United States Department of the Interior, J. A. Krug, Secretary
Fish and Wildlife Service, Albert M. Day, Director

Fishery Leaflet 212

Chicago 54, Ill. December 1946

A RAPID TEST FOR VITAMIN A STABILITY

By F. B. Sanford,* R. W. Harrison,** and M. E. Stansby*

Vitamin A is a relatively unstable substance. It is especially susceptible to destruction by oxidation. In fish liver oils, the destruction does not take place gradually. For a certain initial time (known as the induction period) unfavorable storage conditions produce little or no decomposition, but then a sudden and rapid diminishment in potency occurs. The relative stability of the vitamin A in various fish liver oils depends upon a number of factors, such as the presence or absence of natural antioxidants (substances which when present with vitamin A diminish its tendency to oxidize), the care with which the fish livers were handled prior to processing, and the particular rendering methods by which the oil was extracted from the livers.

If the seller knew in advance that a certain oil had low vitamin A stability, he could take steps to improve the stability so that the vitamin would not be easily lost after the oil reached the ultimate consumer. To prevent such losses, the dealers have needed a rapid laboratory method for predicting the stability of vitamin A oils. This need is increasing, because large quantities of fish livers are being imported from tropical regions where high storage temperatures may render the oils especially poor in vitamin stability.

Certain laboratory procedures have been used by fish liver oil producers and others to predict stability, but very little on this subject is to be found in the literature. However, many procedures are available for rapidly determining the stability of oil with respect to rancidity, and this report describes an adaptation of one of these to the determination of the stability of vitamin A oils. This test consists essentially of subjecting a small portion of the vitamin-bearing oil to rapid oxidation by bubbling air through the oil at an elevated temperature. The time required for the destruction, under controlled conditions, of a certain percentage of the initial vitamin A content serves as an index of the relative stability to be expected.

The oxidation is carried out in the bubbling tube shown in Figure 1 on p. 17. This apparatus consists of a test tube 22 millimeters in diameter by 230 millimeters long, through the side of which is sealed a delivery tube, 7 millimeters in diameter, leading to within 5 millimeters of the bottom of the test tube. The assembly is made entirely of non-actinic glass.

The bubbling tube is placed in a constant temperature bath, usually a steam bath at 100° C., and air is pumped through the delivery tube at the rate of about 350 milliliters per minute. A 10 milliliter portion of the oil to be tested is added to the tube and the time noted. Small samples are then withdrawn periodically by means of a clean thief made of glass tubing 3 millimeters in diameter by about 260 millimeters long.

The vitamin A concentration of the original oil and each subsequent sample is determined and plotted against time. The length of time required for decomposition of 50 percent of the initial vitamin A is estimated from the graph.

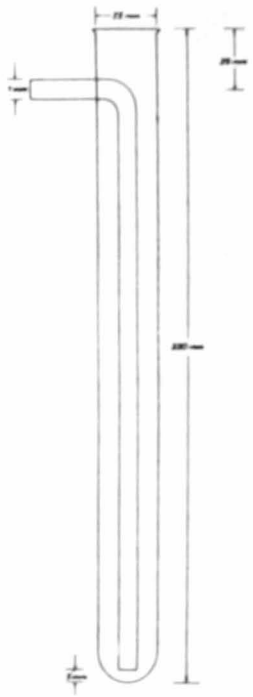
*Chemists, Seattle Fishery Technological Laboratory.
**Formerly a chemist of the U. S. Fish and Wildlife Service.

Note: This leaflet supersedes Sep. 129, a reprint from Commercial Fisheries Review, March 1946, pages 1-3.

In this work, the air for the bubbling tubes has been furnished by an air compressor and passed through a reducing valve to a manifold, 1 inch in diameter, fitted with 4 petcocks, by means of which the proper amount of air has been fed through rubber tubing leading to 4 individual bubbling tubes. In controlling the volume of air used, it has been found convenient to connect a U-tube flowmeter,[1] containing SAE 30 motor oil, to each individual feed line. The flowmeters are calibrated so that the differential in the heights of the oil columns will indicate the volume of air movement.

FIGURE 1

BUBBLING TUBE



DISCUSSION: The vitamin A content of the oil decreases slowly during the induction period, the end of which is signalized by rapid increase in the rate of decomposition. In most oils, the induction period has ended by the time that 50 percent of the initial vitamin A has been decomposed, so that at this point, oxidative destruction is proceeding rapidly, and large changes in the amount of vitamin A in the oil take place quickly. If a graph is constructed in which the percentage of vitamin A remaining is plotted against time, the slope of the curve at the 50 percent point will be very steep, and even though the absolute measurements of vitamin A content are subject to some error, the point at which 50 percent of the vitamin A has been destroyed can be located quite accurately. As a consequence of these considerations, the time required for destruction of 50 percent of the vitamin A content has been chosen by this laboratory as an index of relative stability.

Obviously, the choice of this criterion is somewhat arbitrary, but it has the advantage of giving results that are closely reproducible. For example, in one instance, the stability of an oil at 100° C. was determined on four successive days with the following results:

Day of Examination	Number of Tests Run	Stability: Minutes required for 50 percent loss of vitamin A content
1	1	98
2	4	90; 90; 90; 90
3	2	90; 90
4	1	89

Before the adoption of the procedure given above, preliminary experiments were run to determine the effects of various factors on the destruction rate. These experiments showed the following:

It is of great importance that the oil temperature be constant during the aeration, as each decrease of 10 degrees centigrade in the temperature increases the time required for 50 percent destruction by a factor of

1/ Scientific Supplies Company, Seattle, Washington, Catalog No. 55775.

approximately 2.1. A water or steam bath is convenient for controlling the temperature.

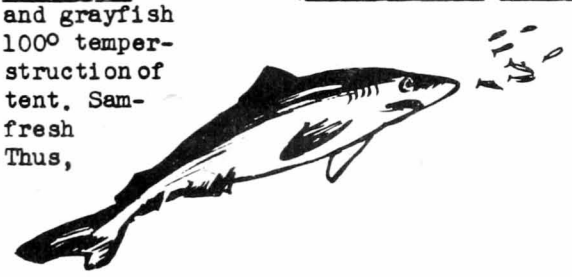
The rate at which the air is bubbled through the oil is less critical. In one instance, when the rate was between one and two bubbles per second, 48 hours were required for 50 percent decomposition, as compared with 35 hours when the bubbling rate was as high as could be used without blowing the oil from the tube.

The relative humidity of the air stream is unimportant. Thus in one test, when the air was dried by passing it over calcium chloride, the 50 percent decomposition time was 88 minutes, as compared with 90 minutes with ordinary moist air taken directly from the compressor.

Variation of the volume of oil in the bubbling tube had little effect. In one case, when an initial volume of 3 ml. of oil was used, the 50 percent decomposition time was 83 minutes, as compared with 90 minutes when 12 ml. of oil was used initially. It would thus appear that inaccuracies in pipetting or volume changes due to the withdrawal of samples are not serious, especially if the initial volume of oil is adequate; e.g., at least 10 ml.

The material used for the bubbling tubes is very important. Preliminary work was done by using one-inch by eight-inch test tubes painted black on the outside and containing a cork stopper through which the glass tube carrying the air extended to the bottom of the test tube. Somewhat erratic results were obtained by using this apparatus. Much more reproducible results were obtained with test tubes constructed of non-actinic glass and containing an air bubbling tube permanently sealed through the side, as shown in Figure 1 on p. 17.

Most commercial samples of liver oil from the soupfin shark (Galeorhinus zyopterus), halibut (Hippoglossus hippoglossus), lingcod (Ophiodon elongatus), and grayfish (Squalus suckleyi) have required, at the 100° temperature, from 50 to 150 minutes for destruction of 50 percent of the initial vitamin A content. Samples prepared in the laboratory from fresh livers were considerably more stable. Thus, one sample of grayfish liver oil so prepared required 744 minutes before 50 percent of its vitamin A was decomposed.



In working with the more stable oils, it is desirable to perform the aeration at a temperature of 100° C. in order to complete the test in one day. However, with oils of low stability, the destruction is so rapid that is is often more convenient to work at a lower temperature such as 55°. In the case of grayfish liver oil, the point of 50 percent vitamin A decomposition is reached at 100° about 28 times as fast as at 55°.

It has not been definitely established that the results obtained in this accelerated oxidation test consistently correlate with the stability of the oil under the storage conditions normally prevailing. Tests to determine the extent of such correlation are planned, but since they involve long storage periods covering many months, the results are not available for presentation at this time.