STUDIES ON DETERIORATION OF VITAMIN A IN FISH LIVERS AND LIVER OILS 1/

PART I - LOSS OF VITAMIN A AND STABILITY IN GRAYFISH LIVERS IN STORAGE

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ABSTRACT

Ground grayfish (Squalus suckleyi) liver samples stored from 4 to 7 weeks under three different conditions: room temperature (23°C.), in ice (0°C.), and in frozen storage (-22.5°C.), were tested at varying intervals. Results of these tests showed no significant change in the vitamin A potency. However, with increase in temperature of storage, the free fatty acid content tends to rise, and the vitamin A stability rapidly decreases.

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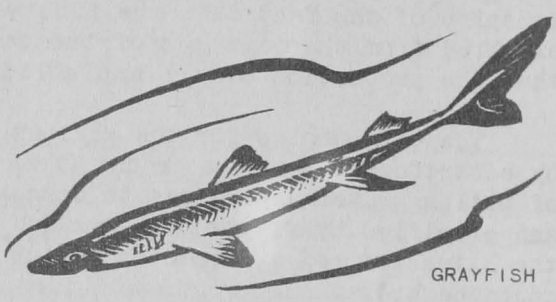
The fish livers from which vitamin A oils are commercially derived are often held for a considerable period of time before being processed. The fishing vessels, in their voyaging for sharks and other fishes rich in vitamin A, range over great distances, and trips of 2, 3, or even 4 weeks' duration are not uncommon. After the livers are landed, a further delay may be encountered before they are processed. Plant facilities are not always adequate to handle the volume of livers brought in at the peak of the season. Economic considerations may also delay the processing of the livers, as, for instance, when they are held for the development of a more favorable market or are shipped to a distant processing plant.



GRAYFISH

Unfortunately, vitamin A is chemically unstable under certain conditions. Knowledge of the effect of storage on the vitamin A content of fish livers is essential, therefore, to the most efficient utilization of this raw material.

In the Pacific Northwest, the grayfish (Squalus suckleyi) is one of the principal sources of vitamin A. The livers from this species are usually stored in 5-gallon cans or 55-gallon drums. The temperature of storage varies widely. Aboard ship, the livers are usually kept iced; on shore, they are ordinarily kept under refrigeration at temperatures from 0° to 20° F. Occasionally, when a boat runs out of ice or refrigeration facilities are crowded, the livers may stand for some time at temperatures considerably above freezing.

An experiment to determine the effect of storage on the vitamin A content of grayfish livers involves a number of practical difficulties. The livers are expensive; a 5-gallon can of them may be worth $16.00 or more. It is difficult to get a representative sample for analysis. There is wide variation in vitamin A potency from one liver to another; and, unless the sampling is carefully controlled, errors in the estimation of the vitamin A content may be large.

To lessen the cost and make uniform sampling easier, the livers can be ground, stirred until homogeneous, and then stored in small containers. It should be

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1/ This is the first of a series of articles on this subject. Others will appear in later issues.

understood that this procedure would not be identical with general commercial practice, as livers are not ordinarily ground prior to storage. However, since the grinding exposes a large surface to the air and thus increases the possibility of oxygen absorption, the conditions of such an experiment are, in all probability, more severe than those encountered commercially. Consequently, no greater vitamin A losses would be anticipated in ordinary practice than would be observed in a test of this kind.

In the present study, approximately 30 pounds of grayfish livers were used. The fish had been caught off Cape Beal[2] an estimated 3 days earlier, and the livers were iced during transit to the laboratory. The livers were then passed through a meat grinder, stirred until homogeneous, and filled into pint Mason jars to within ½ to 1 inch of the top. Rubber rings were placed on the jars, and the lids screwed down tightly. The jars were then divided into three groups, one for storage at room temperature (23° C.), a second for storage in ice (0° C.), and a third for frozen storage (-22.5° C.).

Periodically, three jars from each of the three groups were opened for examination. As could be anticipated, the livers stored at room temperature spoiled rapidly and were definitely putrid by the end of the first week. So much gas was generated that a small amount of liver material was forced out of the jars in spite of the fact that the lids were tightly secured. However, the material expelled from the jars was of the same consistency as that inside the jars, so that the proportion of oil and solids inside the jars remained about the same.

The oil samples for the various organoleptic and other tests were separated by centrifugation of the ground liver after it had been mixed with an equal weight of boiling water and heated to coagulate the protein. They were then dehydrated and clarified by filtration through cotton and anhydrous sodium sulfate. With the livers stored at room temperature, a stubborn emulsion was encountered which could be broken only by the addition of salts such as ammonium sulfate to the mixture of water and liver material.

Oil prepared from the liver material that had been stored 4 weeks at room temperature had a slightly putrid odor and taste, but was not necessarily nauseating or repulsive, although its after-taste persisted for about 30 minutes. Visually, the color of this oil was not significantly different from the color of the oil rendered at the start of the test.

In contrast to the liver material held at room temperature, the materials stored in ice at 0° C. and in the refrigerator at -22.5° C. remained in good condition throughout the entire period of the test. When the jar lids were removed, there was only a slight ammoniacal odor.

In addition to being tested organoleptically, the liver material was analyzed for its oil and vitamin A content. The method used was a slight modification of that recommended by the Joint Government and Industry Committee on Vitamin A that met at San Francisco in June 1944. Optical density readings were made on a Beckman quartz spectrophotometer at a wave length of 328 mmu., and the values of $E\ {}^{1\%}_{1\ cm.}$ (extinction coefficients) were converted into vitamin A units by multiplication by 2000.

In order to determine the changes in the quality of the oil rendered from the stored livers, analyses were made for free fatty acid, and the vitamin A stability

2/ Cape Beal is located on the west coast of Vancouver Island, B. C., Canada.

was estimated. The free fatty acid was determined by the A.O.A.C. VI method,[3] and the vitamin A stability was estimated by the procedure described by Sanford, Harrison, and Stansby.[4]

Results of these experiments appear in Table 1. Since the method of vitamin A analysis is uncertain to the extent of about 2 percent, the fluctuations appearing in the column indicating vitamin A potency are too small to be significant.

Table 1 - Effect of Storage on Ground Grayfish Livers

Item	Storage Period	Amt. of Oil in Livers	Vitamin A Potency of Liver Oil	Free Fatty Acid in Liver Oil	Vitamin A Stability of Liver Oil
	Days	Percent by Weight	U.S.P. Units per Gram	Percent as Oleic Acid	Hrs. to Affect 50% Decomposition by Aeration at 100° C.
Livers stored at room temperature. (18°C. to 27°C., Average = 23°C.)	0	73.52	14,215	0.15	17.55
	7	72.38	14,380	0.48	14.13
	14	72.35	14,288	0.63	7.50
	21	71.53	14,316	0.50	8.72
	28	71.74	14,504	0.68	3.61
Livers stored in ice. (0°C.)	0	73.52	14,215	0.15	17.55
	15	-	-	0.24	12.40
	22	71.22	14,309	0.24	10.83
	29	71.94	14,437	0.23	9.65
	43	72.20	14,350	0.21	13.36
Livers stored in refrigerator. (-28°C. to -20°C., Average = -22.5°C.)	0	73.52	14,215	0.15	17.55
	23	72.41	14,356	0.17	15.76
	49	72.59	14,400	0.14	16.65

Regardless of the type of storage, the amount of vitamin A decomposed was too small to be determined by the analytical methods employed. The possibility existed that a drop in vitamin A potency may have been masked by an increase in extraneous absorption. To check this, the E values for various wave lengths between 300 and 370 mmu. were determined. Ratios of the E values at these various wave lengths to the E value at 328 mmu. (Table 2) indicate that, during the test period, no significant amount of any substance was formed that absorbed light in the region

Table 2 - Optical Density Ratio for Oils Rendered from Ground Grayfish Livers

Item	Storage Period	Optical Density Ratio D_λ/D_{328} for Wave Lengths in mmu. (λ)									
		300	310	320	325	328	330	340	350	360	370
	Days										
Oils rendered from livers stored at room temperature. (18°C. to 27°C., Average = 23°C.)	0	.667	.842	.972	.991	1.000	.980	.806	.570	.352	.180
	7	.622	.872	.959	.997	1.000	.996	.820	.576	.345	.183
	14	.651	.835	.957	1.000	1.000	.989	.815	.572	.355	.181
	21	.649	.849	.958	.998	1.000	.977	.810	.569	.348	.181
	28	.654	.842	.959	1.003	1.000	.984	.812	.569	.354	.181
Oils rendered from livers stored in ice. (0°C.)	0	.667	.842	.972	.991	1.000	.980	.806	.570	.352	.180
	22	.643	.841	.961	.998	1.000	.988	.818	.572	.357	.182
	29	.650	.850	.958	.998	1.000	.976	.816	.571	.355	.181
	42	.652	.839	.956	1.003	1.000	.981	.814	.560	.356	.178
Oils rendered from livers stored in refrigerator. (-28°C. to -20°C., Average = -22.5°C.)	0	.667	.842	.972	.991	1.000	.980	.806	.570	.352	.180
	23	.653	.847	.955	.998	1.000	.978	.813	.560	.346	.182

3/ Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists, Sixth Edition, 501 (1945).

4/ F. B. Sanford, R. W. Harrison, and M. E. Stansby. "A Rapid Test for Vitamin A Stability." Commercial Fisheries Review, March 1946, pp. 16-18. Also F.L. 212.

of the spectrum that was used for estimating vitamin A. Thus, there can be little doubt that the vitamin A analyses reported in Table 1 represent the actual vitamin A values.

The percent of oil recovered from the fresh livers appeared to be somewhat higher than that obtained after the livers had been stored. This is probably an artifact, since the oil assays of the livers stored at room temperature showed no greater changes than did the livers stored on ice. This supports the conclusion arrived at by visual inspection that the ratio of oil to solids remained nearly constant in the jars stored at room temperature despite the previously mentioned loss of putrefying liver material through the seal.

Lowering the temperature of storage appeared to decrease the rate at which free fatty acid[5/] was formed. In no case, however, was the change in free fatty acid large, since even in the oil rendered from the liver material stored at room temperature the increase was only about $\frac{1}{2}$ of 1 percent.

The vitamin A stability[6/] measurements were somewhat erratic. Until further studies have been completed, it would be difficult to determine the exact cause of these variations. Although each stability datum represents the average of duplicate or triplicate measurements, a good proportion of the variation was, no doubt, due to analytical errors, since duplicate measurements deviated one from another by 7.4 percent on the average. However, with the simple method of rendering used, it may well be that the proportions of antioxidants and pro-oxidants extracted differed greatly from one time to another. Variable though the results were, they are sufficiently precise to show clearly that lowering the temperature of storage substantially increased stability. Storage of the livers in ice was a definite improvement over storage at room temperature. The small drop in stability shown during 7 weeks of refrigerated storage was probably within the limits of error of the method of analysis.

In summary, the results of the experiment confirm earlier observations at this laboratory that the vitamin A in grayfish livers is quite stable. The results also indicate that, when these livers are held in well-filled containers with tightly fitting lids, there will be no measurable loss of vitamin A, even if the livers are stored at room temperature for as long as a month. However, other changes taking place in the livers make this temperature of storage definitely unsatisfactory. At room temperature, the livers soon became putrid, and an undesirable odor and taste is imparted to the oil rendered from them. Other deleterious changes also take place. Free fatty acid tends to increase, and vitamin A stability drops rapidly.

5/ The free fatty acid content has long been used as an index of quality, since oils which have been rendered from inferior materials or those which have been carelessly treated usually have a high free fatty acid content. Liver-plant operators have found also that, where the free fatty acids are high, difficulty is encountered in rendering the oil from the livers and that, as a consequence, plant efficiency is lowered.

6/ This stability test is an index of the resistance of the vitamin A contained in an oil toward oxidation. Such information is of importance to the oil buyer as he wants to know not only how much vitamin A he is buying but how well the vitamin will hold up during storage. For example, when a vitamin A oil is mixed with a meal for animal feeding, the oil is spread over an enormous surface under conditions which favor rapid oxidation. Studies already reported in the literature indicate that, under these circumstances, the losses of vitamin A may be both rapid and large. A second aspect of the problem concerns the fate of vitamin A after it has been ingested by the animal. If the vitamin is oxidized before it has been assimilated, the vitamin can then serve no useful purpose.

The more serious of these difficulties can be minimized if the livers are properly iced. Not only is there less formation of free fatty acid and less drop in vitamin A stability; but, over a storage period as long as 6 weeks, the livers are not so apt to become putrid. Where practical, refrigerating the livers at subfreezing temperature is a definite advantage, even if the method cannot be utilized during the entire storage period, since the rate of formation of free fatty acid and the drop in vitamin A stability are greatly decreased as the temperature is lowered.

FATS AND OILS

In the first 6 or 8 months of 1947, particularly in the late spring and summer, supplies of food fats and oils, other than butter, probably will be even smaller than in the corresponding period of 1946. The anticipated output of edible vegetable oils from 1946 crops is about the same as that from crops harvested in 1945. With controls on use of oils and fats in edible products removed in late October 1946, vegetable oils moved into consumption at a substantially faster rate in the last 3 months of 1946 than in the previous year. Factory and warehouse stocks of corn, cottonseed, soybean, and peanut oils on December 31, 1946, totaled only 490 million pounds, 282 million pounds less than a year earlier. There may be some increase in the use of imported oils in food products in 1947. Demand for food fats will remain in excess of supply.

Fish oils will be in very short supply for most of 1947. The California pilchard fishery, which normally contributes approximately one-half of the total fish oil produced in the United States, has suffered the most severe slump in its history.

Production of fish oils during 1946 totaled about 20 million gallons, 4½ million gallons less than the 1945 production and 15.7 million gallons below the 1935-39 average.

--Fishery Leaflet No. 215