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

PART III-LOSS AT 37° C. OF VITAMIN A FROM LIVER OIL IN PRESENCE AND ABSENCE OF GRAYFISH LIVER TISSUE

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ABSTRACT

Under similar storage conditions, the loss of vitamin A from the livers of the grayfish (Squalus suckleyi) was essentially the same as from the extracted oil. The vitamin A was found to be quite stable whether in the liver or in the oil.

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This laboratory was called on to determine whether, under similar storage conditions, the loss of vitamin A would be more rapid from grayfish (Squalus suckleyi) livers than from the extracted oil. The results of the following experiments indicate that the losses in each case are small and not significantly different.

Two types of grayfish liver oils were investigated. "Water-separated oil" was prepared by the "steaming method;" that is, the liver material was cooked briefly in boiling water and then centrifuged. "Solvent-extracted oil" was prepared by washing the ground liver with petroleum ether. These two oils were tested because it was considered possible that the amount of natural antioxidants and associated substances present might be different and thereby lead to contrasting data.

To prepare the liver and oil for the first test, 5 gallons of recently landed, iced, grayfish livers were passed through a meat grinder and then stirred vigorously to obtain a homogeneous material. Twenty-four wide-mouthed, 4-ounce, glass jars were filled with this material to a depth of $\frac{3}{4}$ inch. This exposed to the air a surface area which was large in proportion to the size of the sample. A second series of 24 such jars was filled in a similar manner with the oil extracted by the "steaming method" from a portion of the homogenized livers.

The two lots of samples were then placed in an air oven adjusted to a temperature of 37° C. This temperature was chosen because earlier studies had shown that at temperatures much lower than this the loss of vitamin A was so slow that an inconveniently long storage period would be needed before significant changes could be observed. The liver and oil samples were placed alternately adjacent to one another on the oven shelves so that any temperature gradient in the oven would affect the liver and oil samples equally. Before the start of the test, the liver material and the prepared oil were assayed for their vitamin A content.

1/This is one part of a series of articles on this subject. Part II will appear in a later issue. Part I of this series appeared in the February 1947 issue of Commercial Fisheries Review.

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For the second test, another 5 gallons of recently landed, iced, grayfish livers from a different shipment was ground as before; and portions of the fluid material were placed in similar jars in the same manner. From a second portion of the fluid material, oil was prepared by extraction with petroleum ether by the following method: To each of four pint jars half full of ground liver were added a liberal amount of anhydrous sodium sulfate and about one-third of a pint of petroleum ether. The jars were tightly covered and agitated vigorously on a shaking machine for one-half hour. The contents were allowed to settle, the solvent-oil layer was decanted, and the liver residue was re-extracted with a second portion of fresh solvent. The solvent-oil extracts were then combined, filtered, and placed on a steam bath to remove the solvent. The last trace of petroleum ether was removed as completely as possible by heating and stirring the oil on a steam bath for a short period. This oil was cooled and placed in wide-mouthed glass jars in the same way as before. The jars of liver and oil were then placed in the 37° C. oven for storage. As before, the liver and oil samples were assayed for vitamin A at the start of the storage period.

Shortly after the beginning of the storage test, the liver samples developed a foul, putrid odor and showed evolution of gas. The oil samples, however, showed no visible change during the storage period. One of the oil samples was used as a "pilot" to indicate when a measurable loss of vitamin A had occurred, and aliquots from this jar were assayed at weekly intervals. It was not until three weeks had passed that a definite loss in vitamin A was observed, and then the decrease was only 2.5 percent.

After 30 days of storage, the 24 jars of liver material and 24 jars of oil constituting the first test were removed from the oven. The jars of liver were divided at random into four groups of six jars each. The jars of liver oil were grouped in a similar manner. The samples of the second test involving the solvent-extracted oil were handled similarly.

In all the various assays of the liver material, the oils used for the assays were extracted from the material as follows: The sample was mixed thoroughly and a 3- to 5-gram aliquot was transferred by means of a large-bore pipet to a 2-ounce, narrow-mouthed, tared centrifuge bottle provided with a tight-fitting, extracted cork, and the sample weight determined to within 0.001 gram. Approximately 5 grams of pulverized, neutral, anhydrous sodium sulfate and exactly 25 ml. of petroleum ether (Skellysolve F, or equivalent grade) was added to the sample, which was immediately stoppered tightly and shaken until the vitamin A and oil were in complete equilibrium with the solvent phase.

When equilibrium was obtained, the solvent layer was inspected; and, if found to be free from all turbidity, aliquots for vitamin A and fat were drawn. If turbidity persisted in the solvent phase, the entire mass was centrifuged until a clear solvent layer was obtained.

A 1-ml. aliquot was transferred to a 100-ml. volumetric flask and diluted with "anhydrous" isopropanol for spectrophotometric examination.

A 5-ml. aliquot was pipetted into a tared flask and rendered free of solvent by heating on a water bath.

The percentage of oil in the sample was calculated by the following formula in which S = sample weight and W = weight of oil in aliquot:

$$\% \text{ oil} = \frac{W(25 \times 100)}{S(5 - \frac{W}{0.92})}$$

The sample weight used in calculating E (1%, 1 cm.) was one-fifth of the weight of oil derived from the 5-ml. aliquot.

The optical densities of the various oils were measured with a Beckman quartz spectrophotometer over a series of wave lengths ranging from 300 to 370 mmu. to determine the content of vitamin A and to what extent non-specific, light-absorbing substances might have developed during storage. Only the two ratios, E at 300 mmu. to E at 328 mmu. and E at 350 mmu. to E at 328 mmu., are given in Table 1, since they illustrate sufficiently the changes observed.

Table 1 - Comparison of E Value (Extinction Coefficient) Ratios of Grayfish Liver Oils: For Fresh and Stored Oils and for Oils Extracted from Fresh and Stored Livers

Material	Storage Period Days	E at 300 mmu. / E at 328 mmu.	E at 350 mmu. / E at 328 mmu.
Ground liver No. 1	0	.704	.563
	31	.744	.571
Water-separated oil	0	.704	.563
	31	.739	.557
Ground liver No. 2	0	.703	.558
	30	.724	.562
Solvent-extracted oil	0	.731	.554
	30	.785	.547

Note: Samples were exposed to the air and held at 37°C. during the storage period.

Since only a slight increase occurred on storage in the ratio of the E value at 300 mmu. to the E value at 328 mmu., it is believed that the increase in spurious absorption at 328 mmu. during the period of the test was small. Therefore, the changes shown by the data are believed to represent the true loss of vitamin A. The difference in vitamin A loss during storage between the "solvent-extracted oil" and the liver material from which it was prepared amounts to only 0.78 percent and is not statistically significant.

In Table 2 is presented a comparison of the vitamin A content of the ground liver and the liver oils at the inception and termination of the storage period.

Table 2 - Change in Vitamin A Content of Grayfish Livers and Liver Oils During Storage

Material	Average Vitamin A Content of Oil		Loss of Vitamin A during storage
	Start of test	End of test	
	U.S.P. units per gram	U.S.P. units per gram	Percent
Ground liver No. 1	14,532	13,366	8.0
Water-separated oil	9,574	8,798	8.1
Ground liver No. 2	8,439	7,596	10.0
Solvent-extracted oil	7,979	7,120	10.7

Note: Samples were exposed to the air and held for 31 days at 37°C.

It can be noted from the table that the vitamin A potency of the initial "water-separated oil" is considerably below that of the corresponding "analytically extracted" sample. This is probably due to incomplete rendering of all the oil in the liver tissues. However, the percentage loss of vitamin A during storage was about the same for both oil and liver. Therefore, it was concluded that the extractions of antioxidants and associated substances by the "steaming method"

and by the analytical method had been closely similar even though the vitamin A values were different.

Since the loss of vitamin A during storage at 37° C. was essentially the same in the ground liver tissue as in the oil, it can be concluded that any substances which might have accelerated or inhibited the oxidation of the vitamin A in the grayfish livers were about equally active in the extracted oil.

Another conclusion to be drawn from the data is that the vitamin A of grayfish livers is quite stable whether in the ground liver or in the extracted oil. Even at the elevated temperature of 37° C., with the ground liver and the separated oil exposed freely to the air, the loss of vitamin A in 30 days of storage was only 8 to 10 percent.

In earlier experiments[1/] at this laboratory, it was observed that the rate of oxidative destruction of vitamin A in grayfish liver oil follows the well-known rule of approximately doubling with each 10-degree rise in centigrade temperature. Assuming that the same rule holds under the conditions of the experiment reported in this paper, we can estimate that at 0° C. (the temperature of melting ice) the loss of vitamin A in grayfish liver oil would be less than one percent per month.

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

METHODS OF NET MENDING--NEW ENGLAND

Through an oversight, the March 1947 issue of Commercial Fisheries Review did not credit Boris Knake, author of the feature article, "Methods of Net Mending--New England," also as illustrator. The numerous illustrations accompanying the article were his work.