VOL. 9 JANUARY-DECEMBER 1971 NOS. 1 & 2

THE PHILIPPINE JOURNAL of FISHERIES

Published semi-annually by the
BUREAU OF FISHERIES AND AQUATIC RESOURCES
Intramuros, Manila
1975

The PHILIPPINE JOURNAL OF FISHERIES

Official Publication of the Bureau of Fisheries and Aquatic Resources
Intramuros, Manila 2801, Philippines

Vol. 9 January-December 1971 Numbers 1 & 2

PRELIMINARY STUDIES ON THE METHODS OF EXTRACTION AND DETERMINATION OF VITAMIN A CONTENT OF LIVER OILS OF COMMON PHILIPPINE MARKET FISHES*

By

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ABSTRACT

Comparative studies on the percentage of oil and Vitamin A content of livers of each of eleven Philippine market fishes using direct, as well as indirect (standard AOVC method) extraction methods, show that the direct method presents more advantages than the indirect method because the former eliminates the tedious step of solvent extraction. Percentage recovery of oil and Vitamin A content is relatively higher in the direct method.

INTRODUCTION

Protein deficiency still exists as a serious food problem in the Philippines. Likewise Vitamin A deficiency is also regarded as an important problem in most parts of the world. According to family dietary surveys, sources of Vitamin A is relatively inadequate.

In the Philippines, utilization of fish liver oils is not being exploited in spite of its potential nutritive, medicinal and pharmaceutical values. Fish livers, included in the entrails of fishes which are regarded as waste by housewives, could be utilized in dietary preparations. Fishermen could increase their income by selling fish livers to processors for the production of various pharmaceutical preparations.

Previous works as mentioned by Birosel (1932) on the Vitamin A content have been done colorimetrically by Carr and Price—based on the measurement of the unstable blue color formed by the interaction of Vitamin A and antimony trichloride ($SbCl_3$) and the results checked with the biological assay of Vitamin A by Underhill. Similar studies on color reactions of fish body and liver oils were done by Norris and Danielson, with Norris and Church further carrying out their studies with the non-saponifiable substances of cod-liver oil. The results of their colorimetric assay compared within the limits of the biological test. Moore and Wokes confirmed the colorimetric method prefering $SbCl_3$ since the required color persists longer than the other reagents tested. Alcohol studies on liver oils by Birosel and Sison (1932) confirmed the colorimetric assay as less expensive and faster. Likewise, Isabelo Concepcion (1947) in his work on Vitamins commented that biological assay using animals is time consuming, hence the colorimetric method, is preferable. However, this method requires speed in its determination due to the rapid fading of the required blue color and the corrosive nature of reagent $SbCl_3$ demands careful use and the methods' extreme sensitivity to moisture and interfering substances requires much care in its operation. Since spectrophotometric determination shows better result than the colorimetric method, the former was used in the determination of Vitamin A in this study.

This preliminary work is confined to the comparative study of the percentage oil and Vitamin A content of liver by two extraction methods: indirect method—extraction of unsaponifiable portion from fresh oil, the latter obtained by solvent extraction; and the direct method—extraction of unsaponifiable portion from the fish liver itself.

MATERIALS AND METHODS

Fish liver oils have been extracted from livers of eleven species of Philippine market fishes and analyzed for percentage of oil and Vitamin A contents. The livers were purchased from the local markets; others have been requested from fish dealers and housewives who would have thrown them out as waste products.

I. Indirect Method:

Standard methods of Vitamin assay of the Association of the Vitamin Chemists (AOVC) were used in the extraction and determination of Vitamin A of fish liver oils. Part A of the method involves three processess, namely; the preparation of the oil by sol-

* Technical paper read at the IPFC 10th Session, Seoul, Republic of Korea, 10-25 October, 1962.

vent extraction; saponification and extraction of the unsaponifiable portion. Part B involves the spectrophotometric determination of the samples obtained from part A.

Part A:

Preparation of the oil by solvent extraction: Fish liver oil was extracted from fish liver by the solvent extraction process. Liver sample was homogenized; anhydrous sodium sulfate (Na_2SO_4) was added to absorb excess water from the sample. With diethylether solution, 250 ml. volume of fresh oil was extracted from the sample.

Dilutions to suitable concentrations for spectrophotometric reading of Vitamin A were made from this solution. The percent oil content was also determined.

Saponification: An aliquot portion of the 250 ml. fresh oil obtained by solvent extraction was treated with ethanol and potassium hydroxide solution. The resulting mixture was evaporated until the ether was removed and then saponified.

Extraction of the unsaponifiable oil portion. After saponification, the resulting mixture was transferred to a separating funnel and extracted with ether five times to extract completely the unsaponifiable matter. The ether extract which is the unsaponifiable portion was washed with distilled water until the washings were free from alkali as determined by testing with phenolphthalein. After the removal of the final water the ether extract containing Vitamin A was filtered through anhydrous Na_2SO_4 into a 250 ml. flask. The water-free filtrate is the pure unsaponified oil.

From the three processes done, two samples were obtained: the fresh oil as a result of solvent extraction, and the unsaponified portion obtained through extraction from saponified fresh oil.

Part B:

The Vitamin A of the fresh oil and the unsaponified oil was determined by direct spectrophotometric analysis.

Spectrophotometric analysis:

The Beckmann spectrophotometer was used in the Vitamin A determination of all samples. The procedure consisted of dissolving the liver oils in a suitable solvent like isopropyl alcohol and the

amount of ultraviolet light absorbed by the sample under test was determined quantitatively. Spectrophotometric readings were made at wave length of 325 mμ where maximum absorption occurs. To correct for extraneous materials which also absorb in this region, readings were also made at 310 mμ and 334 mμ followed by mathematical adjustments.

II. *Direct Method:*

Another portion of the fish liver sample was homogenized in a Waring blendor and the weighed sample was directly saponified, and unsaponified oil extracted, without passing the preliminary solvent extraction process. The procedure followed in this experiment was similar to the indirect method in the sense that the procedure for saponification process and extraction of the unsaponified portion was also followed. The main difference, however, was that the solvent extraction process was omitted.

The percentage of oil and the Vitamin A content of the unsaponified oil sample obtained was also determined, the latter by spectrophotometric analysis.

RESULTS AND DISCUSSION

Table I shows the percent oil extracted and Vitamin A content of fish liver oils under study. Chemical analysis revealed that livers of Philippine market fishes contain appreciable amounts of Vitamin A.

Comparatively, as shown in Fig. 1, the amount of Vitamin A present in each sample under the direct method is correspondingly higher than those under the indirect method. Although the extraction of unsaponifiable portion from fresh oil in the indirect method compares favourably with the direct method, the latter is preferable since direct saponification without passing the fresh oil stage lessens the possibility of the presence of interfering substances. Results show that the amount of Vitamin A is not determined by the percentage of oil.

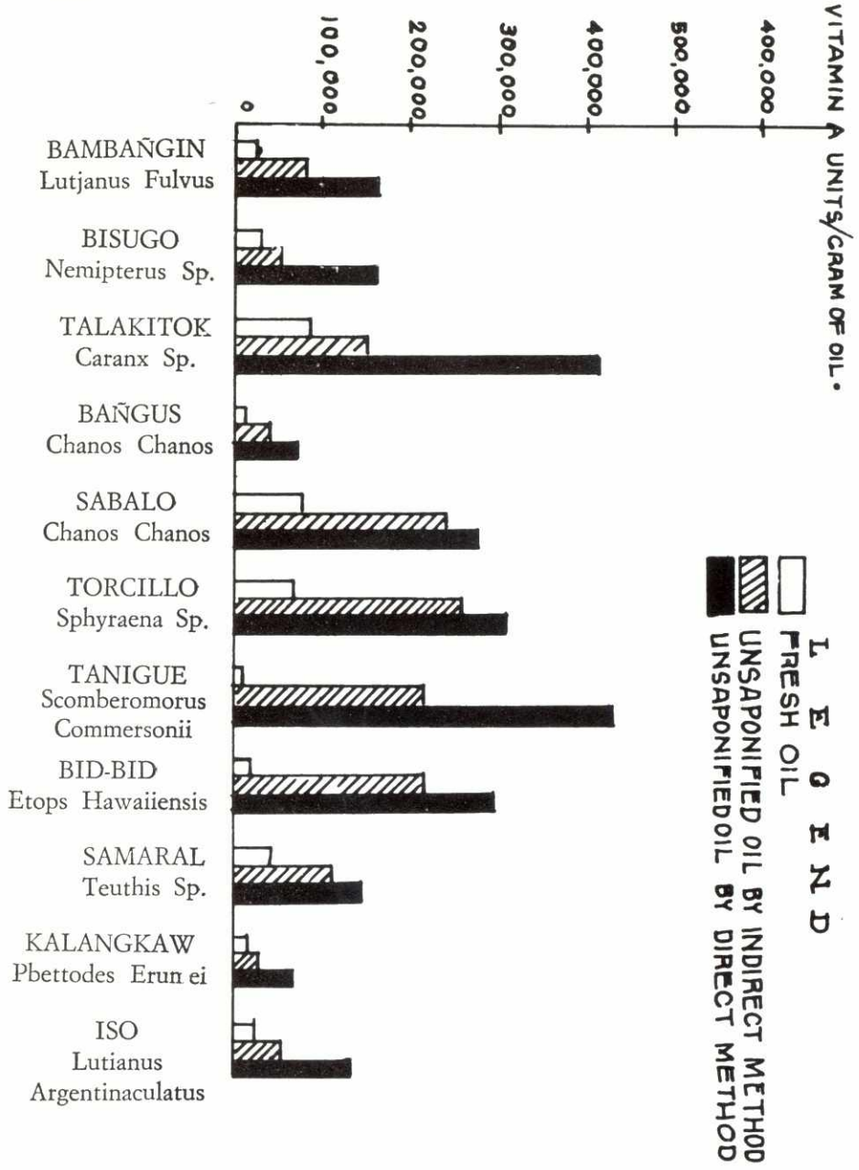
Continuation of the determination of Vitamin A content of livers using the direct method is still in progress. Research is underway to increase the efficiency of this method.

TABLE I. *Vitamin A Potency of Liver Oils of Philippine Market Fishes.*

Local Name of Fish	Method of Extraction	Oil content %	Vitamin A, International units/gram of Oil
1. Bambangin (*Lutjanus fulvus*)	Fresh Oil	13.28	24,870.50
	Unsaponifiable fraction from fresh oil	1.21	82,653.00
	Unsaponifiable fraction (Direct)	2.15	165,817.00
2. Bisugo (*Nemipterus* sp.)	Fresh Oil	4.72	37,290.00
	Unsaponifiable fraction from fresh oil	0.710	57,060.00
	Unsaponifiable fraction (Direct)	1.51	164,090.00
3. Talakitok (*Caranx* sp.)	Fresh Oil	5.88	86,740.00
	Unsaponifiable fraction from fresh oil	0.506	156,585.30
	Unsaponifiable fraction (Direct)	1.29	419,515.00
4. Bangos (*Chanos chanos*) Brackish water	Fresh Oil	7.62	18,085.01
	Unsaponifiable fraction from fresh oil	0.442	55,352.10
	Unsaponifiable fraction (Direct)	1.44	76,091.70
5. Sabalo (*Chanos chanos*) Marine form	Fresh Oil	8.44	83,930.00
	Unsaponifiable fraction from fresh oil	0.775	246,106.00
	Unsaponifiable fraction (Direct)	1.44	280,296.00
6. Torcillo (*Sphyraena* sp.)	Fresh Oil	7.58	78,382.00
	Unsaponifiable fraction from fresh oil	0.535	263,299.00
	Unsaponifiable fraction (Direct)	2.15	312,625.00
7. Iso (*Lutianus argentimaculatus*)	Fresh Oil	6.69	24,997.00
	Unsaponifiable fraction from fresh oil	0.515	54,834.00
	Unsaponifiable fraction (Direct)	1.54	135,912.00

TABLE I. (*Cont'd.*)

Local Name of Fish	Method of Extraction	Oil content %	Vitamin A, International units/gram of Oil
8. Tanigue (*Scomberomorus commersonii*)	Fresh Oil	11.33	6,249.00
	Unsaponifiable fraction from fresh oil	0.906	217,720.00
	Unsaponifiable fraction (Direct)	1.56	434,391.00
9. Bidbid (*Elops hawaiiensis*)	Fresh Oil	30.86	22,968.55
	Unsaponifiable fraction from fresh oil	0.68	218,253.60
	Unsaponifiable fraction (Direct)	2.34	295,186.70
10. Samaral (*Teuthis sp.*)	Fresh Oil	6.62	43,666.00
	Unsaponifiable fraction from fresh oil	0.63	118,690.00
	Unsaponifiable fraction (Direct)	2.165	151,054.00
11. Kalangkao (*Psettodes erumei*)	Fresh Oil	2.34	17,022.15
	Unsaponifiable fraction from fresh oil	0.198	32,477.70
	Unsaponifiable fraction (Direct)	0.860	71,324.00



RELATIONSHIP OF VITAMIN A CONTENT IN FISH LIVER OIL USING DIRECT AND INDIRECT METHODS

CONCLUSION

The direct method of oil extraction presents more advantages than the indirect method because the former eliminates the solvent extraction process. The omission of this process lessens the possibility of losing the Vitamin A present in the sample during the saponification and extraction of the unsaponified oil.

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