



Title	STUDIES ON THE EXTRACTION OF FISH LIVER OIL. : . EXAMINATION ON VITAMIN A CONTAINING FISH LIVERS AND ITS METHOD. With 3 tables
Author(s)	MURATA, Kiichi; SUZUKI, Matsuo
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STUDIES ON THE EXTRACTION OF FISH LIVER OIL.

I. EXAMINATION ON VITAMIN A CONTAINING FISH LIVERS AND ITS METHOD.

By

Kiichi MURATA and Matsuo SUZUKI

(Faculty of Fisheries, Hokkaido University)

With 3 tables

As the result of the simplification in the manner of determining vitamin A and D in liver oil it is possible to produce liver oil from other fishes as well as from cod which had been the only raw material for this purpose until rather recently. The writers attention was directed towards other fishes for liver oil production in which the extraction is easy bringing about remarkable progress in the liver oil industry.

Although there are many methods used in the manufacturing of liver oil, the one used here is the alkaline method the most commonly adopted in Japan. The authors proceeded to ascertain from which fish liver and by what method of extraction they could produce the highest vitamin content, maximum quality liver oil. This paper reports the results of several of these tests which are intended to make clear which fish contains higher or the highest unit vitamin A in its liver oil.

I. Relation between Changing pH with Oil Extraction and Vitamin A Unit.

METHOD:.....Cut fish liver finely with a chopper. In the case of flounder *Reinhardtius matsuurae* Jordan et Snyder, all internal organs are used instead of liver only. Take a part of this finely minced material into a large container, after mixing thoroughly, and then 20 gr. of sample into a 100 c.c. Erlenmyer's flask. Add 20 c.c. of distilled water and mix well. By adding different quantities of 10% NaOH solution to the samples, different water solutions in value of pH are produced. Place the sample on a water bath, under constant shaking heat it to 50° C., which it to be maintained for one hour in the same condition, then raise the temperature to 85° C., and heat it at this temperature for another one hour.

The livers or organs are then completely dissolved. Take it off and cool it. Transfer the content into a separating funnel and refined peroxide-free ether. After thoroughly shaking, separate the upper and the lower layers from each other, the former into a Erlenmyer's flask. Repeat this operation several times and add all the upper layers together. The combined upper layers are then to be dehydrated with anhydrous Na_2SO_4 . In separating, when the separation of ether layer from water layer is not clear, drop in a small quantity of ethyl alcohol. Shake a few minutes, set it aside, the layers will separate from each other completely. Next filler it into a weighed Erlenmyer's flask, evaporate the ether from the filtrate by warming it on a water bath at $30^\circ\text{--}40^\circ\text{C}$., the residual Na_2SO_4 is washed with ether using SbCl_5 , dissolve in CHCl_3 solution other indicator until the washing ether is colorless. The ether used for washing is added to the filtrate and the mixture is heated to drive away ether. Any ether and alcohol that may remain are freed from the filtrate by using vacuum pump.

RESULTS:.....Table I shows the extraction of vitamin A from flounder. When pH value was between 11.3 to 13.0 there was no increasing of vitamin unit with increasing of pH value. On the contrary, oil extraction ratio gradually decreased.

Table 1

Tests No.	Liver quantity gr.	pH value	Oil quantity gr.	Ratio of oil extracted %	Vitamin A unit (U.S.P.U.)	Total vitamin A quantity in extracted oil (G.U.S.P.U.)	Total vitamin A quantity per gr. of internal organs (G.U.S.P.U.)
1	20.0	11.3	2.1	10.5	99100	208110	10405.5
2	20.0	12.0	1.9	9.5	99200	188480	9424.0
3	20.0	13.0	1.8	9.5	99400	178920	8946.0

II. On the Quantity of Liver Oil from Fish Livers and the Vitamin A Units in Those Liver Oils

METHOD:.....(A) Adjust the pH of several different fish livers at values up to pH 11.4, under frequent shaking heat each of them at 50°C ., for one hour and then one hour at 85°C . After complete dissolving, extract the oil with ether. The results are shown in Table II.

METHOD:.....(B) Extraction of oil from livers directly without dissolving it with any alkali. Take 10gm. of finely chopped fish livers into a mortar and add 50 gr. of anhydrous Na_2SO_4 to 5 times its weight. Mix well. Keep in a desiccator for a certain number of hours (about 2 to 3 hours) to dehydrate. Transfer into an Erlenmyer's flask, add ether, shake it, and set it aside. Filter the upper clear portion into a weighed flask. Repeat several times until the filtrate is

colorless when $SbCl_3$ dissolved in $CHCl_3$ solution is added. Put all portions of filtrate together and warm on a water bath at 30° to $40^\circ C.$ to evaporate ether, then under vacuum pump drive away the last traces of any ether. The results are shown in Table III.

RESULTS:.....Results obtained by the above methods are shown in the following tables. Table II shows the results of alkaline method when applied to small tuna, middle tuna *Thynnus thynnus* Linne, swordfish *Xiphias gladius* Linne, flounder and rockfish *Sebastolobus macrochir* (Güntner) together with equal quantities of flounder and rockfish. Table III shows the results from the direct ether extraction method. Raw materials used in this experiment are swordfish, middle tuna, mackerel *Scomber japonicus* Houttuyn and young tuna.

Table II

Species of fish	Liver quantity gr.	Water quantity cc.	5% NaOH cc.	NaOH gr. liver %	pH value	Oil quantity gr.	Ratio of oil extracted %	Vitamin A unit (U.S.P.U.)	Total vitamin A quantity per gr. of livers (G.U.S.P.U.)
Small tuna	10.5	10.0	4.0	1.9	11.4	0.55	5.2	20300	10556.0
Middle tuna	10.0	10.0	4.0	2.0	11.4	0.50	5.0	86000	43000.0
Swordfish	10.0	10.0	4.5	2.2	11.4	1.00	10.0	245400	24540.0
Flounder	10.0	10.0	4.0	2.0	11.4	1.60	16.0	52200	8352.0
Rockfish	10.0	10.0	4.5	2.2	11.4	1.70	17.0	72700	12359.0
Flounder and Rockfish	10.0	10.0	4.2	2.1	11.4	1.70	17.0	67000	11390.0

Table III

Species of fish	Liver quantity gr.	Na_2SO_4 quantity gr.	Oil quantity gr.	Ratio of oil extracted %	Vitamin A unit (U.S.P.U.)	Total vitamin A quantity per gr. of livers (G.U.S.P.U.)	Extinction ratio E 300/328
Swordfish	10.0	50.0	1.50	15.0	33500	5025.0	173.6
Middle tuna	20.0	100.0	1.10	5.5	142200	7821.0	108.8
Mackerel	20.0	100.0	1.05	5.3	111100	5888.3	—
Young tuna	10.0	50.0	0.60	6.0	46800	2808.0	—

As clear from the above tables tuna are classified as large tuna, middle tuna and small tuna, the vitamin A unit of the former is much higher than that of the latter. The vitamin A unit of flounder is relatively low, yet its oil extracting ratio is much great. The vitamin A units of mixture of equal quantities of flounder and rockfish indicate that its vitamin A unit seems to be more abundant in comparison with the results of extracting them separately.

From Table III, the oil extraction ratio of swordfish is seen to be high while the others show no difference. Materials used in Table II and that used in Table III are not the same which makes the comparison of data in these two tables impossible. The different conditions of livers, for instance their freshness and the

method of treatment used, will influence the results greatly even though the livers are of the same kind of fish.

III. Conclusion

(1) When the extraction is applied to livers dissolved with alkalis, i. e., when the solution is adjusted at pH 11.3 to 13.4, the vitamin A unit of the liver oil will increase with the increasing pH value, while contrariwise the oil extracting ratio decreases at the same time.

(2) Livers of tuna, rockfish and swordfish and internal organs of flounder contain high vitamin A unit.

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