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A RAPID METHOD FOR DETERMINING THE VITAMIN A POTENCY OF FISH LIVERS 1/

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A rapid method of estimating vitamin A potency is a primary requirement for buying fish livers on a potency basis, because any delay in pricing the livers tends to work a hardship on the fishermen.

The proposed method directly determines the vitamin A content of the livers and differs from the usual methods in that neither the oil content nor the oil potency are determined. Specifically, a weighed sample of the liver is shaken with a measured quantity of petroleum ether; an aliquot of the petroleum ether solution is diluted with isopropanol, and the optical density of the resulting solution determined with a spectrophotometer. Liver potency is then calculated directly by means of the following formula:

where

- P = liver potency in millions of U.S.P. units per pound.
- d = optical density of vitamin A solution (wave length = 328 m. mu.)
- e = ml. of petroleum ether used to dissolve oil and vitamin A from the liver sample.
- s . weight of liver sample.
- f = average percentage of oil in livers of the species under examination.
- 1 = length of absorption cell.
- v = dilution of petroleum ether aliquot (i.e., volume of the isopropanol-petroleum ether solution).

As an example of the use of the formula, suppose that 3.00 g. of grayfish liver is shaken with 100 ml. of petroleum ether until equilibrium is established and that a 5 ml. aliquot of this is diluted with isopropanol to 100 ml. in a volumetric flask. If the optical density of this solution is 0.600 when measured in an absorption cell 1.005 cm. long, then the potency of the liver can be calculated thus (assuming that grayfish livers average 70 percent oil):

1/ This leaflet supersedes Sep. 100, a reprint from <u>Fishery Market News</u>, April 1945, pages 7-8.

$$P = \frac{(2000)(0.600)}{(100)(1.005)(3.00)(5/100)(10^6)} = 3.69 \text{ million units per pound}$$

The equation is somewhat cumbersome in its present form, but simplification can be effected by combining the constant terms. For example, where a single species is being analyzed and the same dilution is used for each analysis, the formula can be reduced to

$$\frac{P = kd (e + 0.01 sf)}{s}$$

where k is a constant,

(2000)(454)(100) 1v 10⁶

The volume of the petroleum ether-oil solution (e + 0.01 sf) can be combined with the constant, if a calibrated thief $\underline{1}$ / or a similar device is employed to extract approximately uniform samples for each analysis, thus permitting the simpler form

 $P = \frac{K^{\perp}}{K} d$

Obviously, potencies thus determined are subject to slight inaccuracies due to the uncertainty as to the oil content of the livers, but the use of a large volume of petroleum ether and a small sample renders the error negligible. For example, the oil content of grayfish livers will seldom, if ever, fall below 50 percent. In the illustration just given, if the true liver oil content had been 50 percent, the assumption of 70 percent oil would result in an error of less than 0.6 percent.

When the liver potency has been determined, there is no great advantage in knowing the exact potency of the oil if it is under 40,000 units of vitamin A per gran. However, if reasonable estimates are to be made of the monetary value of livers yielding oils of higher vitamin A potencies, the latter must be approximated quite accurately. To accomplish this, a graph of "oil potency vs. liver potency" based on former records could be used by the analyst to estimate oil potency sufficiently accurately for most practical purposes.

1/ F. B. Sanford, G. C. Bucher, and W. Clegg, "Some Time and Labor Saving Techniques in Vitamin A and Oil Analyses," <u>Fishery Market News</u>, August 1944, pp. 6-8.