent effectiveness of the two dispersing agents.

Table 1 - The Apparent Oil Concentration in Liver of Low-Oil-Content (Rockfish, Sebastodes sp.) as Determined From 5- or 18-Gram Samples, Using Varied Quantities of Powdered Pumice or Powdered Anhydrous Sodium Sulfate to Disperse the Liver Material

Weight of liver sample	Volume of pumice added	Volume of anhydrous sodium sulfate added	APPARENT CONCENTRATION OF OIL IN SAMPLE								
			Replicate Sample Number								Average of replicates
			1	2	3	4	5	6	7	8	
Grams	Milliliters	Milliliters	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent
5	25	0	11.2	14.5	16.0	16.3	15.3	16.0	16.7	16.3	15.3
18	25	0	3.9	16.1	15.8	16.2	14.9	10.2	17.1	13.1	14.8
5	50	0	17.1	17.4	17.1	17.1	17.2	17.1	16.8	16.7	17.1
18	50	0	11.4	12.8	16.0	15.6	15.9	11.9	12.6	11.0	13.4
5	100	0	17.5	16.0	16.2	15.2	15.3	16.9	12.8	16.9	15.6
18	100	0	9.8	8.8	15.7	15.5	12.0	9.4	16.0	11.9	12.4
5	0	25	12.7	12.6	11.1	10.8	13.4	14.6	13.6	11.4	12.5
18	0	25	8.2	2.5	-	1.3	1.9	4.9	1.8	7.8	4.1
5	0	50	14.0	12.3	16.2	14.7	15.6	15.7	15.9	16.7	15.2
18	0	50	7.6	6.6	6.8	8.5	8.6	11.8	5.6	5.8	7.7
5	0	100	13.2	-	12.6	12.8	11.7	14.9	16.2	16.6	14.0
18	0	100	6.0	7.3	12.3	9.6	12.0	10.9	7.0	7.3	9.0

With 5-gram liver samples, 50 milliliters of dispersing agent (pumice or anhydrous sodium sulfate) was better than either 25 or 100 milliliters. With 18-gram samples, the optimum volume of dispersing agent appeared to vary with the type of agent. Thus, with pumice, the use of 25 milliliters appeared to result in better extraction than did the use of 50 or 100 milliliters; on the other hand, the use of 100 milliliters of anhydrous sodium sulfate appeared to result in better extraction than did the use of 25 or 50 milliliters. Inasmuch as the 18-gram liver samples could not be extracted efficiently, no attempt was made to confirm these results.

Instead, with the amount of dispersing agent set at 50 milliliters, the effect of reducing the sample weight from 5 grams to 1 gram was studied. The data for this second series of experiments are reported in Table 2. The results of the experiments are in agreement with those of the first series. In short, the dispersion was better with the pumice than with the anhydrous sodium sulfate, and relatively more extractives were obtained from the 1-gram samples than from the 5-gram samples.

Table 2 - The Apparent Oil Concentration in Liver of Low-Oil-Content (Sole) as Determined from 1 or 5-Gram Samples, Using 50 Milliliters of Powdered Pumice or Powdered Anhydrous Sodium Sulfate to Disperse the Liver Material

Weight of liver sample	Kind of dispersing agent	APPARENT CONCENTRATION OF OIL IN SAMPLE										Average of replicates
		Replicate Sample Number										
		1	2	3	4	5	6	7	8	9	10	
Grams		Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent
1	Pumice	14.8	14.0	13.4	14.6	15.0	14.7	14.7	14.1	13.8	14.6	14.4
5	"	13.7	14.2	13.8	14.0	13.8	14.2	13.7	14.1	13.9	14.2	14.0
1	Sulfate	11.2	13.3	12.4	12.4	12.7	12.6	11.1	13.7	13.6	13.0	12.6
5	"	10.9	11.5	10.8	11.8	11.7	11.5	10.6	10.3	9.5	11.2	11.0

In the present experiments, neither the amount of the non-oil impurities nor the completeness of the oil extraction was determined. For this reason, the oil concentrations reported in Tables 1 and 2 have been designated as "apparent."

The large deviations among the replicates show that the results were affected by variables other than those being directly investigated. Observations indicated that one cause of the uncontrolled variation was the clumping of the liver material after it had been introduced into the shaking bottle. The clumps were especially noticeable when anhydrous sodium sulfate was used. Clumping would account for at least a part of the variation because the aggregation of the liver particles obviously would reduce the completeness of the extraction. Therefore, in extracting oil from liver material by means of petroleum ether and the shaking method, care should be taken to obtain small, dispersed liver particles.

SUMMARY

Low-oil liver samples, 1, 5, or 18 grams in weight, were shaken in square, 180-milliliter bottles with 50 milliliters of petroleum ether and, depending upon the experiment, 25, 50, or 100 milliliters of powdered pumice or powdered anhydrous sodium sulfate. It was found that:

(1) Relatively more oil was extracted from the small samples than from the large ones.

(2) Judging both from the appearance of the liver particles when dispersed by powdered pumice or by anhydrous sodium sulfate and from the relative amounts of oil extracted when each of these materials was used under similar conditions, pumice was a more effective dispersing agent than anhydrous sodium sulfate.

(3) With the 5-gram liver samples, 50 milliliters of dispersing agent was more effective than 25 or 100 milliliters.

(4) For efficient oil extraction, care must be taken to obtain small, well dispersed liver particles.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the assistance of Mr. Alvaro Bonilla A. and Mrs. Mabel A. Edwards.

LITERATURE CITED

ANONYMOUS

1947. Preliminary procedures for the analysis of vitamin A in fishery byproducts. Commercial Fisheries Review, vol. 9, no. 1, pp. 40-2.

SANFORD, F. B., and MANALO, A. D.

1949. Studies on methods of extracting vitamin A and oil from fishery products. Part II - Experiments on the solvent extraction of low-fat livers. Commercial Fisheries Review, vol. 11, no. 2, pp. 18-22.

PACKAGING FROZEN FISHERY PRODUCTS

Packaging is of importance in retarding oxidative changes in fish. Oxidation of the fat that is present in fish is a factor which has much todo with the period of time that fish--particularly those designated as fatty--can be maintained in a satisfactory condition in frozen storage. The fat contained in fish is much more susceptible to oxidation than is the fat found in other animal or vegetable foods. Oxygen is rapidly absorbed by this fat and will soon cause the loss of fresh flavor and the development of rancidity. Bleaching and fading of the natural color of the fish may also occur and in extreme cases the fat will darken, causing the fish to assume a brown color. These changes can be retarded by packaging tightly with essentially air-tight wrappings to prevent ready passage of air to the fish.

--Fishery Leaflet 324