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

PART II - EXPERIMENTS ON THE SOLVENT EXTRACTION OF LOW-FAT LIVERS[2/]

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ABSTRACT

Using the shaking method for the solvent-oil extraction of low-fat livers some of the variables investigated were: the type of solvent, weight of sample, amount of drying agent, type of dispersing agent, and blendering time. Lyophilization to remove moisture from the sample prior to solvent extraction was also studied. A complex inter-relationship exists between the variables. For example, whether or not a drying agent or a dispersing agent is of critical importance depends upon the nature of the solvent used. Ethyl ether requires a drying agent while a dispersing agent is necessary with petroleum ether. In fact, the solvent actions of petroleum ether appear to be reduced when a drying agent is employed.

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INTRODUCTION

In the analytical determination of vitamin A and oil in livers and viscera, two methods are employed. One is used with materials containing less than 40 percent oil, while the other is used when the oil content is greater. In the low-oil method, the material is repeatedly extracted with portions of fresh solvent, while in the high-oil method, the material is simply placed in a bottle in contact with the solvent and shaken until equilibrium is established. The low-oil method is tedious and cumbersome when a large number of analyses are run at one time. In contrast, the high-oil method is relatively simple and ideal for mass production. From earlier work, it appeared that the high-oil method might be adapted to low-oil materials if the type of solvent could be improved. The purpose of the present experiments was to find a basis for choosing the proper solvent.

In the analysis of liver or viscera for oil and vitamin A, usually a quart sample of the material is submitted to the laboratory. Here the sample is homogenized by a Waring-type blender. This process reduces the liver, or viscera material, to small particles, and it also liberates oil. The essential problem in solvent-extraction is to dissolve from the particles the oil and vitamin A not set free in the blendering process.

An assumption in the present work was that the moisture associated with the particles forms one of the principal obstacles to the action of the solvent. For

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1/Part I - "Vitamin A Potencies of Oils 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. Available as Separate No. 186.

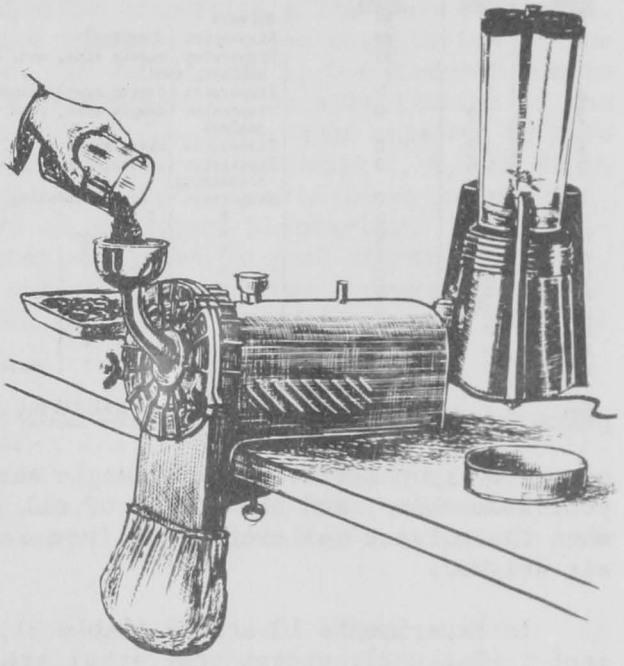
2/This paper was presented at the Western Session of the 114th national meeting of the American Chemical Society at Portland, Oregon, on September 15, 1948.

this reason, solvents were arbitrarily divided into three groups: those insoluble in water, like petroleum ether; those partially soluble, like ethyl ether; and those completely soluble, like acetone.

The general procedure was to compare the results obtained with these three types of solvents and to see what difficulties were encountered. Due to limitations in time, only the oil aspect was considered, and the problem of vitamin A extraction was ignored. Further, to save time, portions of the same liver material were used throughout the experiments. This was accomplished by homogenizing some low-oil liver, sealing the resulting material in cans, and preserving it by refrigeration. The general procedure of extraction was that followed in the high-oil method. In order to obtain an independent estimate of the amount of oil in the material, a proximate analysis for ash, protein, and moisture was made, and the oil content was estimated by difference.

EXPERIMENTAL

In the preparation of the samples, 30 pounds of rockfish livers were passed through a meat chopper and further disintegrated and homogenized by means of a propellor-like device having sharpened edges and rotated by an electric motor. The resulting material was sealed, under atmospheric pressure, in half-pound flat cans. (So much air had been beaten into the material that it frothed over the sides of the containers when a vacuum pack was attempted.) To keep the liver from spoiling, the flats were placed in a cold room maintained at 0° F. Before use, each can was left overnight in a refrigerator held slightly above freezing so that the material in the can could thaw.



The technique for oil extraction was to remove the liver from the can and to homogenize this material further by means of a Waring-type blender. A portion was transferred to a square bottle of 160 ml. capacity and weighed. A measured volume of solvent was added; and, in certain experiments, anhydrous sodium sulfate was used as a drying agent. The sulfate was always added after the solvent, as the reverse procedure tends to cause the liver material to clump into balls. After stoppering the bottle by means of a cork, the bottle was machine-shaken for one hour. Centrifuging the bottle and its contents settled the suspended matter. A 5 ml. aliquot was taken and evaporated in a 50 ml. beaker over an air bath. Three minutes after the solvent was gone, the beaker was removed, allowed to cool to room temperature, and weighed.

The proximate analysis for ash, protein, and moisture (Table 1) followed the regular procedures established by the Association of Official Agricultural Chemists.

In Experiment 1 (Table 2), petroleum ether was used as the solvent. Experiment 2 differed only in that the mass which tended to form on the side of the bottles (the bottles lay on their sides in the shaking machine) was broken up by shaking the bottles occasionally by hand. In Experiment 3, there was more sulfate, a smaller sample was taken, and sand was used as a dispersing agent. In Experiment 4, the sulfate was omitted. In Experiment 5, the sample was again reduced in size and pumice was used. In Experiment 6, kieselguhr was substituted for the pumice. In Experiments 7 through 9, the time of blendering was varied. In Experiments 10 through 16, different amounts of water were used as a dispersing agent. In Experiment 17, a small amount of sulfate was added to the water. In Experiment X, the free moisture was removed from the sample by lyophilization, the sample was refluxed with a measured quantity of the petroleum ether, and the amount of oil present was estimated from data obtained when the solvent was evaporated from an aliquot and the resulting oil residue was weighed.

Table I - Data Obtained in Proximate Analysis[1]

Constituent	Relative Amount of the Constituent % by Weight
Ash	1.12
Protein	13.42
Moisture	67.33
Oil (by difference)	18.13

1/Analyses made by Virgil Uyenco

Table 2 - Data Obtained in the Extraction of Oil from Low-Fat Liver by Means of the Shaking Method[1] and the Use of Petroleum Ether as a Solvent

Identity of the Experiment	Approx. Wt. of Samples	Approx. Wt. of Anhydrous Sodium Sulfate Used	Variables Investigated	AMOUNT OF OIL EXTRACTED Replicate 1	2	3	4	5	6	Mean of Replicates
Symbol	Grams	Grams		Percent	Percent	Percent	Percent	Percent	Percent	Percent
1	20	30	Solvent	4.83	3.63	3.65	4.98	5.04	6.48	4.77
2	20	30	Dispersion (clumping)	12.32	12.35	15.05	13.30	12.70	16.30	13.67
3	7	40	Dispersion (sample size, amt. of sulfate, sand)	15.45	15.38	15.63	14.11	12.79	14.09	14.57
4	7	0	Dispersion (drying agent, sand)	17.20	17.13	17.17	17.19	16.89	17.07	17.11
5	2.5	0	Dispersion (sample size, powd. pumice)	17.41	17.36	17.15	17.31	17.52	17.28	17.34
6	2.5	0	Dispersion (kieselguhr)	15.88	16.47	15.41	15.19	14.00	14.60	15.26
7	1.6	0	Dispersion (pumice, 1 min. blendering)	17.58	17.50	17.55	17.52	-	-	17.54
8	1.6	0	Dispersion (4 min. blendering)	17.40	17.32	17.45	17.49	-	-	17.42
9	1.6	0	" (12 " ")	15.80	15.96	15.80	-	-	-	15.85
10	2	0	" (0 ml. water)	5.77	5.43	6.25	-	-	-	5.81
11	2	0	" (1 " ")	9.38	8.67	8.71	-	-	-	8.92
12	2	0	" (2 " ")	10.09	9.51	9.62	-	-	-	9.74
13	2	0	" (4 " ")	15.81	15.79	17.41	-	-	-	16.33
14	2	0	" (8 " ")	16.91	16.91	16.91	-	-	-	16.91
15	2	0	" (16 " ")	17.08	16.51	16.41	-	-	-	16.66
16	2	0	" (32 " ")	16.85	17.01	16.95	-	-	-	16.93
17	2	3	" (16 " " , sodium sulfate)	17.08	17.19	17.04	17.10	-	-	17.10
X[1]	5	0	Penetration (lyophilization)	16.76	16.80	-	-	-	-	16.78

1/Results in Experiment X were obtained by lyophilization instead of by the shaking method. Made by Dirk Verhagen.

In Experiments 18 and 19 (Table 3), the conditions were the same as in Experiment 1 (Table 2), except that ethyl ether was used instead of petroleum ether.

Table 3 - Data Obtained in the Extraction of Oil from Low-Fat Liver by Means of the Shaking Method[1] and the Use of Ethyl Ether as a Solvent

Identity of the Experiment	Volume of Solvent Used	Weight of Sample	Variables Investigated	AMOUNT OF OIL EXTRACTED Replicate 1	2	3	4	5	6	Mean of Replicates
Symbol	Ml.	Grams		Percent	Percent	Percent	Percent	Percent	Percent	Percent
18	50	20	Homogeneity of samples	17.41	17.65	17.70	17.60	17.70	17.80	17.65
19	50	20	" " "	17.60	17.70	17.68	17.61	17.76	17.60	17.66
20	100	2	Sample size	18.10	17.48	18.50	17.76	17.99	17.35	17.86
21	50	20	Moisture effect (no sodium sulfate)	17.49	17.56	17.53	17.39	-	-	17.49
Y[1]	300	5	" " (lyophilization)	17.23	17.66	17.87	17.88	17.92	18.10	17.78

1/Results in Experiment Y were obtained by lyophilization instead of by the shaking method. Made by Dirk Verhagen.

In Experiment 20, the sample size was reduced. In Experiment 21, anhydrous sodium sulfate was omitted, and in Experiment Y, the sample was lypholized and extracted as in Experiment X (Table 2).

In Experiment 22 (Table 4), the conditions were the same as in Experiments 1 and 18, except that acetone was used as the solvent.

Table 4 - Data Obtained in the Extraction of Oil from Low-Fat Liver by Means of the Shaking Method and the Use of Acetone as a Solvent

Identity of the Experiment	Volume of Solvent Used	Weight of Sample	Variables Investigated	AMOUNT OF OIL EXTRACTED						
				Replicate						Mean of Replicates
				1	2	3	4	5	6	
Symbol	Ml.	Grams		Percent	Percent	Percent	Percent	Percent	Percent	Percent
22	50	20	Solvent	8.64	8.51	8.93	8.32	8.44	8.67	8.58

RESULTS

The proximate analysis (Table 1), indicated that about 18 percent oil was present in the sample. This value was obtained by determining the percentage of ash, protein, and moisture, and subtracting the sum of these figures from 100.

In Experiment 1 (Table 2), using petroleum ether as a solvent, only 4.77 percent oil was extracted instead of 18 percent as indicated by proximate analysis. The bottles lay on their sides in the shaking machine, and in this Experiment, the liver material covered the bottom side in much the same manner as putty. When the bottle was machine-shaken, the solvent slid over the top of this mass. In Experiment 2, shaking the bottles by hand occasionally prevented the putty-like mass from forming and resulted in better oil extraction. In Experiment 3, the essential change was the addition of sand, which aided in the dispersion of the liver particles. In Experiment 4, omission of sodium sulfate resulted in less coagulation of the liver particles and greater oil extraction. In Experiment 5, the powdered pumice gave greater dispersion than the sand. In Experiment 6, the substitution of the kieselguhr appeared to result in less extraction. The kieselguhr appeared to have a tendency to make the liver particles coagulate. In Experiments 7, 8, and 9, it appeared that blendering tends to heat the liver material and to cause coagulation. The result was that less oil was extracted on prolonged blendering. In Experiments 10 through 16, it was found that water was a fairly good dispersing agent. In Experiment 17, the addition of sodium sulfate to the water appeared to result in better oil extraction. In Experiment X, lyophilization of the sample resulted in fair extraction of the oil.

Experiments 18 and 19 were replicates of each other. The solvent used was ethyl ether, and the amount of oil extracted was the same in both Experiments. In Experiment 20, reduction of the sample size appeared to have resulted in slightly more oil being extracted. In Experiment 21, omitting sodium sulfate reduced slightly the amount of oil extracted. In Experiment Y, lyophilization resulted in good oil extraction.

In Experiment 22, acetone was used as the solvent. While the yield of oil was poor, the physical appearance of the sample was good in that there seemed to be no tendency for the liver to coagulate.

DISCUSSION

Due to uncertainties in the analysis of ash, protein, and moisture, and also due to the fact that certain potential constituents such as glycogen, etc., were not taken into account, the figure 18.13 percent arrived at by proximate analysis (Table 1), can be considered only as an estimate as to the amount of oil present.

Using petroleum ether as a solvent (Table 2), one of the principal difficulties appears to be that of penetration. The moisture present in the liver would appear to prevent the penetration of this solvent. Hence, when petroleum ether is used, the liver should be dispersed so as to present as large a surface of liver material

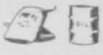
to the action of the solvent as is possible. Shaking the bottles by hand (Experiment 2) helps as does also the addition of sand. Anhydrous sodium sulfate has a tendency to cause the liver to form clumps. Since petroleum ether dissolves almost no water, anhydrous sodium sulfate is useless when this solvent is used and should probably be omitted. Kieselguhr (Experiment 6) does not appear to be as good a dispersing agent as sand or pumice. Blendering (Experiments 7, 8, and 9) tends to increase the temperature which in turn tends to coagulate the liver particles. It would appear that the proper time of blendering is that which will produce optimum homogenization with minimum heating. Water appears to be almost as good a dispersing agent as sand. However, when the action of water on liver is viewed under a microscope, the water seems to have a coagulating effect. The addition of salts, such as sodium sulfate to the water (Experiment 17), may aid dispersion as more oil appears to be extracted in this case. Lyophilizing the sample appears to aid the solvent to penetrate the material, but penetration does not appear to be complete, as only 16.78 percent oil was obtained.

In Experiments 18 and 19 with ethyl ether, the results indicate that the liver material is the same in all cans. In Experiment 20, there is an indication that reduced sample size results in better extraction. Experiment 21 indicates that moisture in the solvent may reduce oil solubility. Experiment Y also supports this view, because the oil extracted appears to increase when most of the moisture is removed.

In Experiment 22, with acetone, the oil extracted is less than half that obtained with ethyl ether. A possible explanation is that anhydrous sodium sulfate may not function effectively with acetone. An increase in the moisture content of the solvent could result in less oil solubility.

CONCLUSIONS

While with the aid of dispersing agents, petroleum ether can be made to function fairly effectively, it does not appear that the liver particles can be dispersed finely enough to result in complete solution of the oil when this solvent is used. Hence, pure petroleum ether does not appear to be a satisfactory solvent. Ethyl ether is better than petroleum ether, but improvements seem possible, either by the use of a more efficient drying agent or by the admixture of another solvent. Pure acetone, under the conditions of the experiments, give poor results. However, a smaller-sized sample or the use of a drying agent more effective than anhydrous sodium sulfate may improve extraction. Apparently what is needed is for the solvent to have sufficient affinity for moisture to allow penetration, but for it not to dissolve so much moisture as to reduce oil solubility.



PACKAGING FROZEN FISHERY PRODUCTS

During storage, fishery products undergo changes of two general types, namely, chemical and physical. Chemical changes include those brought about by the action of bacteria, those due to the action of naturally occurring enzymes, denaturation of the protein and oxidative changes in the fat or oil. The principal physical changes are desiccation or drying out of the flesh and ice-crystal formation.

--Fishery Leaflet 324