A Study of Oil Extraction From Fish Livers

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IF one takes into consideration the fact that fish liver oils, owing to their vitamin A content, command, on the whole, higher prices than other edible oils, one would expect that the technology of fish liver oil extraction would have reached a high level of development in comparison with the extraction of less valuable oils. This however is not exactly the case, and scientific methods in the fish liver oil industry seem to gain ground somewhat slowly. On the one hand there are a number of patented processes in use (1) which may or may not provide good results, and on the other hand many establishments still digest livers with steam or boiling water although the advantages of more up-to-date methods, such as alkali digestion, are steadily gaining recognition. The merits of alkali digestion have been stressed repeatedly (2,3), and yet even some of its adherents seem to be not quite clear with regard to its performance as compared with the steaming method. Lovern, for instance, while stating (3) that alkali digestion gives an almost theoretical oil recovery, maintains at the same time that—

a) "the centrifugal separation of steamed livers also gives nearly theoretical oil recovery," which implies that both methods give equally good yields. The same author also states that—

b) "it is really the recovery of the vitamins rather than of the oil which is desired, but in practice this amounts to about the same thing." Both statements in quotation marks are open to controversy. So also is the opinion expressed by Brocklesby (4)—

c) that alkali treatment of fish livers affects the stability of vitamin A by removing some of the natural antioxidants from the oil and that—

d) dilute soap solutions formed during digestion adsorb preferentially vitamin A from oil (5).

The present investigation, in which diverse digestion methods have been examined strictly from the point of view of vitamin A recovery has been carried out with the view of clarifying the points a), b), and c).

The question of preferential adsorption of vitamin A by soap solutions has been dealt with by the writer in a recent communication (6).

Raw Materials and Details of Methods. The material investigated consisted of shark livers mostly from school shark (*Galeorhinus australis*) and of livers from groper (*Polyprion oxygeneios*). To obtain a uniform basis for experiments 40 lb. of fresh shark livers were finely minced and after a thorough mixing put into small glass jars and kept in a refrigerator at —30°C. The same was done with 20 lb. of groper livers. The latter represented a material with low oil content while the former was rich in oil. Data relating to the species mentioned can be found in studies of Shorland *et al.* (7,8).

For the purpose of estimating the efficiency of various digestion methods, the oil content and the vitamin A content of the livers was established in the following manner. Weighed samples of the minced liver were treated with boiling ethanol, dried in vacuo, and the residue repeatedly extracted with petroleum ether, B.P. 50-70°. The vitamin A content of the oil was estimated, using a Beckman Quartz spectrophotometer, model DU. Although from the yield and the vitamin A potency of the extracted oil the vitamin A content of the livers could be computed with fair approximation, a modification of Moore's (9) procedure was finally adopted as a more convenient method of obtaining the vitamin A content per gram of liver. Samples of liver were saponified with alcoholic potassium hydroxide, the resulting clear solution extracted repeatedly with ethyl ether, the combined ethereal extracts dried in vacuo, redissolved in absolute alcohol, and assayed in the spectrophotometer. The extinction coefficient $E^{1\%}_{1\,cm.}$ at 328 mμ. was multiplied by the factor 1600 to obtain the vitamin A potency in international units per gram (10). A correction of irrelevant absorption suggested recently by Morton and Stubbs (11) was applied, and in each case the corrected coefficient was multiplied by 1800. The same method was adopted when examining oils from various digestion experiments.

Digestion experiments consisted in treating 100-g. samples of minced livers with steam, water, and aqueous solutions of a) caustic soda with and without salt, b) sodium carbonate, c) Na_2CO_3 + CaO, d) pepsin and hydrochloric acid, and e) hydrochloric acid.

The procedure was to mix the sample with twice its weight of water or solutions of chemicals just mentioned and to stir mechanically for ½-2 hours in a water bath. The mixture was subsequently separated in an "International" centrifuge (size 5) for 10 min-

TABLE I

Shark Liver: Vitamin A Content per g. Liver, 57,570 I.U. (Gross), 56,900 I.U. (Corrected); Oil Content 50.1%

100 g. liver digested with	Oil therefrom							
	Yield, %	Total oil recovered, %	Acid value	Lovibond		Vitamin A potency I.U./g.		Vitamin A recovered,* %
				Yellow	Red	Gross	Corrected	
Steam ≃ 200 g. condensate	40.0	79.9	1.0	20	9	105,800	93,000	65.4
200 g. H_2O	36.6	73.1	1.0	21	8.7	105,200	92,500	59.5
200 g. H_2O + 2 g. NaOH	44.8	89.5	0.25	16	1.5	114,100	104,300	82.1
200 g. H_2O + 2 g. NaOH + 10 g. NaCl	46.0	91.8	0.25	16	1.2	113,800	104,000	84.1
200 g. H_2O + 2.7 g. Na_2CO_3	42.2	84.3	0.6	19	5	109,500	102,800	76.3
200 g. H_2O + 2.7 g. Na_2CO_3 + 1.4 g. CaO	47.0	93.9	0.3	17	1.2	113,500	104,000	86.0
200 g. H_2O + 2 cc. HCl conc.	42.1	84.1	1.0	21	9	117,000	105,100	77.8

* Based on the vitamin A content obtained by saponification of livers.

utes, the "stickwater" and oil being decanted from the solid residue and transferred into a separating funnel. After holding the funnel for ½ hour in an oven at 60°C., the water was run off, the oil drained into a weighed flask, the funnel rinsed with petrol ether, and the contents of the flask dried in vacuo. In a few digestions 1,000-g. samples of liver were used, and the separation effected with a laboratory Sharples centrifuge running at 23,000 r.p.m.

The oxidation promoting effect of alkali digestion claimed by Brocklesby was investigated by means of the methylene blue test, originally developed by Greenbank and Holm (12), which is based on the observation that when oils containing the dye are exposed to light, the unsaturated oil constituents are oxidized and the dye reduced. In using a simplified modification of the test, measured amounts of methylene blue in alcohol were added to oil samples from various digestion experiments, and after irradiation with a 100-watt Mazda lamp at 70° C. the gradual disappearance of the blue color was observed in a Lovibond tintometer, and the corresponding times were noted.

Results

Laboratory Digestion Tests. a) *Shark livers:* Data obtained by using various digestion methods for this type of liver are shown in Table I. The 1,000-g. samples of the same liver digested with 2,000 g. of water and 2,000 g. of 1% NaOH solution, respectively, yielded after being put through a laboratory Sharples centrifuge:

	I.U./g.
Water digestion: 41.0% oil with vitamin A potency (corrected)	92,700
Alkali digestion: 47.9% oil with vitamin A potency (corrected)	104,000

b) *Groper livers:* Neither water nor alkali digestion was successful with groper livers, and consequently pepsin and acid digestion was employed, following the technique of Rapson *et al.* (2) but replacing ether extraction with centrifugal separation.

Factory Tests. Further data were obtained from tests conducted in one of New Zealand's fish liver oil factories. Four batches of livers were minced, and each of them was divided, after thorough mixing, into two equal parts. After drawing samples for analysis, part a) (c.f. Table III) was digested with steam and part b) with 1% caustic soda solution. In both cases the ratio of liver weight to water at the end of digestion was approximately 1:2. The separation was effected in a Sharples centrifuge.

Analysis of "stickwater" and of residue collected in the centrifuge bowl from batch 1-a showed 5.3% oil calculated on the weight of livers with a potency of 42,000 I.U./g. and from batch 2-a 7.8% oil with a potency of 43,000 I.U./g., which accounted fully for oil losses during the process.

TABLE II

Groper Livers: Vitamin A Content per g. Liver, 25,200 I.U. (Gross), 24,000 I. U. (Corrected); Oil Content 12.7%

100 g. liver digested with	Oil therefrom				
	Yield, %	Total oil recovered, %	Vitamin A potency I.U./g.		Vitamin A recovered, %
			Gross	Corrected	
100 g. H_2O + 0.5 g. pepsin + 2 cc. HCl conc.	7.3	57.5	200,100	196,400	59.5
100 g. H_2O + 2 cc. HCl conc.	4.2	33.1	198,300	195,500	34.2

Removal of Antioxidants and Stability of Vitamin A. In applying the methylene blue test previously described for measuring the removal of natural antioxidants from the oil, 0.2 cc. of 0.1 methylene blue solution in alcohol was added to 5 cc. of shark liver oil prepared by digestion with water and alkali, respectively, and irradiated. The oil from water digestion showed in the Lovibond tintometer at the beginning of the experiment, 10 Blue, which after 5 minutes of irradiation was reduced to 8 Blue, and after a further 5 minutes to 4 Blue. The oil from alkali digestion was under the same conditions reduced from 10 Blue to 4 Blue after 3 minutes.

To test the stability of vitamin A in the oils from water and alkali digestion 25 cc. of either oil was exposed in open glass dishes to light at room temperature, and the vitamin A potency checked periodically with following results:

Days	Oil from water digestion vitamin A potency I.U./g.		Oil from alkali digestion vitamin A potency I.U./g.	
	Gross	Corrected	Gross	Corrected
0	105,600	92,500	114,800	104,300
5	100,950	90,400	112,100	97,700
11	98,600	87,100	108,400	95,200
20	94,900	79,500 (86.0%)	104,300	90,300 (86.5%)

The original oils, kept in tightly stoppered, amber bottles, showed after 90 days, 105,100 (gross), 92,000 (corrected) I.U./g. for the oil from water digestion, and 114,000 (gross), 104,100 (corrected) I.U./g. from alkali digestion.

Discussion

Both laboratory and factory tests show that the yield of oil and vitamin A obtained by steaming with subsequent centrifuging is below the yield obtained by methods in which the liver protein is hydrolyzed. They also show that when employing different methods, the vitamin A recovery is not proportional to the

TABLE III

	Batch 1		Batch 2		Batch 3		Batch 4	
	Shark livers		Shark livers		Shark livers		Mixed livers (shark, hapuka, etc.)	
	a) Steam digestion	b) Alkali digestion	a) Steam digestion	b) Alkali digestion	a) Steam digestion	b) Alkali digestion	a) Steam digestion	b) Alkali digestion
Weight of livers	200 lb.	200 lb.	392 lb.	392 lb.	360 lb.	360 lb.	400 lb.	400 lb.
Oil content (solvent extraction)	64.1%		58.8%		58.0%		35.7%	
Vitamin A content per gram liver I.U.	33,350		29,000		30,200		14,600	
Oil yield in factory	58.2%	62.5%	51.0%	54.2%	51.1%	55.0%	26.7%	28.2%
% of theoretical oil yield	90.8%	97.5%	86.9%	92.2%	88.2%	95.0%	74.8%	79.1%
Acid value	1.1	0.4	2.0	0.4	2.0	0.6	13.4	1.2
Vitamin A potency I.U./g. of oil in factory	45,500	50,000	44,500	47,000	47,000	47,000	34,000	38,000
% vitamin A recovered *	79.5%	93.8%	78.3%	87.9%	79.6%	85.6%	62.2%	73.5%

* Based on the vitamin A content of livers.

oil yield since oils from alkali digestion have a higher potency than oils rendered by steam. This lends support to the theory that vitamin A in fish livers is not just dissolved in oil globules but at least some of it is bound to the protein.

Of alkaline agents employed, sodium hydroxide was much more effective than sodium carbonate. Instead of sodium hydroxide a mixture of CaO and Na_2CO_3 can be used with good results. The addition of NaCl to the sodium hydroxide solution appeared to offer some advantage in laboratory tests, but this was not confirmed in works practice.

The superiority of alkali digestion over the steaming process became more pronounced with livers of low oil content (25-30%). However livers with an oil content of approximately 10%, similar to the investigated groper livers, had to be treated with pepsin and acid. Digestion of shark livers with acid only gave an oil with slightly higher potency than alkali digestion, but the oil and vitamin recovery was lower. This of course is bound to vary with different livers.

The removal of free fatty acids during the alkali digestion which was previously believed to cause a decrease of the vitamin A potency of the oil is more likely to have the opposite effect, as has been shown elsewhere (6). There is moreover a considerable bleaching effect characteristic of alkali refining. Stale livers with high percentage of free fatty acids may lead to emulsions when digested with alkali. However, from further laboratory experiments in which varying amounts of pure oleic acid have been added to the livers before the digestion and from the factory batch No. 4 (Table III), it appears that livers whose oil contains up to 10% free fatty acids do not offer any difficulty with regard to alkali digestion and give a higher oil yield than could be expected if steaming and separate alkali refining were employed. Partly responsible for this rather surprising result is probably the buffer action of the protein, which protects neutral oil from saponification despite the considerable amount of alkali present.

The adverse effect of alkali digestion on the keeping qualities of vitamin A reported by Brocklesby (4) could not be confirmed under conditions of the present work. Although the methylene blue test indicates that such digestion removes some of the natural antioxidants from the oil, the stability of vitamin A was apparently not affected by their removal. The above findings are in agreement with some of the results published recently by Swain (13), but while more study is needed in this connection, it seems advisable to investigate the sensitivity of vitamin A after alkali treatment on the basis of direct measurements of potency and not in relation to secondary factors such as antioxidants.

Summary

Results obtained by steam, water, alkali, and acid digestion of shark and groper livers have been compared on the basis of vitamin A recovery, and alkali digestion, under the conditions reported, has been found to be the best method for livers with an oil content from 30% upwards. The partial removal of natural antioxidants from oil by alkali treatment does not seem to impair the stability of vitamin A on exposure to daylight and air at room temperature.

Acknowledgment

Grateful acknowledgment is made to F. B. Shorland of the Department of Scientific and Industrial Research, for valuable advice and criticism, and to A. S. Jessop for assistance in the experimental work.

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[Received March 6, 1950]