SOME STUDIES OF THE CHEMISTRY AND TECHNOLOGY OF SOUPFIN SHARK LIVER OIL .

T H E S I S

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by

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C O N T E N T S.

...../1. Antioxidants

GRAPHICAL ILLUSTRATIONS.

* Throughout this work the symbol $E^{1\%}_{1\ cm.}$ has been used to denote $E^{1\%}_{1\ cm.}$ 328 mμ.

I. SURVEY OF INDUSTRY.

During the past ten years the world demand for vitamin A has increased steadily. The increase is due partly to growing popularity of vitamin therapy and partly to the enormous rate at which vitamin A is being used in America for animal feeding. During the war years it has been used extensively for food fortification in England and Europe, and this will probably be continued for several years.

It is very difficult to estimate the annual world consumption of vitamin A, but we know[44] that in America 67×10^{12} International Units (I.U.) were used in 1939. Approximately 60% of this quantity was used for animal feeding. We also know that in England the fortification of margarine is compulsory and that the diet of all school children is supplemented with vitamin A.

The distribution of the world production of vitamin A was seriously disrupted by the war, when the Norwegian and Japanese productions were cut off from their normal destinations. Under these circumstances it is no wonder that the United States, Canada, South Africa, India and Australia made serious and successful attempts to produce fish liver oils, the main source of vitamin A. Argentina produced 25×10^{12} U.S.P. units in 1945[5] and the United States 9×10^{13} U.S.P. units in the same year. The South African production increased from 2×10^{12} I.U., valued at £80,000 in 1943, which year may be regarded as the birth of the South African industry, to 1×10^{13} I.U., valued at £400,000 in 1946-1947.

There are four companies engaged in the production of fish liver oils in South Africa, and the success of this venture has probably provided the biggest impetus to our rapidly expanding fishing industry.

This treatise deals with some aspects of the composition, characteristics, production, refining and storage of soupfin shark liver oil.

II. THE RAW MATERIALS OF THE INDUSTRY.

A. GENERAL DISCUSSION.

The most important sources of raw material for the South African vitamin A oil industry are the soupfin shark, stockfish or hake (merluccius capensis), several species of dogfish, notably the spiny dogfish, snoek (thyrsites atun.), and the seal (arctocephalus pusillus).

The livers and intestines of stockfish, which are caught mainly by trawling operations and is the most important species landed in the Union, are important sources of vitamin A. Dogfish are also caught in the trawl nets while snoek, caught on hand lines, is one of the most valuable species handled by the inshore fisheries. Like the stockfish, snoek is essentially a food fish, and the liver and intestines - sources of vitamin A - can be regarded as by-products.

The Cape fur seal is taken for its skin in the first place, and only quite recently[160] has it been shown that seal livers contain 1 - 11% of oil and 0.053 - 1.75% of vitamin A, and therefore constitute a valuable source of the vitamin.

The soupfin shark (galeorhinus canis), known locally as vaalhaai, is by far the most important source of vitamin A, and is the only species which justifies the equipping of special craft for its capture. It is caught mainly by line, and only the liver is utilised. It belongs to the family carcharinidae of the order plagiostomi and is closely related to the soupfin shark (G. zyopterus) of the north-west Pacific Ocean.

The soupfin shark is found along the African coast from Walvis Bay on the west coast to Algoa Bay on the east coast. Very little is known of its life history and no complete investigation has yet been completed on the

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relationship of sex, size, oil content of liver, locality, and vitamin A potency. A systematic survey has recently been initiated[9] and it is hoped that the results will help to assure commercial exploitation of the shark fishery for many years without seriously depleting the stocks.

The soupfin shark grows up to 7' in length, the liver constitutes on an average 10% of the total weight of fish, and contains on an average 50% of oil. The average potency of the liver oil is about 20,000 I.U./gram or 0.75% vitamin A.

B. VARIATIONS IN CONDITION OF SHARKS WITH SEASON, AGE, SEX, LOCALITY, POTENCY OF THE OIL ETC.[161]

Usually larger sharks are caught during early winter, when the females move into the shallow and sandy False Bay and Mossel Bay areas to give birth to their young. During autumn smaller and immature fish predominate. Males are more common in deep water and most specimens caught by the trawlers are very large males. In the Walvis Bay area males seem to predominate, and no females have yet been encountered amongst the occasional specimens caught there, either in shallow or deep waters. Most specimens from that area are large.

Although locality influences the exact time, autumn and early winter have been found the seasons when the young are born. Gestation has a very marked influence on the stored fat of the soupfin shark. In the case of mature females, the oil content of the liver recedes during pregnancy (Table 3), and the vitamin A potency consequently increases by concentration: During this period the degree of unsaturation of the oil usually decreases. The oil content of the liver seems to reach a minimum just before or at the time of birth of the young. In addition to the decrease in oil content of the liver, the liver itself constitutes a smaller portion of the fish. The results in

...../Table 3

Table 3 show a very significant decrease, during gestation, of the liver oil expressed as a percentage of total fish. Whereas the liver oil constitutes on an average 11.9% of the total fish when fat, it only accounts for 1.6% when the fish is in an advanced stage of pregnancy and very thin.

Vitamin A accumulates in the soupfin shark with age. Embryo liver oil is practically devoid of vitamin A (Table 3c) and the full-grown or large fish provide the highest yields[9].

Large males generally provide much more vitamin A per fish, than females. It is common for a deep-water male to yield a liver oil containing 3% or more vitamin A and to yield 100 million I.U. per fish.[161.9]

C. DISTRIBUTION OF VITAMIN A IN THE SHARK.

In the soupfin shark, the liver is the main site of fat deposition. Head, body and intestine contain negligible amounts of oil. The small oil yields from head and body have been shown to be devoid of vitamin A by Molteno et al.,[1] while the intestine constitutes such a small portion of total weight, and contains so little oil that the reported $E^{1\%}_{1cm}$ values of 9.7 and 6.4 signify negligible quantities of vitamin A per fish.

Analyses were carried out to determine the differences between the vitamin A contents of different parts of the liver and/or different lobes. This was undertaken in order to investigate the possibility of preparing more potent oils for specific purposes, by pre-segmentation of livers. The upper hepatic portion, a transverse section through the middle and the lower portion or tip, were digested and extracted separately, for

(i) a big, fat liver and
(ii) a small, thin liver.

The segments were cut from both lobes. In a third sample, the segments were collected separately for the two lobes. The analytical results are summarised in Table 1.

TABLE 1.

Distribution of oil and vitamin A in soupfin shark livers.

1		2	3	4
Liver		% oil	$E^{1\%}_{1cm}$	Ex % oil.
Large fat liver	(Upper Hepatic	78.4	3.84	301
	(Middle	76.4	3.37	258
	(Tip	79	2.78	220
Small thin liver	(Upper	37.6	52.16	1960
	(Middle	38.6	45.10	1740
	(Tip	38.3	43.51	1670
Average Liver — Bigger Lobe	(Upper	68.8	35.26	2420
	(Middle	67.2	34.48	2320
	(Tip	67.9	34.81	2360
Average Liver — Smaller Lobe	(Upper	63.2	39.18	2480
	(Middle	66.6	34.4	2290
	(Tip	67.4	34.3	2310

From these results it appears that the vitamin A potency gradually falls from the upper portion to the tip. This is in accordance with results previously recorded[10.1], although the range for the fat liver is wider than that reported earlier[1]. From a consideration of the oil contents of the different parts, and the results in column 4, it is obvious that the difference in potencies is not due to a dilution effect, but that the upper hepatic portions are richer in vitamin A. In the more potent oils, the difference is too slight, however, to warrant the trouble and cost of pre-segmentation of livers. Note the high value of $E^{1\%}_{1cm}$ of the oil from the upper hepatic of the smaller lobe of the average liver.

D. CHEMICAL COMPOSITION OF SOUPFIN SHARK LIVER OILS.

1. Preliminary.

It has been found by Molteno et al.[1], that soupfin shark foetus liver and yolk sac oils are more highly unsaturated than the liver oil of the parent. In addition, the foetus oil has a much higher content of

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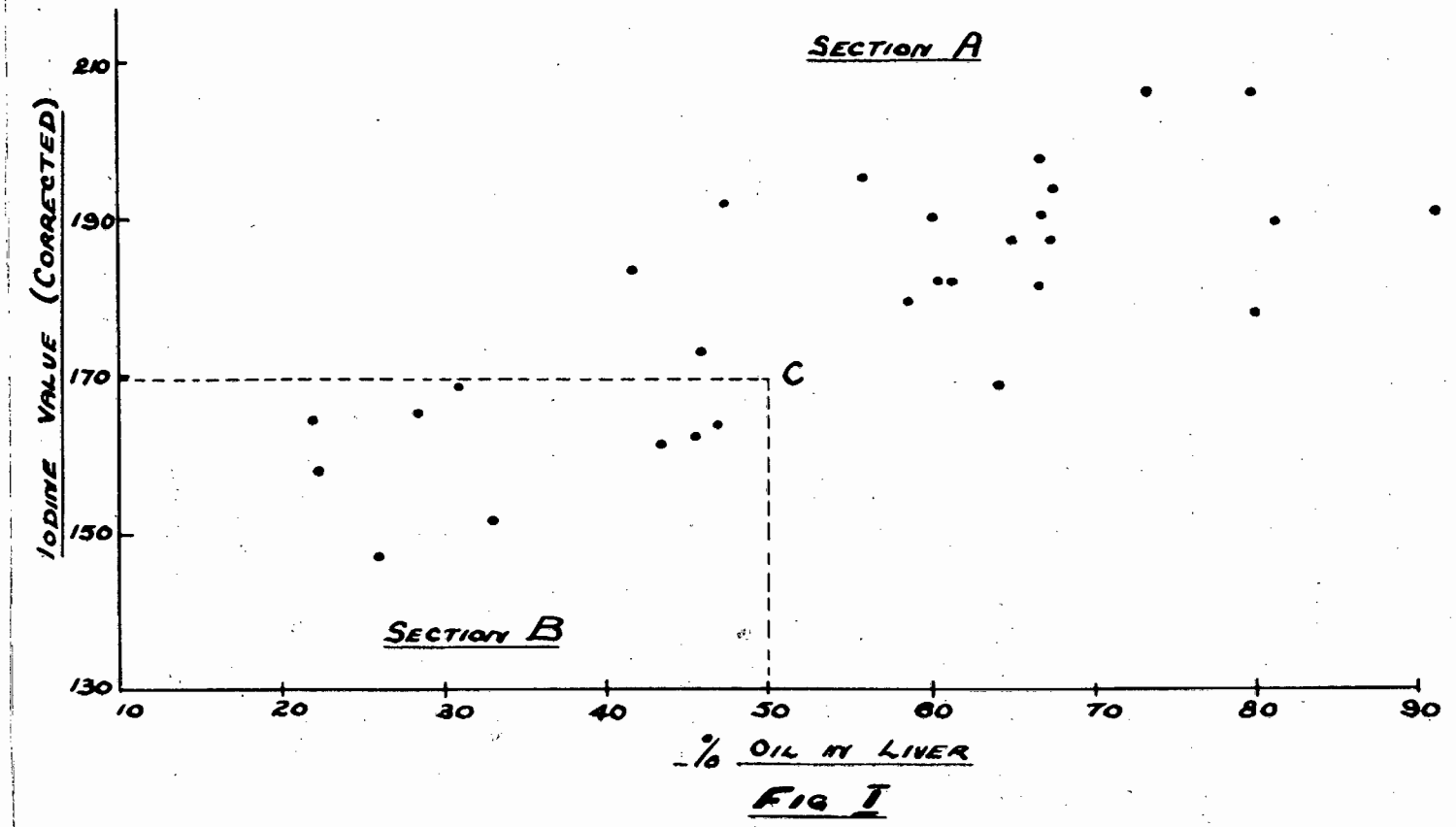

FIG I

VARIATION OF IODINE VALUE WITH CONDITION OF FISH

unsaponifiable matter, and these facts mark it as very different from the liver oil of the mother. The Author's investigation (Table 3(c)) of four batches of embryo liver oil representing 343 specimens, confirmed the very high degree of unsaturation previously noticed in embryo liver oil. These high iodine values are all the more interesting if one considers that these oils are practically devoid of vitamin A (I.V.356) which usually accounts for a considerable increase in iodine values of very potent oils (note difference between observed and corrected I.V.'s in Table 3(B)). In the light of this experience it was decided to undertake a comparative chemical study of embryo and parent liver oils.

A further interesting observation was made[9] vis. that a roughly linear relationship seems to exist between oil content and iodine value (corrected to exclude vitamin A) of the liver oils of, thirty specimens collected (Table 2 and figure 1). These specimens varied from very thin to very fat fish and covered a range from 12 - 92% oil in liver and iodine values from 128.9 to 205.5. When these figures are plotted on a graph, they present a very scattered picture (fig. 1). Some rough linear relationship is apparent and at point C(I.V.170, 50% oil) the graph can arbitrarily be divided in two sections A and B. The points in section B can be regarded as representing thin fish while those in section A represent fat fish.

Upon consideration of this phenomenon, it was decided to extend the proposed chemical analysis and compare the constituents of embryo oil with those of thin and fat female liver oils in order to study the effect of condition of fish on composition of liver oil at the same time.

2. <u>Component acids of fat and thin female soupfin shark liver and embryo liver oils.</u>

In this analysis, an attempt was made to prepare composite samples of oil representative of very fat and very thin female sharks. At the same time embryos were

TABLE 2.9

VARIATION OF IODINE VALUE WITH % OIL IN LIVER OF SOUPFIN SHARK.

No.	% oil in liver	1% E_{1cm}.	I.V. on whole oil	Corrected I.V. on non-vitamin A fraction
1	12.2	313.2	172.9	128.9
2	22	120	178.6	164.4
3	22.3	86.8	168.7	158.1
4	25.9	183.2	170.8	147.2
5	28.5	86.5	175.6	165.5
6	31	136	184.1	168.3
7	33.1	66.4	160	151.7
8	41.7	10.3	184.3	183.7
9	43.3	45.4	166.9	161.3
10	45.5	36	166.6	162.3
11	45.8	23.6	175.9	173.1
12	46.8	17.2	165.5	163.6
13	47.3	10.5	193.1	192
14	55.9	10	196	195.1
15	58.5	21	180.8	178.6
16	60.3	16.2	183.6	181.9
17	60	29.24	192.8	189.8
18	61	8.72	182.7	181.8
19	64.1	11.9	169.7	168.4
20	66.8	21.2	183.1	180.8
21	66.8	9.60	197.8	197
22	67.3	24	189.6	187.2
23	65	6.79	187.5	186.8
24	66.9	7.63	190.9	190.1
25	67.5	1.92	193.7	193.6
26	73.5	6.57	206.1	205.5
27	80.3	4.46	178.1	177.6
28	80.0	3.21	206	205.6
29	81.2	5.36	189.8	189.1
30	92	6.81	190.5	189.8

collected from the pregnant fish and their liver oil extracted. The composite sample of fat shark oil was prepared from the samples listed in Table 3, after due consideration of the results in Table 2 and figure 1. Specimens were picked from section A of figure 1, and they were oils constituting more than 65% of the liver, and 8 - 15% of the total weight of the fish. Likewise the composite sample of thin shark oil was prepared from specimens in section B of figure 1, i.e. oils constituting less than 46% of the liver, and in no case more than 2.9%of total fish (Table 3).

Details of these composite samples are given in Table 3.

The characteristics of these specimens can be correlated with sexual condition. In the fat females the uterus was either undeveloped or only partly developed, with only one exception, a fish that carried 13 small embryos with large yolk sacs. The livers were large, constituting on an average 16% of total weight of fish. Note too that the liver oil constituted 8% or more of total fish in all specimens (Table 3A). The thin specimens on the other hand, were in an advanced state of pregnancy, carrying well developed embryos in most cases. Specimen 2 was spent. Note that these sharks had small livers, constituting on an average 4.7% of the total weight, while the oil never constituted more than 2.9%, and on an average only 1.6%, of total fish.

The embryo liver oil was obtained from four batches of young, three of which were practically fully developed, while the last batch was made up of much smaller embryos.

These composite samples representing the fat condition (early pregnancy), secondly the thin condition i.e. the time when the young are ready to be born, and finally the embryo liver oil, were saponified, and the free fatty acids

...../recovered

TABLE 3^2

Analytical details of oils used for Acid Studies.

No.	Date caught	Weight (Kg.)	Sexual Conditions	Liver as % of fish	% oil in liver	Liver oil as % of fish	$E^{1\%}_{1cm}$	I.V.	I.V. on non-vit.A portion.
A. Fat Female Shark.									
1	1. 3.46	10.3	Uterus undeveloped	15.43	67.5	10.3	1.92	193.7	193.6
2	22. 3.46	25	Eggs not yet in uterus	19	81.2	15.4	5.36	189.8	189.1
3	21. 3.46	7.7	13 Embryos with large yolk sacs	16.4	67.3	11.1	24	189.6	187.2
4	24. 4.46	11.4	Uterus partly developed	12.2	71.6	8.8	2.93		
5	24. 4.46	18.2	Large eggs in ovary	17.8	74.6	13.3	5.25		
6	3. 5.46	17.3	Uterus partly developed	16.2	75.8	12.3	3.84		
Average		15.1		16.1	73	11.9	6.77	195	194.3
B. Thin Female Shark									
1	24. 4.46	14.3	16 Small embryos with large yolk sacs	4.23	33.1	1.41	66.4	160	151.7
2	22. 2.46	14.7	Spent	5.01	28.5	1.43	86.5	175.6	165.5
3	21. 3.46	8.0	16 Embryos with large yolk sacs	6.75	43.5	2.94	45.4	166.9	161.3
4	28. 3.46	12.1	Pregnant. No yolk sacs	4.57	46.8	2.12	17.2	165.5	163.6
5	25. 2.46	17.2	Pregnant - 10 embryos	4.77	22.3	1.06	86.8	168.7	158.1
6	22. 3.46	16.4	Pregnant	3.04	12.2	0.37	313.2	172.9	128.9
7	22. 2.46	24.2	16 Embryos with yolk sacs	4.77	31	1.48	136	184.1	168.3
8	28. 3.46	14.5	Pregnant - 16 embryos	5.29	45.8	2.43	23.6	175.9	173.1
9	15. 2.46	16.8	Pregnant - 16 embryos	4.73	22	1.05	120	178.6	164.4
10	22. 2.46	22.1	18 Embryos	5.17	45.5	2.36	36	166.6	162.3
11	22. 2.46	20.8	21 Embryos. Only 2 yolk sacs	4.02	25.9	1.05	183.2	170.8	147.2
12	24. 4.46	14.3	16 Embryos, large yolk sac	4.23	33.1	1.41	66.4	160	151.7
Average		16.3		4.7	32.5	1.6	86.04	167.75	157
C. Shark Embryo.			Average Length						
57 Embryos	24. 4.46	5.75	30.1 cm.	5.4	51.7	2.78	0.22	216.2	
29 do.	15. 2.46	3.31	31.5 cm.	5	42.1	2.1	0.14	214.8	
57 do.	21. 2.46	7.89	32.7 cm.	5.6	39.9	2.22	0.12	214.8	
200 do.	28. 3.46		12 cm.		22		0.23	203.3	
Average				5.3	39	2.37		215.6	

recovered after extraction of unsaponifiable matter from the soap solution. The acids were separated into "solid" and "liquid" fractions by the lead-salt separation method[3], and these fractions were re-esterified with methyl alcohol, while the methyl esters were analysed by the Hilditch ester fractionation procedure[3]. Experimental details and calculated compositions are summarised in Tables 4 - 12, which will be found in the back of the book, while Table 13 (included in the text) gives a condensed summary of the calculated components of all three oils.

The composition of individual fractions were calculated from their determined saponification equivalents and iodine values and use was made of the computation forms set up by Rapson et al.[4]

Discussion of calculations:

(a) "Liquid" fractions containing C_{18} and C_{20} or C_{20} and C_{22} unsaturated esters only. The Charnley[5] mathematical method was used with Rapson's computations to determine composition and average unsaturation in fractions L6 - L14 for the fat female oil (Table 4) fractions L7 - L15 for the thin female oil (Table 7), and fractions L5 - L13 for the embryo oil (Table 10). Average unsaturation was determined for every two adjacent fractions and the mean for every set taken.

(b) "Liquid" fractions containing only C_{16} saturated and C_{16} and C_{18} unsaturated components. These are calculated by the same method, assuming the C_{16} as monoethenoid and using the determined unsaturation for C_{18}. In this way fraction L2 - L6 for thin female oil (Table 7) and L2 for embryo were calculated (Table 10). Fractions L2 - L4 for the fat (Table 4) were calculated to an average unsaturation of -3.5H for C_{16} and -3.9H for C_{18}. The latter figure of course was obtained from the calcuations of the C_{18}

...../ and C_{20}

and C_{20} fractions as discussed under (a), while the former figure was obtained from the calculation of fraction L5 for fat female oil. This fraction, which should consist essentially of C_{16} and C_{18} esters (from a consideration of its S.E. and I.V., gives a negative result for one or the other constituent when calculated to C_{16} saturated and C_{16} and C_{18} unsaturateds. It was therefore assumed to contain only C_{16} and C_{18} unsaturated constituents, and calculated according to the Charnley formulae. A mean unsaturation of -3.5H was then obtained for the C_{16} esters, and this value was used in the calculation of fractions L2 - 4 of the fat female oil. Fractions L3 and L4 in the embryo oil (Table 10) likewise gave negative values for some of the constituents when calculated to C_{16} saturated and C_{16} and C_{18} unsaturated esters. The only assumption leading to positive results is that the fraction contains only palmitic and C_{18} unsaturated esters, and an almost identical example is quoted by van Rensburg et al.[6] who advance the suggestion that the cutting of such fractions apparently arises from the fact that unsaturated esters tend to distil before saturated esters of the same carbon content.

(c) Fractions L1 for thin female and embryo oils (Table 7 and 10 respectively) were calculated to C_{14} and C_{16}. Saturated and mono-ethenoid constituents, assuming the S.E. of the saturated portion of each fraction to be the same as that of the whole fraction. This assumption leads to very little error in the final analysis and does away with the necessity for isolating the saturated fraction. Fraction L1 for the fat female (Table 4) was calculated in the same way, only using the determined mean unsaturation for the C_{16} constituent.

(d) The composition of final fractions ("liquid") L15, L16 (Table 4) L16, L17 (Table 7) L14, L15, L16 (Table 10)

were assessed on the basis of saponification equivalents only. Oxidation and/or polymerisation usually occur during distillation, resulting in a drop in iodine values of the last fractions, which makes them insignificant. In the absence of any method of evaluating the mean unsaturation of the C_{24} esters in these last fractions, they are taken as C_{24} - 1OH.

(e) The early "solid" fractions S1 - S6 for fat female oil (Table 5), S1 - S6 for thin female oil (Table 8) and S1 - S8 for embryo oil (Table 11) were calculated to C_{14} and C_{16} saturated and mono-ethenoid esters, making the same assumption as in (c) above, c.f. van Rensburg[3].

(f) Fraction S8 - S13 for fat female oil (Table 5), S8 - S11 for thin female oil (Table 8) and S9 - S12 (Table 11) for embryo oil, have been calculated to a mixture of C_{16} and C_{18} saturated and mono-ethenoid acids. There is no reason to assume greater unsaturation than -2H for the unsaturateds, as Hilditch[3] points out that polyethenoid acids of any carbon extent should pass into the "liquid" acid group during the lead-salt separation.

(g) Fractions S14 (Table 5) S12, S13 (Table 8) were calculated to C_{18} and C_{20} saturated and unsaturated acids, while S14 (Table 11) and S15 (Table 8) were calculated to C_{20} and C_{22} saturateds and unsaturateds. All "solid" fractions involving C_{20} and higher unsaturated acids, are assumed to have the same mean unsaturation as that determined for them in the "liquid" portion. Most calculations only become possible if this assumption is made; this has also been the experience of van Rensburg[6] and Guha[7] et al.

(h) Fraction S7 for both fat and thin female oils (Tables 5 and 8 respectively) were examples of borderline fractions, almost identical with an example quoted by van Rensburg et al.[6] They give negative values for

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some components if calculated to C_{14} and C_{16} saturated and mono-ethenoid acids. This condition apparently arises in "solid" fractions when the C_{14} saturated and unsaturated esters have almost disappeared. These fractions gave positive results when calculated to C_{14} and C_{16} saturated and C_{16} mono-ethenoid esters.

(i) Fractions S15 (Table 5) S14 (Table 8) and S13 (Table 11) could only be calculated to C_{20} saturated and C_{20} and C_{22} polyethenoid esters. These examples are analogous to that found by van Rensburg[8] who points out that unsaturated esters tend to distill before the saturated ones of the same carbon content. In all three these fractions the observed S.E.'s are less than 326 and in such cases the presence of C_{22} saturated esters is excluded.

(j) Finally, the residual fractions S16, S17 (Table 5) S16 (Table 8) and S15, S16 (Table 11) were calculated to C_{22} and C_{24} unsaturated components only. In all five fractions the observed I.V.'s were lower than in the previous fractions, so that they were calculated on the basis of S.E. only. In view of the high I.V.'s they were assumed to consist of unsaturated esters only.

3. <u>Discussion of results, and comparison of fat and thin female shark and shark embryo liver oils (see Table 13)</u>

The marked differences in chemical composition between fat and thin shark liver oils, serve to confirm earlier observations[177] that analytical data for single specimens can be very misleading as a result of considerable variations in composition caused by condition of the fish. These variations have been demonstrated to be related both to mode and degree of fat storage.

(a) The following general observations can be made from

...../Table 13

TABLE 13.

COMPARISON OF THE COMPONENT ACIDS OF LIVER OILS OF THE FAT FEMALE SHARK, THIN FEMALE SHARK AND SHARK EMBRYO.

Acid	Fat Female	Thin Female	Embryo	-
	195	157	215.6	I.V.
	301.2	327.95	329.8	S.E.
SATURATED:				
C_{14}	3.28	3.47	3.26	
C_{16}	17.71	17.34	18.45	
C_{18}	1.58	3.55	2.17	
C_{20}	0.71	1.22	0.52	
C_{22}	-	0.36	0.08	
UNSATURATED:				
C_{14}	0.53 (-2H)	0.99 (-2H)	0.65 (-2H)	
C_{16}	9.41 (-3.1H)	8.63 (-2H)	6.33 (-2H)	
C_{18}	25.30 (-3.7H)	23.11 (-2.6H)	17.46 (-3.4H)	
C_{20}	24.37 (-8H)	17.21 (-6.5H)	20.88 (-8.3H)	
C_{22}	15.89 (-10.3H)	17.52 (-9.8H)	25.54 (-10.6H)	
C_{24}	1.20 (-10H)	6.38 (-10H)	4.64 (-10H)	
C_{26}	-	0.20 (-10H)	-	
TOTAL	99.9	99.9	100.0	

Table 13:-

(i) The fat female and embryo liver oils are characterised by greater total unsaturation (iodine values) than that of the thin female liver oil. This is explained by the higher content and higher degree of average unsaturation of C_{18}, C_{20} and C_{22} components.

(ii) The embryo and thin female oils are distinguished from the fat female oil by their much higher saponification equivalents (330 and 328 as against 301). This is explained by the higher C_{22} acid content of the embryo oil and the presence of relatively large amounts of C_{24} unsaturated acids in embryo and thin female liver oils. In fact, the presence of so much C_{24} acids and even a trace of C_{26} unsaturated acids in the thin female liver oil is unusual.

(iii) In main features, the composition of soupfin shark liver oils appears to agree with a few examples of Elasmobranch fish (skate, angel fish, thresher shark, spotted dogfish) whose liver oils contain less than 10% unsaponifiable fraction[178]. The average content of C_{20} unsaturated acids in shark liver oils appears to be slightly lower than the average of the cited examples (ca. 20% as against ca. 27%) while the average mean unsaturation is slightly higher (ca-8H as against -6.5H).

While the cited examples contained no C_{20} or C_{22} saturated, or C_{24} unsaturated acids, the shark liver oils contain small percentages of C_{20} and C_{22} saturated acids, and appreciable quantities of C_{24} unsaturated constituents.

(b) <u>Comparison of fat and thin female shark liver oils.</u>

The effects of mode and degree of fat storage in relation to the chemical composition of the fat, have been described for the Cape John Dory[179], a species with a diffuse system of storage. The present observations

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are of significance in that they refer to a fish with fat storage localised in the liver. In such cases the suggestion has been made[177] that the liver oil should approximate in composition to the body and head oils of species with diffuse storage.

On the basis of this hypothesis, the composition of shark liver oil should show -

(i) a decreased content of high molecular weight unsaturated acids with decreased fat content of the liver;

(ii) an increase in degree of average unsaturation of the higher acids with decreased fat content of the liver.

The results of the present studies indicate considerable departure in the elasmobranch species from what might have been expected on the basis of the above-mentioned experience.

In the first place, depletion of fat stores during pregnancy, results in an all-round and considerable decrease in unsaturation of the different component acid groups. A similar effect has been reported[180] in the oil from the flesh of herring, as far as C_{18} and C_{20} unsaturated acids are concerned. As far as the soupfin shark is concerned, the average unsaturation of the component acids groups of the fat female oil is very definitely higher, it should be noted that it has even been possible to determine the degree of unsaturation of the C_{16} acids, which is most unusual, as the C_{16} unsaturated acids are normally assumed to be mono-ethenoid.

A second observation in the thin female liver oil composition is the increased content of C_{18}, C_{20} and C_{22} saturated acids.

Depletion of fat stores during pregnancy leads to a small decrease in C_{16} and C_{18} unsaturated acid contents, a marked decrease in C_{20} acid content, but increased

...../contents of

contents of higher molecular weight unsaturated acids. While the C_{22} unsaturated acid content is but slightly higher in the thin female oil, its C_{24} acid content is very markedly higher, while a trace of C_{26} unsaturated acid is also present. This state of affairs is a further contradiction of the suggestion described earlier[177].

In general then, depletion of reserves of liver fat during pregnancy, results in -

(i) general decrease in degree of average unsaturation of the acids, and

(ii) an increase in higher molecular weight components, especially unsaturated acids, where C_{24} acids are synthesized at the expense of C_{20} acids.

(c) Comparison of embryo and mother shark liver oils.

As indicated in Table 3, the embryo exhibits similarity with the thin female shark in that its liver oil constitutes less than 3% of the total weight. The embryo is therefore also a "thin" fish and we might expect a similar relationship between condition and composition, to that which exists in the mother liver oil.

The preferential deposition of C_{20}, C_{22} and C_{24} unsaturated acids in the embryo liver oil, and the high average unsaturation of these acids, account for the high observed iodine values of embryo liver oils.

The component acids of embryo liver oil are distinguished from those of the mother oil by their higher degrees of average unsaturation, approximating as they do the average unsaturation of the corresponding component acids of the fat female liver oil.

Embryo liver oil is further characterised by its considerably lower content of C_{18} unsaturated components and considerably higher content of C_{22} unsaturated acids, and it is clear that there is preferential deposition of highly unsaturated higher molecular weight acids, particularly of the C_{22} group.

...../4. Unsaponifiable

4. Unsaponifiable components of soupfin shark liver oils.

When a liver oil is saponified and the resultant soap solution extracted with a suitable solvent, the vitamin A is extracted together with any other unsaponifiable constituents that the oil may contain, and constitutes the major portion of the unsaponifiable fraction, especially in the more potent oils.

The vitamin potency of shark liver oils varies from about $E^{1\%}_{1cm}=5$ when the fish is fat, to over $E^{1\%}_{1cm}=300$ when the fish is thin (Table 3), while very high potencies are also observed in the liver oils of large male fish[161].

The ratio $\frac{E^{1\%}_{1cm}}{16}$ gives percentage vitamin A in the oil, so that the abovementioned range of $E^{1\%}_{1cm}$ values represents a vitamin A content of 0.3% to over 18% in the oil. It can therefore be seen that unsaponifiable values, commonly known as non-sap. values of over 18% of the oil will be encountered. In such oils the major constituent of the unsaponifiable fraction is vitamin A.

Normally, however, the average $E^{1\%}_{1cm}$ value of shark liver oil is 12.5, or 0.78% vitamin A, and the average unsaponifiable content is about 2.5% so that two-thirds of the non-sap. consist of constituents other than vitamin A.

A detailed investigation of the unsaponifiable fractions of marine animal oils has been carried out during the last two years[181]. For the sake of completion of the present review on the components of soupfin shark liver oils, a brief abstract of the results that have been obtained to date, will be included in this work[46].

The unsaponifiable fractions from the oils of a few specimens have been examined for squalene and α-glyceryl ethers, with the following results :-

(i) Squalene.

Unsaponifiable matter content of oil	2.68%
Squalene (as % of unsaponifiable matter)	1.8%

So that the actual squalene content of the whole oil is very low indeed.

(ii) Glyceryl Ethers.

Table 14.

Material.	Unsaponifiable matter content of oil (%)	α-Glyceryl ether content of unsap. (%)
Vaalhaai liver oil 1	1.85	17.7
" " " 2	3.00	34.4
" " " 3	1.87	24.2
Embryo liver oil	8.82	0.4
Yolk sac. oil	10.30	2.1

Note the very low α-glyceryl ether content of the unsap. fractions of embryo and yolk sac oils.

The bulk of the unsaponifiable fraction appears, from crystallization observations, to be steroid in nature. A detailed examinsation of a bulk sample will be carried out at a later stage.

The high unsaponifiable content of embryo liver oil is not due to vitamin A, as pointed out in Table 3c.

III. PRODUCTION OF LIVER OILS.

The production of shark liver oils is a technological undertaking to which many problems are attached.

The most important steps are collection and preservation of livers, liberation of the oil from the tissue cells, separation of the oil in a pure state and storage of the oil.

Since the sharking boats are too small to house refrigeration units, and remain out at sea for days at a time, sometimes for weeks, the preservation of livers constitutes a serious problem. Many micro-organisms can operate at low temperatures and cause lipoxidases to be formed, which cause rancidity in oils[47] or serious losses of vitamin A. The action of lipoxidase to cause destruction of vitamin A has been strikingly demonstrated[48] by comparing the oils from 3 sterilised livers with their unsterilised counterparts, which had been stored for 10 days at 39°F (4°C). The oils from the unsterelised samples lost 11.3, 9.8 and 25.7% of vitamin A respectively.

(a) Preservatives:

It becomes essential therefore to aim at processing the livers as fresh as possible, as stressed by Drummond and Hilditch[49], and to use the best possible preservatives, when immediate processing is impossible. There is a dearth of information on this subject in the literature. Sulphur dioxide, normally a powerful bactericide, has been found to produce unpleasant odours in the oil[50]. The author has confirmed this finding. An excellent review on preservation of fish and fish products is given by Tarr & Sunderland[51], who found sodium nitrite an excellent bactericide, functioning only at a pH below 6[52]. Hess and Gibbons[53] found a 2% solution of caustic soda, a chlorine solution containing 5,000 p.p.m. available chlorine, and formaldehyde solution

...../(1 in 1,000)

(1 in 1,000) effective. Tarr[54] found equally effective bactericidal action by a 2½% solution of 40% formalin, a 1% solution of sodium hypochlorite and a solution containing 5% sodium nitrite and 1% acetic acid. Formalin is corrosive but this can be counteracted by adding 1 part in 2,000 sodium nitrite to it. Brocklesby and Green[55] propose the use of borax at a concentration of 2%, or 10% common salt.

Most of the work quoted above was done on fish products other than livers or intestines. It was decided to test the suitability and effectiveness of all these preservatives for use with fish liver. Unfortunately there is no single, and entirely satisfactory, chemical or physical test by which the efficiency of any preservative can be accurately and quantitatively measured. For the purpose of this investigation the following criteria were considered :-

(i) Appearance of the liver after storage in contact with preservative i.e. whether firm, coagulated hard, or disintegrating;

(ii) Smell;

(iii) Yield of oil and vitamin potency and smell of the oil;

(iv) Effect of preservative on metal containers;

(v) Oil solubility of the preservative and its physiological effects.

It was attempted to carry out bacterial counts in conjunction with the Union Department of Health, but even the well preserved samples were so badly infested that this course was abandoned.

Organoleptic tests were made with the assistance of several other people.

The test was conducted by obtaining a fresh liver from a shark directly after it had been caught, cutting portions from it and dipping under preservative solution.

...../A blank

A blank was stored under 95% alcohol. After six days the samples were removed, examined and the oil extracted by sodium sulphate dessication and ether extraction[182]. Borax was used dry, the requisite quantity being sprinkled over the liver, while the preservatives were dissolved in brine solution as indicated in Table 15.

Table 15[56]

Preservation of liver A. Substrate for preservatives -5% common salt solution, except for borax. Period of contact 6 days.

No.	Preservative	Smell of Liver	Condition of Liver	Colour of extracted oil	Odour of oil	$E^{1\%}_{1cm.}$	Acid in oil (% oleic)
0	Alcohol (control)	X	firm	XX	good	10.86	0.26
1	(brine only)	XXXX	firm	XXX	fair	9.86	2.41
2	Borax	X	firm	XX	bad	10.06	1.37
3	0.1% formalin (40%)	X	very firm	X	very good	10.03	2.14
4	1% formalin	X	very firm	X	very good	9.5	0.6
5	0.2% $NaNO_2$	XX	firm	XX	good	9.75	1.71
6	0.5% $NaNO_2$	XX	firm	XXX	bad	10	0.8
7	0.5% sodium sulphite	XXX	firm	X	fair	10.23	3.5
8	5% sodium sulphite	XXX	firm	X	good	10.45	1.03
9	No preservative	XXXX	disintegrated	XX	fair	9.21	3.81

(1) Smell of liver
X - as fresh
XX - slightly off
XXX - bad
XXXX - very bad indeed.

(2) Colour of oil
X - light straw
XX - darker yellow
XXX - brownish yellow.

From these results it is apparent that formalin preserved the livers well and produced light coloured and good smelling oils. 0.1% Formalin in the substrate is probably too little (consider high acid figure) but 1% may

...../be too

be too much, as there was a very distinct smell of formaldehyde in the preserving solution. The borax-preserved sample yielded a darker and more rancid oil. The liver was well preserved though. Sodium sulphite did not preserve the liver well, and the samples were almost as bad as the unpreserved samples. Nitrite was fair, but not as good as formalin or borax. The apparent losses of vitamin A may be adventitious, considering the fact that vitamin A is not distributed evenly throughout the liver (See Chaper I.C and Table 1). Later tests were carried out on minced liver.

Note the development of free fatty acids. In the well preserved samples, i.e. with 1% formalin 0.5% sodium nitrite, the development of free acidity is almost negligible. Note too the high free acid contents of the unpreserved sample and those where sulphite was used.

In order to eliminate sampling errors, a further preservation experiment was carried out by mincing and mixing very well the livers of five sharks. Aliquots of two pounds each were weighed out for the preservation test. In this case there was no opportunity to study the physical appearance of the liver at the end of the period of storage, but the smell can be regarded as a fair guage of quality. Shark livers putrefy very quickly and once putrefaction has set in, it is impossible to produce quality oils from such livers. So that organoleptic tests on liver and oil and vitamin A assays on the recovered oil, were used to determine the efficiency of the preservatives. While such methods can not produce quantitative data, they serve to distinguish between good, indifferent and poor preservatives. In the following experiment, unless otherwise stated, all preservatives were dissolved in sea water, or stronger brine solutions, prepared from sea water. It has been found very satisfactory

...../to use

to use the preservative in the form of a pickling solution and to submerge the liver completely, and this method is recommended strongly for use by the industry. It is the only method that ensures perfect contact and reduces the danger of atmospheric oxidation to a minimum. It is also the simplest method as far as the crew of a fishing boat is concerned.

After one week, the samples of liver were alkali-digested and the oil recovered by centrifuging. The results are summarised in Table 16.

General discussion (Table 16).

(i) The lightest oils in colour were obtained from sulphite preserved liver. There was a rancid smell and taste noticeable in them however.

(ii) It was again confirmed that formaldehyde and sodium nitrite are excellent preservatives. Oils with good odour and taste were obtained from these samples, although the colour of the nitrite preserved samples were darker than those from the formaldehyde. Formaldehyde was used as a 40% formalin solution.

(iii) Note the failure of sample 11, in which butyl gallate was used together with the sulphite.

(iv) Borax is probably very effective, but it is difficult in practice to ensure proper contact with all parts of the liver. This is demonstrated by the improvement when using it in solution viz. No. 7. During long contact, borax sometimes causes partial saponification and emulsification during processing.

(v) To sum up, the following preservatives in order of preference, are suggested for fish liver:

(a) 0.1% Formaldehyde (0.25% of a 40% Formalin) dissolved in sea water. To the solution should then be added 1 part in 2,000 of sodium nitrite to prevent corrosion.

(b) 1% Borax, dissolved in sea water.

(c) 0.1% Sodium nitrite dissolved in sea water.

...../Since

TABLE 16

Vitamin potencies and quality of oils from samples of liver preserved in different ways.

(Period of storage - 7 days)
(Preservative expressed as % of weight of liver used)

No.	Preservative	Condition	$E^{1\%}_{1cm}$ of oil	Odour of oil
1.	1% Dry Borax sprinkled over liver	Not well preserved	29.6	rancid
2.	0.1% Formaldehyde in 3% brine (sea water)	Liver very well preserved. Sweet oil produced	27.9	sweet
3.	0.1% Formaldehyde in 10% brine	do. do.	29.2	sweet
4.	0.1% Sodium nitrite in 3% brine	do. do.	28.0	sweet
5.	0.1% Sodium nitrite in 10% brine		29.7	sweet
6.	0.1% Sodium nitrite plus 0.1% Hydroquinone in 3% brine	do. do.	29.9	sweet
7.	1% Borax in 3% brine	Much better than No. 1	29.8	slightly rancid
8.	0.1% Borax in 3% brine	Much worse than No. 1	28.6	rancid
9.	0.5% Sodium Sulphite in 3% brine	Decayed	28.0	rancid
10.	0.5% Sodium Sulphite in 10% brine	Better than 9, same as 7	28.3	slightly rancid
11.	0.5% Sulphite plus 0.1% Butyl Gallate in 3% brine	Decayed, same as 12	27.7	very rancid
12.	Liver unpreserved, room temp.	Very badly decayed	29.6	very rancid
13.	Liver unpreserved, stored at 0°C	Slightly poorer than 2, 3, 4 & 5	28.3	sweet
14	Oil extracted immediately (blank)		28.5	sweet

Since it is being concluded that formalin is an excellent preservative for fish livers, the effect of formaldehyde on vitamin A has been investigated. Also, since tin has been claimed to be a very suitable material for the construction of fish liver oil processing plant (see this chapter, (b) - Recovery of oil from liver) the effect of formaldehyde on tin was determined. The results are given in Tables 17 and 18 respectively:-

Table 17.

Effect of formaldehyde on vitamin A.

No.	Test.	% loss of vitamin A after 6 days.
1	Sample of liver oil ($E^{1\%}_{1cm}$ 40) with equal volume of commercial formalin (40%). Mixture shaken up well, twice a day	11
2	Sample of same oil mixed with equal volume of 5% formaldehyde solution, treated as No. 1	2.2
3	Sample of oil mixed with equal volume of 2½% formaldehyde solution, treated as No. 1	0
4	Duplicate of No. 2	0

Air was excluded from these tests, so that no oxidation could take place to mar the results. It is apparent that if an equal volume of 5% formaldehyde does not cause destruction of vitamin A, it is most improbable that the low concentrations used in preparing germicidal sprays and solutions, can affect the potency of any liver oil. Contact of six days was allowed because that is normally the maximum period of storage of liver prior to processing.

The effect of a concentrated solution of formaldehyde on tin was determined by submerging a piece of tin in commercial 40% formalin, weighing the tin at intervals, and watching for any signs of reaction. After two months no detectable changes could be noticed. The formalin solution did not develop colour, as happens when iron is

...../left

left in contact with it, and no signs of corrosion could be detected on the surface of the metal. The variation in weight of the tin is tabulated in Table 18.

Table 18.

Decrease in weight of tin left in contact with 40% formalin solution.

Date	wt. of metal	Loss of wt. (%)
27/11/1944	6.5730	-
1/12/1944	6.5733	-
18/12/1944	6.5724	0.01
20/1/1944	6.5720	0.015

In practice such concentrated solutions will never be used and period of contact will be much shorter so that the corrosive effect of formaldehyde on tin may be considered negligible.

A further point about pre-treatment of liver that has been found important in this investigation is that the quality of oil is greatly improved by removing the gall bladder and rinsing the whole liver in sea water before adding preservative. If this simple procedure is carried out, the livers are never slimy and can be preserved in a much better condition.

(b) Recovery of oil from liver.

Before discussing the methods of recovery, it must be emphasised that absolute cleanliness of all plant equipment is essential[49]. Secondly, the influence of metals on the oil, and particularly with reference to destruction of vitamin A, is important in selecting the materials for plant construction. Many writers[57, 58] have described the pro-oxidative effect of various metals. In Table 19 the rate of destruction of vitamin A is indicated when samples of the same liver oil were heated in contact with various metals at 100°C. The oils were

...../stirred

stirred slowly and in each case 100 ml. of oil were placed in contact with 1 square inch of well-cleaned and dried metal. Light was excluded from all samples, but they were open to the atmosphere.

Table 19.

Destruction of vitamin A, during contact with various metals at 100°C.

Metal	% Vitamin A destroyed.	
	After 6 hours	After 18 hours
Lead	24.6	48
Iron	20	46.6
Copper	20	43.6
Aluminium	17	38.5
Chrome nickel steel	12.1	27.1
Chrome steel	9	22.6
Tin	5	27.5
No metal (blank in glass)	4	26

From these figures it appears that tin and stainless steel are the least active, being no worse than glass, the material of construction of the container used in all these tests.

If stainless steel and tin appear to be very suitable metals for construction of liver oil plant, particularly digestion vessels, the action of hot caustic soda solutions on them must be determined. Normally the highest concentration of caustic soda in the digester is 2%, but for this purpose, carefully cleaned and weighed strips of the various metals were immersed in boiling 10% caustic soda, and the loss of weight determined after five hours. The results are given in table 20.

...../Table 20

Table 20

Loss of weight of various metals, boiled for 5 hours in 10% aqueous caustic soda.

Metal	% loss of weight
Lead	0.75
Copper	0.214
Mild steel	0.034
Chrome Steel	0.0275
Chrome Nickel Steel	0.008
Tin	0.0

The rather unusual inactivity of tin may possibly be explained by virtue of its position in the electrochemical series. Tin has a relatively low negative electrode potential, and in addition has a high overvoltage[183]. It must be borne in mind that steam was escaping from the boiling caustic soda solution all the time, so that no air could enter.

These results indicate that tin is a suitable metal for constructing liver digestion vessels.

In the following experiement, aliquots of the same sample of liver were processed in different ways, and the destruction of vitamin A determined upon storage at room temperature for six months.

Table 21.

Storage loss of vitamin A, in oils processed as follows:-

A. Liver digested in glass and oil stored in glass.

B. Liver digested in old and pitted mild steel vessel that had been cleaned well. Oil stored in glass container.

C. Liver digested in same container as B, and stored in contact with iron.

	Date	Sample A	Sample B	Sample C
$E^{1\%}_{1cm}$	11/10/1944	71.7	70.1	70.1
"	27/10/1944	69.7	69.1	67.4
"	7/11/44	69.5	68.7	65.7
"	18/12/44	68.2	66.2	51.8
"	10/1/45	68	66	48.3
"	9/2/45	67.5	65.1	45.1
"	11/3/45	67.1	64.2	43.3
"	12/4/45	66.7	64.05	42.01
Total loss		7	10.8	41.3

Samples B and C soon developed colour and odour, while A changed very little, if at all. After six months B was dark yellow and rancid, while C was dark brown and foul in odour.

This is perhaps an exaggerated test, but it does indicate the danger of processing liver or storing oil in old and corroded iron containers, and the advisability of using stainless steel, tin, or tinned steel for storage and processing of liver and storage of oil.

There are several methods for extracting the oil from the livers[55]. Some of these are very laborious and require intricate plant. The only methods used in South Africa are solvent extraction for material of low oil content, and the alkali-steam digestion process followed by centrifugal separation of the oil, which is the only method used for processing shark livers. The liver is minced and diluted with double the quantity of water, containing 1% of caustic soda. The mass is heated to boiling with live steam, boiled for half an hour and passed through a centrifugal separator while hot. Clear oil is usually obtained directly, but occasionally the oil contains small amounts of moisture and soap, and a second passage through the centrifuge is necessary. Occasionally frothing occurs during steaming, or else the mass tends to emulsify during separation, so that thick water-in-oil emulsions emerge from the centrifuge. Both these effects have been overcome in the present investigation by a slight but important modification of the alkali-steam digestion process. The mixture of liver and water (1:2) is brought to boiling point with live steam. Caustic soda, to the extent of 2% of the weight of liver is added after about 10 minutes, to the gently boiling mass, either as dry flakes or as a 20% aqueous solution. Boiling is continued for 15 minutes and the

...../mixture

mixture is fed into the centrifuge. This procedure has never produced frothing or emulsification. It has also been the author's experience that steam should be passed into the liver-water mixture through one or two relatively large apertures, and that a steam coil with a multitude of small openings, tends to cause emulsification.

The alkali-steam digestion process has been demonstrated by Rapson et al.[59] to ensure full recovery of the oil and vitamin A.

The process yields a clear lemon-yellow oil, prime in every respect if all precautions are taken in handling the livers properly and keeping the equipment clean.

The alkali digestion process has only been in use for a few years, but all reports state that it is becoming more popular all over the world. An interesting development[61] is its use for extracting avocado oil.

Different concentrations of caustic soda and different periods of digestion have been tried, and the resulting oil yields and potencies compared. The stabilities of all the recovered samples of oil were determined, since it has been claimed[60] that rendering and refining methods destroy the natural antioxidants of oils.

% Caustic soda denotes percentage of the weight of liver.

Table 22.

The effect of concentration of caustic soda and period of steaming on yield of oil, potency and stability (Induction period).

No.	Details of process	% oil	$E^{1\%}_{1cm}$	I.P.
1	1% NaOH) Digest for	48.6	19.71	3 hrs.
2	2% ") 30 minutes	49	20.25	3.5 hrs.
3	5% ")	48.75	20.34	3 hrs.
4	2% NaOH Digest for 15 minutes	49.7	20.03	3 "
5	2% NaOH Digest for 1 hour	48.2	20.14	3.5 hrs.
6	Liver dessicated with sodium sulphate and oil extracted with ether	49.35	19.61	3.5 "

...../Sample 1

Sample 1 gave some trouble. It was definitely not digested properly and frothed very much during aeration when the induction period was determined.

These results seem to indicate that ½% caustic soda is insufficient, and that more than 2% is superfluous, they also indicate that steaming for more than 15 minutes is unnecessary (measured from the time that the mass reaches boiling point) while steaming for up to an hour causes no apparent harm. It is furthermore noticeable that the dessicated and solvent extracted sample had a lower potency than the digested samples and corresponded with the poorly digested sample 1. From a consideration of the induction periods it looks as if the natural antioxidants of fish liver are not of such a nature that they are destroyed by dilute hot caustic soda solutions.

IV. REFINING OF PRIMARY OILS.

The main object in refining of oils, whether vegetable or animal, is to improve the appearance, taste and odour. A clear distinction must be drawn between characteristic odour and flavour, and rancidity caused through spoilage. In fish oils the characteristic odour is very pronounced, and is not permanently removable[70] by even the most drastic steam deodorisation. According to Davies[71] fishiness is associated with the presence in the oil of nitrogenous compounds and highly unsaturated glycerides, and results from chemical combination of the two during oxidation of the glycerides, which reaction can never be inhibited completely. The characteristic fishy odour is not objectionable but develops very quickly into more rancid odours, due to oxidation and/or other causes. In most cases it is relatively easy to tell the difference between rancid and non-rancid oils or fats by smell, and the term "sweet" is generally used to denote the latter class. The object of refining of fish oils is therefore to remove the rancidity or odoriferous materials in excess of that which may be considered to cause the characteristic odour.

A second objective is the removal of colour, which usually develops in rancidifying oils, while a final object sometimes is to produce a clear oil containing no "stearin" i.e. the solid or semi-solid particles of fat that separate from an oil upon cooling. The most important methods of refining will now be described briefly.

(a) Use of Alkalis.

Of all the methods used for refining vegetable and animal oils, the use of alkalis is most common. A comparison of prevailing methods is given by Manderstam[62], Brocklesby[63] and many others. There is no detailed description of a generally applicable procedure, since

...../experience

experience, and the characteristics of the oil under consideration, will determine the details of operation. Jamieson[64] states: "Caustic soda refining is an art that can be acquired only through experience ....."

Caustic soda and lime are the two most widely used alkalis. Caustic soda is usually used as a 12 - 20% aqueous solution, depending on the amount of free acidity in the oil to be refined. Some commercial producers of vegetable oils still use the "floating potato" standard to determine the strength of the caustic soda solution, and this is indicative of the haphazard way in which this process is being carried out. The caustic soda is usually added to the oil, which is heated by closed steam coils, at a temperature of 24 - 30°C, the oil being stirred gently. After 10 - 30 minutes the oil is heated to 43 - 49°C, and the aqueous soapy layer separates out[64]. The oil may be washed with warm water and dried.

This general procedure was tried on fish liver oils, using caustic soda, sodium carbonate and lime. The results were discouraging and no improvement could be detected. This is probably due to the fact that fish liver oils, produced by alkali digestion of the livers, contain only small amounts of free acid, while in vegetable oils, often containing very high percentages of rancid free acids, the improvement is much more noticeable.

Various modifications of the alkali method were tried out and eventually the following method was adopted and found to give very good results. Again it must be emphasised that this is a general method and the plant operater will find by experience how to modify it to the best advantage in treating individual oils. It should be pointed out that refining becomes necessary due to the fact that rancid and highly coloured primary liver oils are often produced from liver that had been stored for

...../long periods

long periods or from badly preserved material.

The method as developed then consists of heating the oil with live steam to 100°C, at which stage 1% of caustic soda, as a 20% aqueous solution, is added. Steaming is continued, keeping the oil in rapid motion by escaping steam, for 15 - 30 minutes. The steam is now shut off and the aqueous layer of soap and excess alkali settles to the bottom, and is tapped off as completely as possible. The volume of caustic soda solution added is known, so that approximately the same volume is drained off. This alkaline drainage is not discarded, but run to the liver digestion tanks, and put to good advantage. The steam is turned on again, and when the oil reaches a temperature of 100°C, an equal quantity of boiling water is added, and steaming continued for 10 - 15 minutes. If the acid content of the crude oil was not abnormally high, and if the soap-alkali layer had been removed completely, this aqueous-oil mixture is now ready to be passed through the centrifuge. No harm is done however, and no oil is lost, if the aqueous layer is run off and used for liver digestion and the oil steamed with a further quantity of boiling water, after which it can be fed into the centrifuge.

It has been found most essential to add the caustic soda solution to hot oil (100°C), and to add boiling water to the hot oil for washing. These are claimed as special features of this process, to ensure complete removal of free fatty acid and soap, and to prevent emulsification during processing or separation.

Sometimes oils with very high free acidity are encountered and it has been found advantageous to use ammonia (20 - 30% solution) in refining them. The danger of emulsification is then ruled out, but the ammonia, which is driven off by the steam, may cause tainting of

...../other oils

other oils in the vicinity. Moreover, ammonia does not give the bleaching effect obtained with caustic soda.

The final wash before separation of the oil can be given with a boiling 1% solution of common salt. This procedure has been found to result in easier separation of the oil. However, the normal method described above, when properly carried out, has given such satisfactory results, that the brine wash may be omitted in most cases.

The efficiency of this process, as in any refining process, can be judged by considering:

(i) loss of vitamin A and loss of material;
(ii) degree of refining obtained;
(iii) palatability of refined product;
(iv) expense and trouble involved;
(v) stability of refined product.

In Table 23, analytical figures will be given for a few alkali-refined oils to illustrate points (i), (ii) and (iii).

Table 23

Analysis of liver oils before and after alkali-refining.

Material		$E^{1\%}_{1cm}$	Peroxide value	Free fatty acid (% oleic)	Odour	Colour
Liver oil 1	Before	9.91	1.6	0.51	foul	very dark
	After	9.96	0	0.003	sweet	light yellow
Liver oil 2	Before	8.91	1.45	0.81	bad	brownish
	After	8.90	0	0.002	sweet	yellow
Liver oil 3	Before	15.63	0.93	0.03	bad	dark yellow
	After	15.60	0	0.001	sweet	pale yellow
Liver oil 4	Before	20.95	1.16	1.06	very rancid	brown
	After	20.92	0	0.01	sweet	yellow
Liver oil 5	Before	25.26	2.4	0.56	bad	dark
	After	25.36	0	0.003	sweet	lemon
Liver oil 6	Before	11.43	1.62	0.92	rancid	dark yellow
	After	11.27	0	0.004	sweet	lemon

...../It is apparent

It is apparent that there is no loss of vitamin A. Oil recovery is 99 - 100% depending on free acid content. The degree of refining obtainable is remarkable, yet difficult to indicate by actual figures. Improvement in taste and smell is very distinct and sweet oils, with only the characteristic fishy odour, but not at all rancid, are produced. Bleaching is effected to a considerable extent. The alkali-soap layer is usually very dark brown, often black, indicating the extent to which colour bodies, and possibly polymerised adehydes and ketones, are removed. It is readily understandable why this refining method should produce such good results. During the digestion of livers, the oil is liberated but remains in contact with large amounts of nitrogenous and odoriferous degradation materials, and it is to be expected that some of these will dissolve in, or be separated with the oil. During alkali-refining, a relatively pure material is being treated, with much more hope of removing trace constituents that cause rancid flavours. The caustic soda is present in more concentrated form, excess of wash water is used, and many impurities like acids, aldehydes or ketones, which are steam-volatile, are driven off. Several of these alkali-refined samples of oil have been stored for a period of 18 months and have shown no colour or odour reversion and can definitely be classified as first class after such long storage.

Additional features of this process, are firstly its inexpensiveness, as even the caustic soda-soap effluent is re-used, the only additional cost being that incurred by re-separation of the oil, and secondly the fact that no additional equipment is required. Any liver oil factory equipped for alkali-digestion of liver, can practice alkali-refining on poor quality oils, using the same equipment.

...../In order

In order to prove that alkali-refining does not hydrolyse the vitamin A, estimations of vitamin A alcohol and esterified vitamin A were carried out on several of these oils, according to the method of Reed et al.[65] The following results illustrate that the vitamin is not hydrolysed under these conditions.

Table 24

Ratio of free and esterified vitamin A in primary and alkali-refined liver oils.

Material		% Vit. A present as ester	% Vit. A present as alcohol
Oil 1	(Primary	96.0	4.0
	(Refined	95.5	4.5
Oil 2	(Primary	96.3	3.7
	(Refined	96.6	3.4
Oil 3	(Primary	97.5	2.5
	(Refined	97.0	3.0

Disadvantage of alkali-refining process:

In accordance with earlier observations[66], it has been noticed that alkali-refined oils exhibit delayed "stearin" deposition. The delay is from 2 - 7 days. After that however, the rate of "stearin" deposition is rapid and complete, and leaves a very clear top layer of oil, which may be decanted, should a clear oil be required for specific purposes.

(b) Use of bleaching agents.

Since vitamin A is very susceptible to oxidation, bleaching by chemical means, as practised with vegetable or animal oils and fats, are out of the question. The use of nascent chlorine[68] is ruled out by the high degree of unsaturation of fish oils in general. The use of peroxides, sodium and potassium dichromates, air etc., widely used[67, 69] in decolourising vegetable fats, are ruled out because of the danger of oxidising vitamin A.

Physical methods, i.e. absorption of colour bodies and/or odoriferous substances, are described in many

...../publications

publications[72, 73], and have been used mainly in refining vegetable and mineral oils. Activated carbons and natural or activated bleaching earths are commonly used. The optimum quantity of bleaching agent, time of contact, and temperature, vary with every oil, and must be determined by trial and error methods. Hinners et al.[74] state that few, if any, types of bleaching earths are suited equally well for refining all kinds of oil. A particular product may be very effective for a certain oil and at the same time ineffective for others.

Under these circumstances, the bleaching of fish liver oils could only be carried out by trial and error, using as many different agents as possible and noting the results obtained. Colour comparison of untreated and treated products becomes a very difficult matter, as fish oils fall in the class of materials containing varying amounts of red, yellow and green pigments, which fact makes it impossible[75] to obtain a satisfactory match by the Lovibond method, the usual method of matching colours of oils. The pigmentation of fish liver oils depends on such factors as the presence of carotenoid pigments, method of processing, method of liver preservation, presence of metals etc.

It was decided to carry out a few tests with available bleaching agents and earths, found suitable for mineral oils. The tests were carried out according to the official method of the American Oil Chemists' Society[76], in order to obtain uniformity. The figures in Table 25 provide a summary of the results obtained. Activated Bentonite was prepared by boiling with concentrated hydrocloric acid for six hours. In order to minimise the danger of oxidation by the large volumes of air usually absorbed on active carbons and earths, these materials were pre-treated by repeatedly evacuating at

...../100°C

100°C and admitting carbon dioxide. By this means it was hoped to replace most of the air by carbon dioxide. Oil, similarly de-aerated, was then added to the bleaching agent, and a layer of carbon dioxide was kept on the surface of the mixture.

The results obtained were judged mainly by detectable improvement in colour and odour, while a careful check was kept on the vitamin potency.

Discussion (Table 25).

As far as the carbons are concerned , good decolourisation and improvement in odour were obtained with Merck's Weinkohle, Clarocarbon and animal charcoal, but serious losses of vitamin A resulted ranging from 4 to 8%. Blood charcoal and animal charcoal did not decolourise at all and oxidised vitamin A so rapidly that they were ruled out completely. Carbon has previously been quoted[72] as having a tendency to cause oxidation of the oil to which it is added. The amount of colour removed by the first three agents (Table 25), although not measured quantitatively, is remarkable, but in view of the destruction of vitamin A, in spite of most careful precautions, the method is not favoured. Such elaborate precautions would be unpractical on plant scale.

As far as the earths are concerned, fair decolourisation resulted from the use of bentonite (straight and activated), colonial clay and kieselguhr, but "retrol", the bleaching agent par excellence for mineral oils, produced no visible bleaching of a dark liver oil, and caused very rapid destruction of vitamin A. On the whole, smaller losses of potency resulted from active earth bleaching, than from carbon treatment. Both methods involve a filtration operation after treatment, and as this is always a costly and troublesome procedure, the results obtainable should be encouraging enough to warrant

...../the extra

Table 25

Loss of vitamin A during refining of liver oils by bleaching, steam-vacuum deodoristion and alkali-refining.

No.	Method of refining	% Loss of vitamin A	
		Oil A ($E^{1\%}_{1cm}$=10)	Oil B ($E^{1\%}_{1cm}$=22)
	(a) Decolourisation:		
1	(i) Merck's Weinkohle 1%	5.3	6.8
2	(ii) Clarocarbon Merck C 1%	6.5	7.95
3	(iii) Animal charcoal 1%	5.16	4.14
4	(iv) Blood charcoal 1%	36.3	42.5
5	(v) Nuchar C 1%	41.6	57.8
6	(vi) Clarocarbon Merck C 2%	6.0	8.10
7	(vii) Bentonite clay 5%	0	0
8	(viii) Colonial clay 5%	4.30	4.64
9	(ix) Kieselguhr B.D.H. 5%	-	2.1
10	(x) Kieselguhr 5% plus animal charcoal 1%	2.0	3.66
11	(xi) Diatomite Merck 5%	1.9	3.1
12	* (xii) "Retrol" clay 5%	49.8	63.5
13	(xiii) Acid activated Bentonite 5%	6.32	4.8
	(b) Steam-vacuum deodorisation		
14	(i) 200°C for 2 hours	5.0	5.58
15	(ii) 200°C for 30 minutes	5.1	5.1
	(c) Alkali-refining (new method)		
16	(i) -	0	1.1
17	(ii) -	0	0
18	(iii) -	0.2	0.5

* Commercial product extensively used for refining mineral oil.

the extra expense and trouble. In these experiments it has not been the case, and the author does not consider bleaching methods worth while as far as fish liver oils are concerned.

(c) Steam-vacuum deodorisation:

This method of refining is usually an essential step in vegetable oil production[64, 72], whether other methods of refining are used or not.

The basic principle is the passage of a fine steam of superheated steam through the oil at normal or reduced pressure. Andrews[77] recommends a temperature of 200°C and a vacuum of less than ½" of mercury.

This method has been recommended[78] for refining cod liver oil, when steam, superheated to 200°C, is passed into the oil which is contained under a few m.m. pressure, in iron, aluminium, or tinnediron deodorisers, but Hilditch[79] states that this procedure results in destruction of vitamins.

Several attempts have been made to deodorise shark liver oil in this way. Temperatures of 200 - 250°C were employed at a vacuum of 15 m.m. mercury. Without exception, the oils darkened upon treatment. This was at first thought to be due to charring, but an experienced vegetable oil producer stated that the same phenomenon is often experienced with some vegetable and animal oils, and in such a case the steam-vacuum deodorisation should be followed by a bleaching step.

In addition to darkening of the oil, a more or less constant loss of vitamin, to the extent of 5%, was experienced (Table 25) and this fact alone makes one very dubious about the applicability of this process to fish liver oils.

...../(d) Cold-clearing

(d) Cold-clearing of fish liver oils.

It is often necessary to produce medicinal liver oils that will not deposit "stearin" or solid particles at certain specified temperatures. The reason for this is difficult to understand, unless appearance is regarded as very important.

Cold-clearing involves cooling the oil at the most suitable rate, to cause all the solids to deposit, and removing the solids by filtration.

Rate of crystal formation, and all the factors influencing the rate of deposition of solids, are complex problems, commanding the attention of many research workers all over the world.

The author has not made a careful study of this problem, but has noticed that the rate of stearin formation is influenced by such factors as:

(i) Type of preservative used with the liver. In other words, to what extent the formation of degradation products, which may influence stearin formation, is inhibited.

(ii) Method of processing.

(iii) Refining methods.

(iv) Presence of soap particles in the oil.

(v) Presence of metals.

(vi) Presence of other foreign bodies e.g. added antioxidants.

In general it has been noticed that the oils from well preserved liver samples deposited "stearin" more rapidly and more completely (leaving a clear top layer with no specks) than oils from poorly preserved samples. This phenomenon was clearly observed in the samples described in Table 25.

Alkali-refining causes inhibition of stearin deposition, but once crystal formation sets in, the deposition

...../is rapid

is rapid and complete. This agrees with the findings of Brocklesby and Denstedt[81] that alkali-refining greatly increases size and degree of perfection of the crystals formed.

As regards antioxidants it was found that the rate of stearin formation and settling was much greater in samples protected with hydroquinone than in samples with butyl gallate and citric acid. In the samples of oil with hydroquinone, the rate of stearin deposition seemed to increase with concentration of antioxidant.

All metals seemed to retard stearin formation and settling, so that after three months the samples of oil stored in contact with metals were still cloudy and the stearin had not settled out completely. This happened during the months September to December, so that the continued cloudiness was not due to a progressive drop in atmospheric temperature.

The filtration of oil is standard procedure and presents no unusual problems. A small laboratory model of a standard pressure filter has been operated successfully, at pressures ranging from 20 - 50 pounds per square inch.

V. STORAGE AND STABILITY OF SHARK LIVER OILS.

In this chapter a review will be presented of the causes of rancidity in oils generally, methods of detecting and estimating degree of spoilage, measurement of the susceptibility of oils to oxidative rancidity, and finally experimental data will be given to indicate the influence of various factors on the stability of shark liver oils.

A. CAUSES OF RANCIDITY AND OXIDATION OF OILS.

The term rancidity is usually employed to denote deterioration in flavour and odour of fats and oils. It is generally accepted that deterioration can be caused by

(i) absorption of foreign odours;

(ii) enzymatic or microbiological action;

(iii) atmospheric oxidation;

or by combinations of these factors. By far the most important factor, from a scientific point of view, is oxidation, causing a type of spoilage known as "oxidative rancidity". To the manufacturers of food, and edible or industrial oils, oxidative rancidity spells disaster. Oxidative rancidity is usually accelerated by such factors as light, heat, metallic or other catalysts and these will be discussed in the following chapters.

The mechanism of autoxidation is not yet fully understood. Lea[12] gives an excellent review of the theories. In fish liver oils, oxidative rancidity or the products formed during autoxidation, cause serious losses of vitamin A. This has been proved by Lea[11] and forcibly confirmed by results obtained by the author (results in following chapter). In addition, rancid flavours caused by microbiological spoilage in the liver before processing, or by oxidation in the oil after processing, seriously affect the quality and value of liver oils to be used in food

...../fortification

fortification or medicinal preparations; in fact rancid fats may even be injurious to health[13].

Organoleptic tests alone are not sensitive enough to detect rancidity, especially in the early stages; moreover it is impossible to assign numerical values to the results of such tests. It becomes necessary then to make use of chemical methods.

Upon subjecting an oil to the presence of air or oxygen, there is usually a period during which no detectable reaction occurs. This is called the Induction Period.(I.P.) After this, autoxidation sets in and reaction or decomposition products are formed which give rise to phenomena that denote spoilage. The problem of measuring the stability of an oil, or its resistance to oxidation, is resolved into finding a method of measuring the induction period i.e. chemical or physical means of detecting reaction products formed immediately after the end of the induction period, or means of detecting the point at which oxygen is being rapidly absorbed. The induction period then affords some means of assessing the relative stability of the oil.

The normal rate of interaction of a fat and oxygen is usually too slow to provide rapid results, often required to assist production or to evaluate any product. Most workers therefore resort to some or other means of accelerating this reaction. Acceleration can be produced by using pure oxygen instead of air, or by increasing the temperature of the reaction, or by using pressures greater than atmospheric, or by making use of the catalytic effects of light or metals. Such an accelerated oxidation should not depart too drastically from ordinary conditions, but on the other hand it should provide a rapid and workable control method for assessing the relative stabilities of oils. Likewise the method for detecting the formation of breakdown products, which indicate the end of the induction, should be rapid, and yet as reliable as possible.

...../B.

B. DETECTION AND ESTIMATION OF OXIDATIVE RANCIDITY AND RELATION BETWEEN CHEMICAL OR PHYSICAL TESTS AND RANCIDITY.

During the autoxidation of a fat the iodine value drops[14] and this has been used as a means of detecting the end of the induction period. The author's experience (Table 29) has been that an advanced state of deterioration, from the point of destruction of vitamin A, is reached before a significant drop in I.V. occurs. Moreover the determination of I.V. is lengthy and cumbersome.

Chirgwin[15] and other workers have shown that changes in refractive index occurring during autoxidation, may be used as a means of determining degree of deterioration. The changes are not significant enough, however, to make the method attractive.

The aldehydes produced at the onset of rancidity can be indicated by the Schiff test, or by the Schipsted method[16] or can be estimated by direct titration with sodium bisulphite[17].

The estimation of peroxidic compounds forms the basis of the most common tests for oxidative spoilage. All theories of autoxidation of fats postulate the primary formation of peroxides[18, 19] and many workers have attempted to make the best possible use of the estimation of peroxides as a measure of the degree of rancidity[20, 21, 24], and rapid peroxide formation as an indication of the end of the induction period. The author's experiments (Table 28) have shown how well the rate of peroxide formation indicates the end of the induction period. Perhaps the best review of relationship between peroxide formation and deterioration is that given by Kerr and Sorber[22], who state inter alia that during oxidation there is development of free acid first of all, then a drop in free acid, then a drop in iodine value, an increase in non-sap., and then fixation of oxygen in peroxidic form.

...../Peroxide

Peroxide value is very conveniently determined iodimetrically by Wheeler's method[23] and peroxide content expressed as "ml. 0.002N thiosulphate per gram of oil" also termed "millimoles of peroxide per kilogram of oil". The method is easy to carry out, requires no special apparatus and has given consistently reproducable results.

Another method which, however, appears to be out of fashion, is the modified Kreis test[25], where a benzene solution of the oil is shaken with conc. hydrochloric acid. A solution of phloroglucinol is added, and a pink or red colour taken to denote rancidity. The colour is matched in a Lovibond tintometer, and some quantitative comparisons are obtainable.

C. MEASUREMENT OF THE SUSCEPTIBILITY OF OILS TO OXIDATION.

(i) General:

As stated earlier, the rate of oxidation of oils and fats is normally so slow that many attempts have been made to devise methods which would permit rapid measurement of resistance, and give relative, if not absolute, values. Basically all methods are similar in that oxidation is accelerated under carefully controlled conditions. Most usual method of acceleration is to increase the temperature in an atmosphere of air or oxygen. The progress of oxidation is then followed by measurement of the oxygen absorbed or by estimation of the products of reaction. The two phases should be in equilibrium and this can be attained by saturating the oil with gas by stirring, or by bubbling the gas through the liquid, or by dispersion of the oil in a thin layer. It is essential to observe certain precautions in all methods. The most important of these is that glass apparatus should be scrupulously clean and that the temperature should be maintained at

...../a constant

a constant level throughout the experiment. The author has found the following cleaning procedure efficient:-

Rinse all glass apparatus with acetone to dissolve the oil. Wash with boiling 20% caustic soda solution. Rinse and wash with soap and hot water, using a bottle brush. Rinse and wash with boiling chromic acid solution. Rinse well and leave full of water overnight. Rinse well next morning, dry with acetone and finally blow out with hot air to remove all traces of acetone. The following result indicates the effect of traces of oxidised oil on stability test and also the danger of spoiling a good oil by admixture with low-stability oils.

Table 26.

Effect of traces of oxidised oil on stability of fresh oil.

Material	Induction period by swift stability test.
Fresh liver oil	$3\frac{1}{2}$ hours
Same oil plus 1% of oil oxidised to peroxide value of 100	$1\frac{3}{4}$ hours

(ii) Summary of most important accelerated oxidation methods:

In the Schaal method[26], a definite amount of oil is incubated in a standard glass container in a hot-air oven at a constant temperature, usually 60°C. Rancidity is detected by smell, or by peroxide value determinations. This method is criticised by Beadle[27] on the grounds that it is tedious and involves large personal errors. Nor could consistent results for the same oil be obtained in this study.

The second important method is the oxygen-absorption method, described by French et al.[28] of which

...../there are many

there are many variations. Basically it consists of maintaining the oil under oxygen pressure at elevated temperature, usually 70°C, until the pressure drops, indicating that oxygen is being absorbed rapidly. The objection[27] to this method is that it is cumbersome to perform and that decomposition products which are not removed, may interfere.

The most widely used accelerated method is the active-oxygen or Swift Stability test[29], as modified[30, 31]. Basically the method consists of passing air at a constant rate through samples of oil which are maintained at a constant temperature, usually 100°C. Several samples of the same oil are started at regular intervals, and when the first sample has become distinctly rancid, all the samples are removed and their peroxide values determined. The point at which rancidity set in can thus be determined. This has been found a suitable method, easy to operate, is rapid, and yields reproducable results.

The Swift stability test, comprising the estimation of peroxides while aerating at 100°C, has been used[34] during the recent war in Great Britain by the team of workers belonging to the Food Investigation Staff of the Department of Scientific and Industrial Research, and in part to the Hannah Dairy Research Institute in Scotland. This team incidentally tried out a new variation of the oxygen-absorption method, using haematin as an accelerator. The Swift test is also recognised by the American Oil Chemists' Society[35], and used extensively in the United States.

Many prominent workers have expressed grave misgivings against all accelerated tests to determine susceptibility to oxidation. Hilditch[32] states: "I believe that the use of accelerated tests at higher

...../temperatures

temperatures e.g. 100°C will increasingly come to be regarded as not wholly indicative of what goes on at the atmospheric temperature. They are so much more convenient owing to rapidity, however, that I fancy they will be accepted for a long time as a rough guide or first appxoimation". Beadle[27] also objects on the grounds that the air bubbled through the samples creates conditions which are drastically different from those of ordinary storage. As against this, Bickoff et al.[33] found agreement between stability tests on carotene in edible oil substrate at 25°C, 40° and 75°C. Lovern[34] correlated his 100°C stability tests with absorption of oxygen, bleaching of carotenoids, and development of tallowy odour and flavour. Corroborative tests were also run at 37°C. Riemenschneider[21] found general agreement between relative stability of lards determined by the King rapid method[29] and storage tests for up to 20 months in the dark at 21°C. In addition Riemenschneider et al.[36] found fair agreement between stability values obtained by the active-oxygen and oxygen-absorption methods, as indicated by protection factors of antioxidants.

(iii) Method used in this study:

The modified Swift stability test has been used, with minor modifications. This course was adopted after the method had been tried out well and the ease of operation and reproducability of results clearly proved. The Wheeler method for estimation of peroxides was used and found very convenient and rapid. Table 27 illustrates the duplication obtained. Aeration was carried out at 100°C.

The figures in Table 27 represent typical oils. Further reference will be made to this table later on. At this stage it is sufficient to note the agreement

...../obtained

TABLE 27

Peroxide formation in duplicated samples of oil.

No.		Peroxide value (ml. 0.002 N. Thio. per gram) at time (hours)										
		0	½	1	2	3	4	4½	5	6	7
1	Shark liver oil A)	1.4	6.1	7.2	11.8	13.4	24.1	-	123	120	167
2	do. (duplicate of 1))	1.3	6.4	7.3	12.3	13.0	23.1	-	130	123	167
3	do. (do.))	-	-	-	-	-	23.6	-	-	-	163
4	Shark liver oil B)	1.4	-	8.4	-	4.6	85.0	93			
5	do.)	1.1	-	7.8	-	24.1	83.6	90.8			
6	do.)	-	-					91.1			
7	Shark liver oil C)	0.95	5.3	8.9	13.3	16.1	18.1	33.6	77.3		
8	do.)	1.0	5.8	9.1	13.0	16.7	18.9	31.8	75.1		
9	do.)	1.0			14.0				79.3		
10	Shark liver oil D	1.6	6.3	7.7	11.6	23.2	43.1				
11	do.	1.5	6.6	8.1	12.2	22.8	46.6				
12	do.	1.5	6.6	7.0	10.7	22.4	48.5				
13	do.	1.5	6.5	7.3	12.4	20.2	45.0				

(a) Nos. 1, 2 and 3 connected in Series i.e. air bubbled through 1 into 2, then through 3.
(b) Nos. 4, 5, 6 were connected in Series.
(c) Nos. 7, 8, 9 connected in Series.
(d) Nos. 10, 11, 12, 13 connected in Series.

obtained in peroxide development in duplicate and triplicate samples during aeration at 100°C.

(iv) Description of modified Swift stability test:

The method employed will be described fully in order that it may be of assistance to others who may find it necessary to determine the relative stabilities of animal or vegetable oils and fats.

Approximately 100 gram samples of oil are placed in 200 ml. gas washing bottles with Jena fritted glass filter plates. (Gallenkamp catalogue d.6560). These bottles are immersed in a boiling water bath, so that the level of oil is below the level of water. The bottles pass through holes in the lid of the water bath and are wedged with cotton waste, so that practically no light enters the interior of the water bath. The row of bottles are connected in series, and air, washed by passage through a 0.1% aqueous solution of potassium permanganate, is drawn through the series of bottles by water-jest pump. The air is drawn in through a calibrated orifice at 2.5 ml. per second. The bath is maintained boiling gently, and at intervals the current of air is interrupted, and samples of about 5 grams each removed from the bottles, using a separate clean pipette for each sample. If the pipettes are used the wrong way round, the whole operation of drawing samples from four separate bottles, can be completed in half a minute or at the very most, one minute. Aeration is resumed, the samples cooled, and assayed immediately for peroxide content according to the Wheeler method. The results are available before the next sampling is due, and any bottle can be short-circuited as soon as the contents attains a peroxide value exceeding that found to indicate the end of the induction period. This figure is 25 for vitamin oils, as will be indicated later on. The sintered glass jet

...../in this

in this type of gas washing bottle distributes the air very finely and causes the sample of oil to be agitated well. Absolute cleanliness of apparatus, and careful temperature control are essential, and poor results can usually be traced to one of these factors.

A temperature of 100°C is used, firstly because it is easy to attain and control (boiling water), and secondly, for rapidity. Comparative aerations at 50° gave induction periods of 90 and 22 hours for oils that show 8 and 3 hours respectively at 100°C. Control becomes impossible if the I.P. exceeds 8 hours, i.e. the normal working day.

No attempt was made to dry the air, as it has been demonstrated clearly by several workers, notably Riemenschneider et al.[36], that little significant difference in stability values results when dry air is used instead of moist air.

Several modifications of the original Swift test have been incorporated in this accelerated method. Most important of these is the arrangement of samples in series, so that the same air passes through all. The advantages are simplicity of construction, and assurance that exactly the same amount of air is being passed through all samples. The main objection is that volatile products may pass from one sample to the next and affect the stability of the second sample. This possibility was investigated and it was found that four samples of the same oil, connected in series, gave almost identical peroxide values and stabilities upon aeration. It has been concluded that if the fourth sample is not affected by the combined volatile degradation products of the previous three, there is no danger in conducting the test in this way. The results in Table 27 illustrate this point rather well. These findings may throw some

...../light on

light on the controversial reports by Greenbank and Holm[41] and Roschen and Newton[42].

A second departure from the conventional procedure was the periodic interruption of the aeration for sampling. This procedure was necessitated by a serious shortage of gas washing bottles, but again the figures in Table 27 indicate that repeated interruptions (for very short periods) did not affect the results. Numbers 3, 6 and 9 represent oils that were only sampled once, or not until the end. The final peroxide values as well as intermediate figures, agree well with the results obtained for corresponding samples of oils where the aeration was constantly interrupted.

The effect of variations in the rate of flow of air through the oil has been checked, to confirm earlier observations[38] that large variations do not affect the rate of oxidation. The amount of air has been varied from 1 to 10 ml. per second without affecting the final result. In conducting this aeration test, it is therefore only necessary to pass a steady flow of air that will give enough bubbles to keep the oil in a good state of agitation.

(v) Criterion for indicating end of induction period:

While the method of accelerated oxidation has been described and defended, it now remains to define some means of correlating the peroxide value at some suitable level with end of induction period. In most of the published work, some suitable peroxide value, found organoleptically to co-incide with rancid taste or smell, has been accepted to indicate the end of the induction period. Lowen et al.[39] found that a peroxide value (P.V.) of 20 - 30 corresponds with a distinctly rancid odour. Riemenschneider[21] accepts a figure of 15 for lard. Findlay and Smith[40] decided on 20, 40, 60 and 80

...../milli-equivalents

FIG II A.

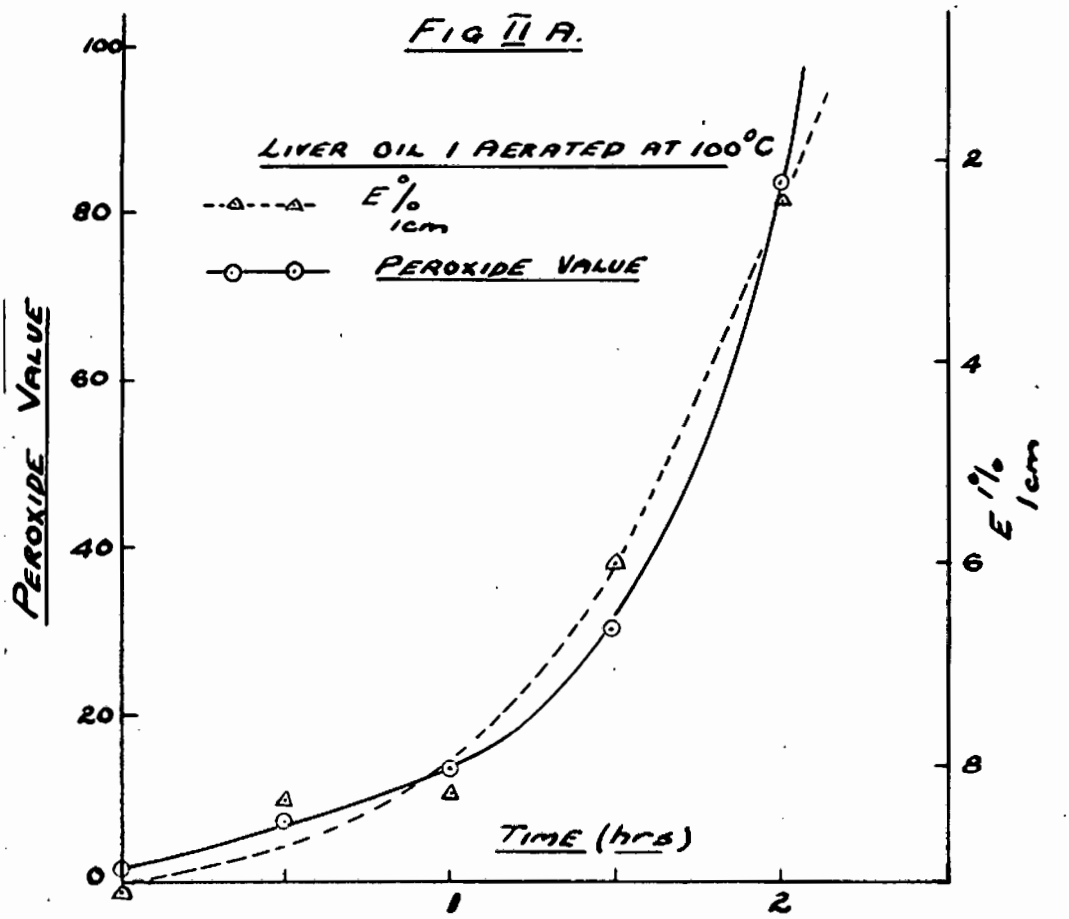
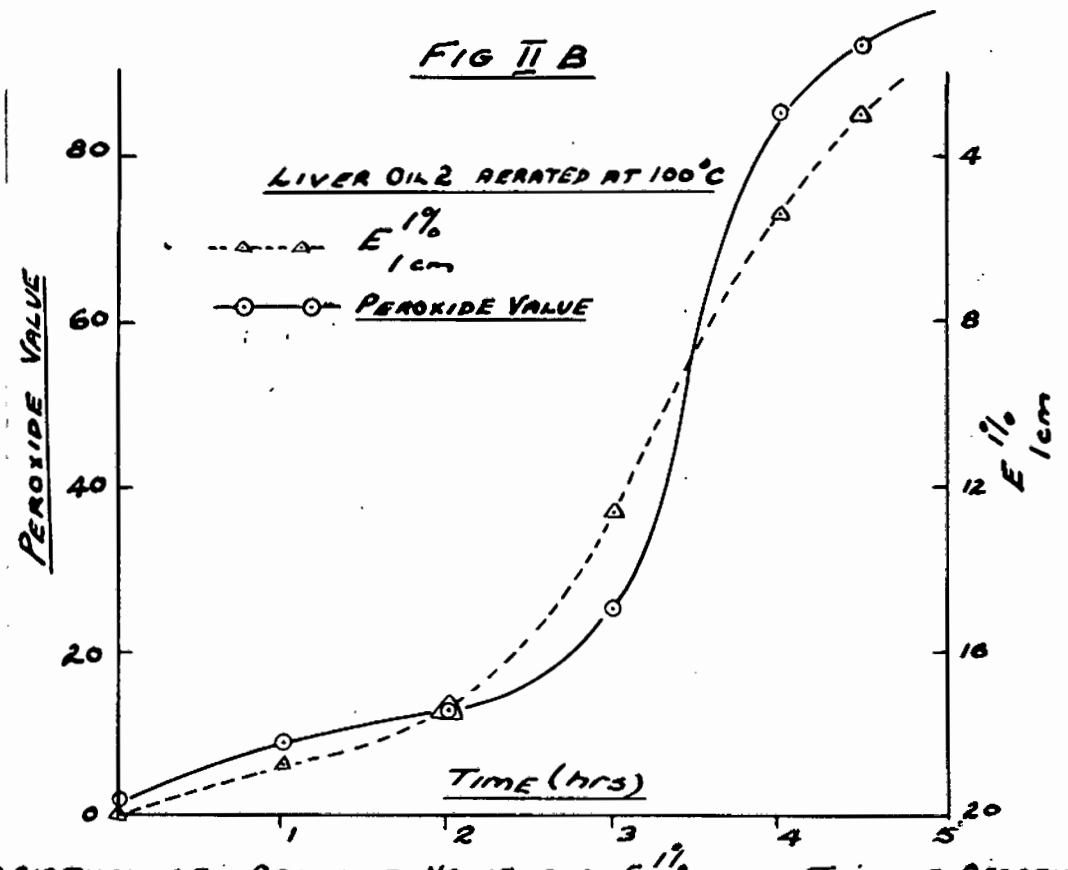


FIG II B



VARIATION OF PEROXIDE VALUE AND $E^{1\%}_{1cm}$ WITH TIME OF AERATION.

FIG II C

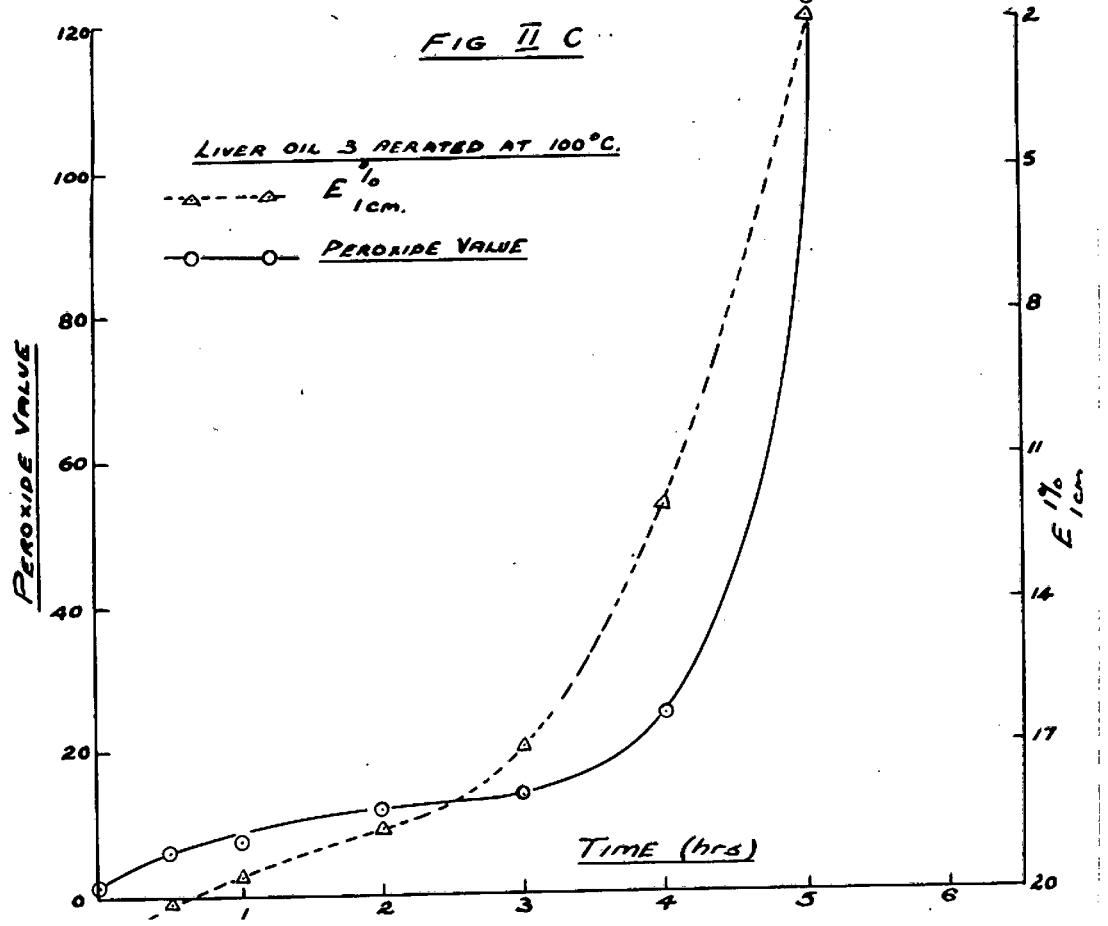
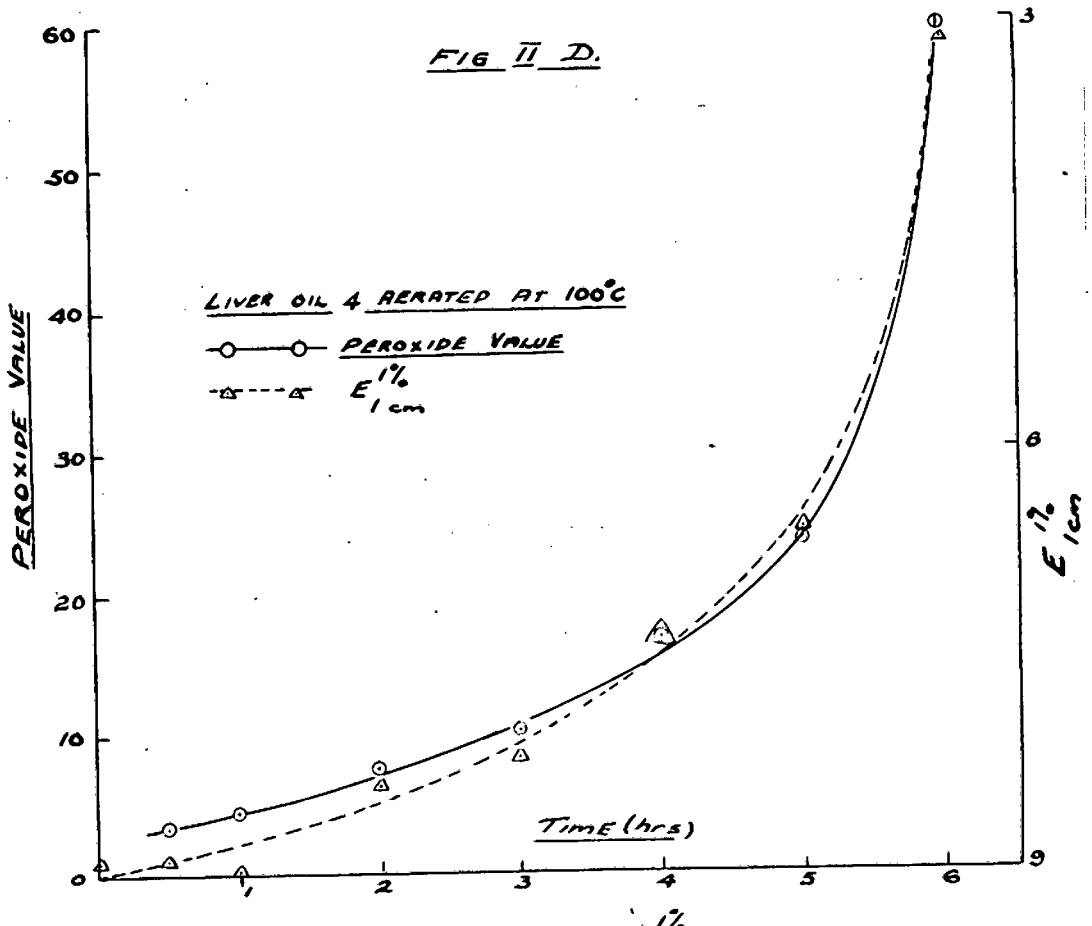


FIG II D.



VARIATION OF PEROXIDE VALUE AND $E^{1\%}_{1cm}$ WITH TIME OF AERATION.

FIG III A.

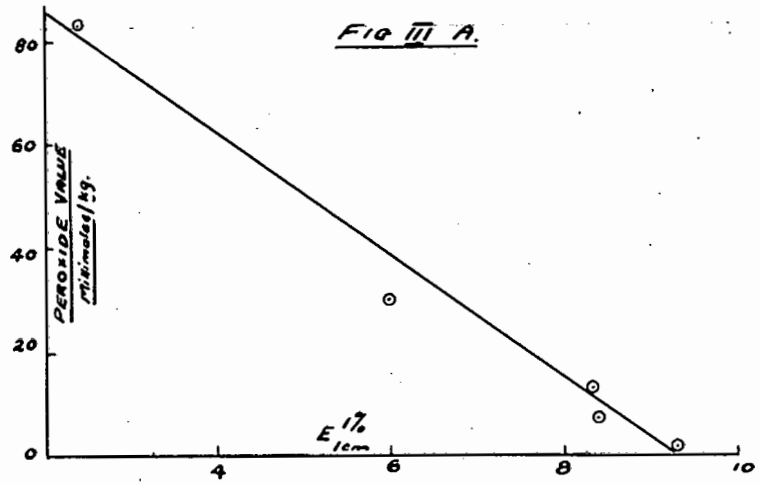


FIG III B

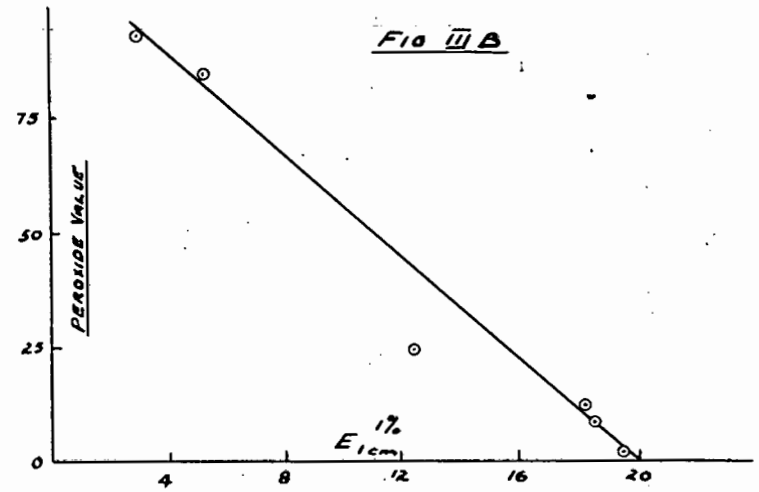


FIG III C

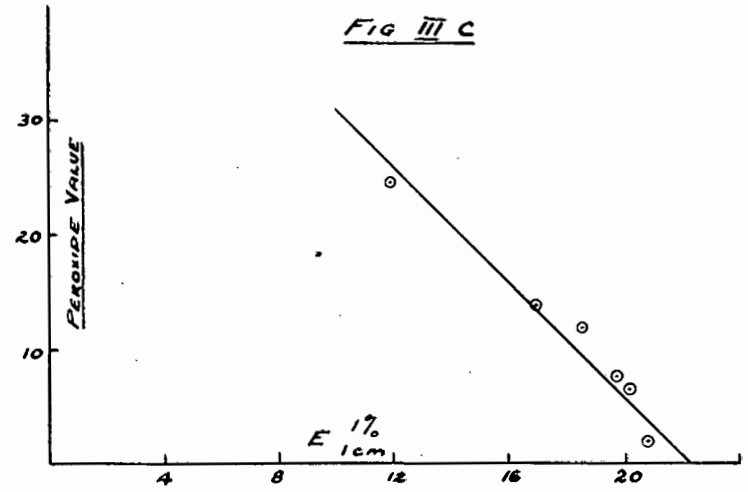
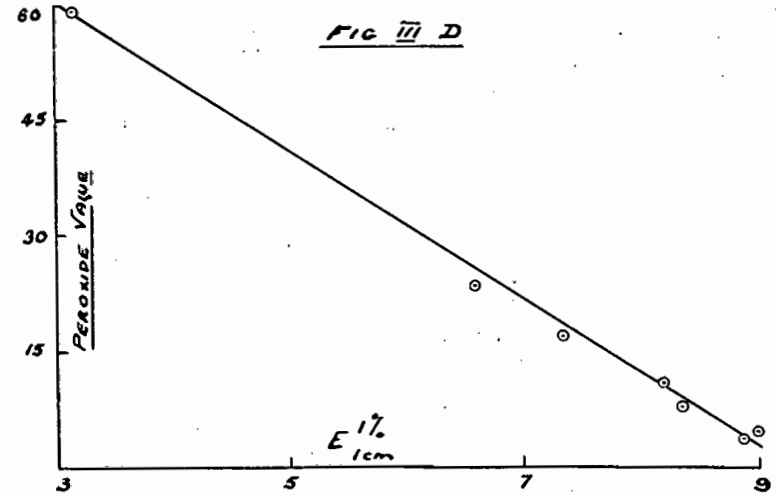


FIG III D



RELATIONSHIP BETWEEN VITAMIN A ($E^{1\%}_{1cm}$) AND PEROXIDE VALUE DURING AERATION AT 100°C

milli-equivalents per 100 g. as the end points for lard, hydrogenated lard, also oils and hydrogenated vegetable oils respectively.

In the examination of fish liver oils, organoleptic rancidity is not the main objection. Oxidative degradation results in loss of vitamin A, and such a peroxide value as will coincide with rapid destruction of vitamin A, will have to be regarded as indicative of the end of the induction period. The relationship between $E^{1\%}_{1cm}$ and P.V. is indicated in Table 28 while some of these examples are illustrated graphically in figures II and III.

From figure II, which illustrates the figures for the first four oils in Table 28 it is clear that the vitamin A curve follows the peroxide curve very closely. This indicates an almost linear relationship between loss of vitamin A and peroxide formation (vix. fig. III). These results agree with earlier work on oxidation of halibut liver oil[39] and cod liver oil[13], in both of which peroxide development is followed closely by loss of potency. It remains then to decide what peroxide value to regard as denoting the end of the induction period. From figure II and Table 28 it is apparent that development of peroxide and corresponding drop in potency proceed progressively but rather slowly, until a peroxide value of 20 - 30 is reached, after which the biggest increase in peroxide value, and decrease in $E^{1\%}_{1cm}$ value, per unit time, are noticed. It seems reasonable therefore, to accept a peroxide value of 25 (ml. 0.002 N thiosulphate per gram of oil) as the end of the induction period for fish liver oils. While this is an arbitrary assumption, it is the most acceptable figure indicated by the results.

For the sake of comparison the changes in iodine

...../value

TABLE 28

Relationship between $E^{1\%}_{1cm}$ and Peroxide Value (P.V.) upon accelerated oxidation at 100°C.

Material	Function	Time of Aeration (hours)															
		0	1½	1	1½	2	2½	3	3½	4	4½	5	5½	6	7	8	8½
Shark Liver oil 1	P.V.	1.3	7	13.4	30	83.5											
	E(1cm.1%)	9.3	8.40	8.36	6.0	2.4											
Shark Liver oil 2	P.V.	1.4		8.4		12.2		24.6		85	93.1						
	E(1cm.1%)	19.8		18.8		17.4		12.6		5.37	3.0						
Shark Liver oil 3	P.V.	1.4	6.1	7.2		11.8		13.4		24.1		123					
	E(1cm.1%)	20.8	20.2	19.8		18.6		17		12		1.9					
Shark liver oil 4	P.V.	–	3.2	4.3		7.3		10		16.6		23.2		59.5			
	E(1cm.1%)	8.9	8.9	8.99		8.37		8.2		7.39		6.6		3.15			
Shark liver oil 5	P.V	–	5.7	11.1	17.3	33.4	54.5										
	E(1cm.1%)	8.7	8.74	8.09	8.04	6.52	3.78										
Shark liver oil 6 plus antioxidant	P.V.	1.2		7				8.6				11.8			16	26.9	53.9
	E(1cm.1%)	9.4		9.4				9.4				8.5			8.3	5.6	4.1
Shark liver oil 6	P.V.	2.3	16.3	36.8		83.5											
	E(1cm.1%)	9.4	8.5	4.6		2.4											
Shark liver oil 7	P.V.	–	6.4	10.6	13.8	16.7	19	21.2		25.1		32.7			75.8		
	E(1cm.1%)	8.3	8.5	8.32	7.95	7.6	7.23	7.23		7.12		5.4			2.3		
Shark liver oil 7 plus antioxidant	P.V.	–				8.9		12.3		16.9		25.6		50.9	90.3		
	E(1cm.1%)	8.3				8.02		7.71		7.20		6.03		3.75	1.87		
Shark liver oil 8	P.V.	0.9	6.0	8.6	12.3	22.3	36.8	63.1									
	E(1cm.1%)	15.5	15.3	15.1	14.7	11.8	6.15	3.81									
Shark liver oil 8 plus antioxidant	P.V.	–						12.8		14.1		22.8	39.1	93.3			
	E(1cm.1%)	15.1						14.8		14.3		10.9	7.53	2.2			

during aeration, were determined for a few shark liver oils. The results are tabulated in Table 29.

Table 29.

Changes in iodine value (I.V.) of shark liver oil during aeration at 100°C. (Compared with peroxide value (P.V.)).

Material	function	Time of Aeration (hrs.) 0	½	1	1½	2	3	4	5
Shark Liver oil 3)	P.V.	1.4	6.1	7.2	-	11.8	24.1	123	1.34
	I.V.	185.6	188.8	192.6	-	186.4	179	163.1	160.6
Shark Liver oil 1)	P.V.	1.3	7	13.4	30	83.5			
	I.V.	191	191	189.5	187.6	173.1			
Shark Liver oil 12)	P.V.	1.0	5.4	7.7	-	9.8	15.6	26.9	66.8
	I.V.	177.6	178.3	179	-	173.3	171.2	168.6	160.0

These findings are in agreement with the results of Filer et al.[37], Wagner et al.[43] and others, showing first an increase in I.V. and then a gradual drop which becomes very definite at the end of the induction period. The determination of I.V. is much too cumbersome, however, for routine work, and the results are not available while the aeration is in progress, as is the case with peroxide values.

By using the described accelerated aeration test, the influence of various factors on the stability of liver oils could be determined and results obtained very rapidly. The author has investigated the influence of antioxidants, different metals, moisture, refining methods, production methods, storage in inert atmospheres (carbon dioxide and nitrogen) and the effect of different preservatives used with the liver, prior to extraction of the oil.

In the first place, the immediate effects of these factors were determined, and secondly, a storage test covering a period of twelve months has been carried out. At three month intervals, induction periods and vitamin A potencies were determined and in this way the

...../deterioration of

deterioration of fish liver oils, stored under the influence of various factors, could be studied. As far as the author is aware, such an investigation has not been carried out on fish liver oils before. The approach of the problem has since been favourably commented on[32].

Storage has been conducted as room temperature, but in a dark, closed cupboard, and in completely filled glass containers, in order to exclude light and metals as factors. This represents ideal storage conditions, and any deterioration can then be ascribed to the variables which were introduced one at a time. In every series of tests, the same oil was used as substrate for the whole period. Containers were not opened until the samples were due for analysis. At the beginning of the 12 month period, sufficient 6 and 8 oz. samples were made up to last the whole period, and every three months one of these samples was merely removed and tested, leaving the rest intact.

D. DETERIORATION OF SHARK LIVER OILS UPON STORAGE.

1. Drop in vitamin A potency during storage for one year under different conditions (primary oil).

In this experiment, a freshy extracted sample of vaalhaai liver oil of $E^{1\%}_{1cm}$ = 13.3 was used to prepare samples that were stored as described in Table 30. Only the loss of vitamin A was studied.

Discussion of results: (Table 30).

Embru[163] has shown the astounding loss of vitamin A on expcsure to light in clear glass containers, and recommends the use of amber glass containers for storage tests.

The results in Table 30 agree in several respects with figures published recently[164]. The losses are bigger in half-filled containers than in completely filled bottles.

...../The similarity

Table 30.

Loss of vitamin A in samples stored under different conditions.

No.	Method of storage	$E^{1\%}_{1cm}$ after 6 months	% Loss	$E^{1\%}_{1cm}$ after 12 months.	Total Loss
1.	Amber bottle, full) open shelf	12.62	5.1	12.1	9
	Amber bottle, half filled) but no	12.92	2.9	11.83	11
	White bottle, full) direct	12.67	4.8	11.89	10.6
	White bottle, half filled) sun-light	12.64	5	6.36	52
2.	Amber bottle, full) Dark	12.82	3.6	12.08	9.2
	Amber bottle, half filled) cup-board	12.4	6.8	11.7	12
	White bottle, full) room	13.08	1.7	12.01	9.7
	White bottle, half filled) temp.	12.86	3.3	11.54	13.3
3.	Amber bottle, full)	13.03	2	13.01	2.2
	Amber bottle, half filled)	12.95	2.6	11.91	10.5
	White bottle, full) $0^{o}C$	13.16	1.1	13	2.3
	White bottle, half filled)	12.67	4.7	11.66	12.3
4.	White bottle, half filled, not corked, shelf	2.95	78	1.2	91
5.	Amber bottle, half filled, not corked, shelf	1.07	92	-	100

The similartiy between the results of corresponding samples in parts 1 and 2 of Table 30 i.e. stored on an open shelf and in the dark, indicates that the effect of light is not too significant, all other things being equal. An exception is the half-filled white bottle stored in diffused daylight, which lost half its potency in one year. This indicates that light accelerates autoxidation to a marked degree in the presence of excess air.

The similarity between losses in half-filled containers stored at $0^{o}C$, with those stored at room temperature, seems to indicate that a low temperature could not retard the autoxidation that is possible with the amount of air present in these containers.

...../The stability

The stability of the oils stored at 0°C in completely filled containers, indicates that autoxidation caused by dissolved air only, can be retarded at low temperatures.

The results of samples 4 and 5 show clearly the rapid destruction of vitamin A caused by free circulation of air over the surface of the oil.

E. FACTORS INFLUENCING STABILITY OF LIVER OIL DURING STORAGE.

In the following chapters the results will be given of tests that have been carried out to determine the effects of various factors on stability of liver oils, as determined by measurement of induction periods at 100°C (accelerated oxidation).

The immediate effect of each factor was determined, and the relative decrease of induction periods caused by it, during storage for twelve months.

1. Antioxidants.

(a) Historical.

Early observations that certain vegetable oils do not become rancid on exposure to the air, led to the belief that these materials contain some component which protects them from oxidation[89]. Anderegg et al.[90] observed that destruction of vitamins A and E was retarded in vegetable oils of equal or greater unsaturation than fish liver oils. Hilditch and Sleightholme[91] observed that resynthesized glycerides were very much less resistant to oxidation than the original oils from which the fatty acids were obtained. Since that time much work has been done[92, 93, 94] on the separation of naturally occurring oxidation inhibitors, and the use of pure chemical compounds to retard autoxidation, and this work is being continued with unabated energy due to the importance of preserving all oils and fats, or foods containing fats.

...../(b) Mode of action.

(b) Mode of action.

The value of any inhibitor is determined by the extent to which it can increase the length of the induction period. It is still a matter of speculation how this inhibition of oxidation is caused. Lea[95] gives a review of the peroxide theory and chain reaction theory. Neither of these, nor the theory of deactivation of autoxidation catalysts, does complete justice to all the results noticed. Further reference will be made to these theories when the author's results are discussed at the end of this chapter.

The degree to which induction period can be prolonged is illustrated by Royce[96], and Bickoff[97] who found that antioxidants will protect a solution of carotene in oil for periods well over a year, without special precautions being taken, such as refrigeration, or storage under nitrogen, or in evacuated containers.

(c) Classification and review.

Antioxidants are primarily classified into two groups by Mattill[98]. These are:-

(i) o. or p. di-, or poly-phenolic compounds or compounds having a similar electronic configuration; and

(ii) synergists, which have little, if any, action alone, but which can reinforce the antioxidants in group (i) to varying degrees.

Class (i) includes quinol, tocopherol, quinone, nordihydroguaiaretic acid and others, while group (ii) includes the acid type of inhibitors e.g. maleic, malonic, malic, citric, ascorbic acids. Phosphatides like lecithin and kephalin, and di- and poly-phenolic acids like gallic acid, are also included in group (ii).

The inhibiting effect of the phenolic type of antioxidant has perhaps been studied more extensively than any other. The protective effect of various phenolic

...../inhibitors

inhibitors have been reported by Mattill[99], Olcott[100], Greenbank and Holm[101], French et al.[102] The last-named authors found that prolongation of induction period is approximately proportional to amount of inhibitor. More recent publications[103] report excellent results with such compounds as lauryl hydroquinone and tocopherols. Good protection with several phenolic inhibtors has been obtained by Lovern[104, 107], Banks[105] and Lea[106], while several reports[108, 88] mention the synergistic effect of acids and lecithin with phenolic inhibitors. Nordihydroguaiaretic acid or 2,3 dimethyl -1,4- bis (3,4 dihydroxyphenol) butane has been found[82] an excellent inhibitor for carotene.

The protection offered by gallic acid and the gallates is described by Lovern[104, 107], Lea[110] and Hilditch[109] who found ethyl gallate unsuitable for carotene, but active when used with unsaturated glycerides of butter fat. The use of methyl, ethyl, butyl and propyl gallates in fish oils has been patented[111]. Findlay and Smith[112] report on the use of ethyl gallate and ascorbic acid to reduce tallowiness in spray dried milk, while Bucher[113] mentions hydroquinone, gallates and a-naphthol.

The value of citric acid is described by Stirton et al.[114], while the synergistic effects between citric and other acids and wheat germ concentrate[115], or citric acid and mixed tocopherols[106], are discussed in several papers. Maleic acid[101] and ascorbic acid[116, 125] have been found active, while ascorbic acid is also a suitable synergist with tocopherol[82].

The tocopherols have been viewed with much favour as antioxidants. It has been suggested[117] that the stability of rat fat may be primarily related to its tocopherol content. Tocopherols are regarded as the only natural inhibitors present in a fat, and it has been suggested[118]

...../that the

that the poor stability of refined lard is due to destruction of tocopherols during rendering and refining. Further reports on the value of tocopherols are by Lea[38], Lovern[104] and Olcott and Emerson[119], who found that a-tocopherol is two to three times less effective than the β or γ isomers, while Riemenschneider[108] found the order of activity α, β, γ

A review of the literature on the inhibiting effect of tannins, is given by Spannuth et al[120], and this is interesting because of the method employed to extract the active principles of tannin viz. addition of the tannin to the oil and removal by filtration. This procedure has also been carried out by the author in trying to extract active principles from milk powder.

The reports on lecithin as inhibitor are very conflicting. While Bibby[121] found no protection in fatty acids, arguing that lecithin only acts synergistically with traces of natural inhibitors present, Bailey[122] found soya phosphatides effective, and more so when added before deodorisation, which fact also disproves the statement that heating to 65°C destroys the antioxygenic properties of vegetable phosphatides. It is claimed[123] that lecithin will stabilise vitamin A concentrates to be used in margarine. Olcott[124] found lecithin much more effective in cottonseed oil than in lard. Wall and Kelley[126] found that lecithin was ineffective in stabilising carotene in vegetable oils stored at temperatures belowe 37.5°C, but offered good protection during accelerated oxidation at 100°C. The synergistic effect between lecithin and hydroquinone for protection of cod and halibut liver oil has been demonstrated[88].

The lecithin that has been used in these experiments is a wheat-germ extract, sold as a fish liver oil antioxidant. The tocopherol is a 40% solution of a-tocopherol.

...../(d) Experimental

(d) Experimental details.

In the present investigation of the protection obtainable with several antioxidants, the same substrate has been used for all tests. This substrate was a year-old commercial oil, with an induction period of less than one hour at 100°C. This oil had lost practically all stability due to natural antioxidants, and provided a stringent test for any antioxidant. By using the same substrate, and starting all storage tests at the same time, it was ensured that all results would be comparable.

The protection obtained with any antioxidant can be described by simply stating the induction period as measured by the accelerated oxidation method and peroxide development. In the storage tests, the decrease in induction period with time is indicated (Table 32). However, in comparing the immediate effects of various antioxidants, and in order to be able to compare the results with those obtained when using different substrates, a more descriptive symbol must be used. Spannuth et al.[83], Lips and McFarlane[84], Riemenschneider et al.[85], and others use the Symbol I_A, denoting increase in induction period upon addition of antioxidant. Equally suitable is the symbol P.F. or protection factor, being the ratio :-

$$\frac{\text{Induction period of oil plus antioxidant}}{\text{Induction period of substrate.}}$$

The latter is used by Greenbank and Holm[85] and many others, and has been used in this investigation, simply because it appears to be in more widespread use than the former method.

As can be seen from Table 31 several of the reputed antioxidants reviewed above, have been selected for spot testing. The choise of antioxidants was limited by availability of materials. Moreover, the object of this investigation, has not been a search for new antioxidants,

...../in the

in the first place, but an attempt to determine the permanency of the protection offered by a few of the recognised oxidation inhibitors. The spot tests did serve to evaluate a few of the commercially obtainable and much advertised products.

For storage tests hydroquinone was selected because it appears to be one of the most active of the phenolic class of inhibitors cited in the literature[88]. Butyl gallate was selected to represent the gallic acid-, and gallic ester-class, which appears to be one of the most popular types in Britain. Citric acid is an example of the acid class of inhibitors. Citric acid appears to be the most widely used of acidic inhibitors and is being used in South Africa to protect vegetable oils[87].

The antioxidants were added to the oil at four different concentrations each, the range (0.01 - 0.25%) having been decided on after due consideration of the varying levels at which antioxidants are normally added by workers in this field.

Hydroquinone and butyl gallate were dissolved in the oil with the aid of gentle heating on the water bath, and under reduced pressure, while citric acid was added as an alcoholic solution. To minimise errors, all the antioxidants were added to the substrate at a concentration of 0.5% while the solution was then diluted to the required concentrations, using untreated substrate as diluent.

Aerations were carried out at 100°C.

(e) Discussion of results.

(i) Spot tests.

From the figures in Table 31, it is apparent that hydroquinone prolonged the induction period more than any of the other inhibitors which were used. The very old and rancid liver oil was given an induction period of nearly eight hours. Butyl gallate

TABLE 31.

Protection Factors (P.F.) obtained with different antioxidants and synergists (Same Substrate used in all tests).

No.	Antioxidant	Concentration (% on oil)	Induction Period (hours)	P.F.
0	Substrate (year-old shark liver oil)	-	0.8	-
1	Hydroquinone	0.25	7.6	9.5
2	do.	0.10	4	5
3	Butyl Gallate	0.25	5.5	6.9
4	Citric Acid	0.25	2.5	3.1
5	Tartaric Acid	0.25	1.8	2.3
6	Maleic Acid	0.25	1.6	2
7	Fumaric Acid	0.25	1.6	2
8	Phosphoric Acid	0.25	2.2	2.8
9	2,5 Ditertiary Butyl Hydroquinone	0.10	1.5	1.9
10	do.	0.25	4	5
11	α-Tocopherol (40% Solution)	0.01	0.6	0
12	do. do.	0.25	0.8	0
13	do. do.	0.5	0.8	0
14	α-Tocopherol (0.5%) plus Citric Acid (0.25%)	-	2.5	3.1
15	α-Tocopherol (0.5%) plus Hydroquinone (0.1%)	-	4	5
16	Lecithin (From Maize Oil)	0.25	0.8	0
17	do.	0.5	0.8	0
18	Lecithin (0.5%) plus Citric Acid (0.25%)	-	2.4	3
19	Hydroquinone (0.1%) plus Citric Acid (0.1%)	-	12.3	15.4
20	Butyl Gallate (0.1%) plus Citric Acid (0.1%)	-	5.1	6.4
21	Fresh Substrate	-	2.4	-
22	*α-Tocopherol in New substrate	0.5	2	0
23	*Lecithin in New Substrate	0.2	2.3	0
24	*Cereal Extract X	0.2	2.4	0
25	Lecithin (0.25%) plus Butyl Gallate (0.1%)	-	2.0	2.5

* Antioxidants sold commercially.

was a good second, while there is not much to choose between the acids. Citric acid is the most useful of this class, however, followed by phosphoric acid. Maleic and Fumaric acids gave identical results, indicating that there is no effect due to geometrical isomerism.

The protective effect of 2, 5 - ditertiary butyl hydroquinone, much advertised[127] lately, is disappointing, and much better results were expected. At a concentration of 0.25% it is less effective than 0.25% butyl gallate and about as effective as 0.1% hydroquinone. At lower concentrations it appears relatively ineffective.

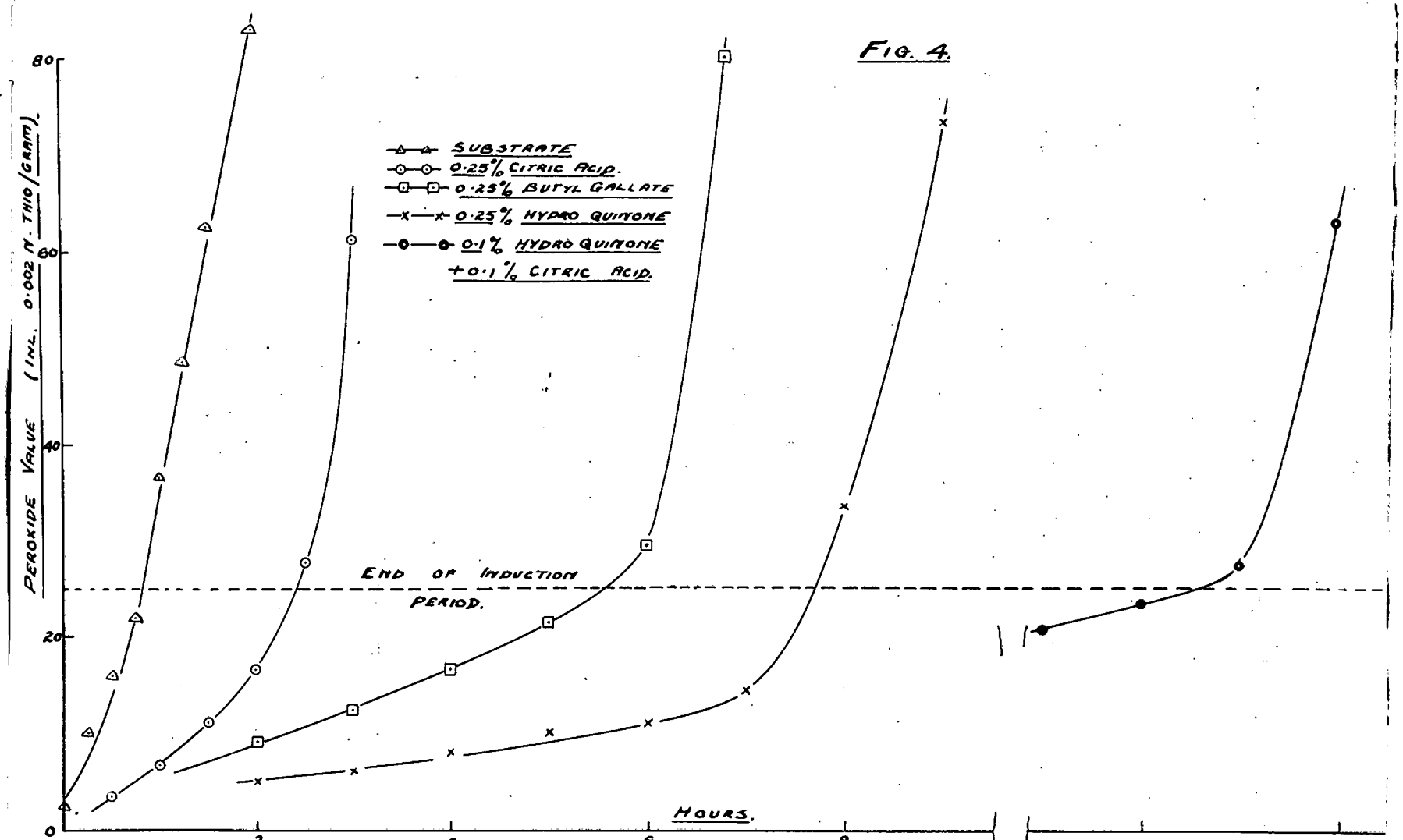
The complete failure of lecithin (Table 31, Nos. 16, 17, 23, 24) to impart any protection at all, confirms earlier reports[121], but the failure to obtain any synergistic effect with lecithin, is noteworthy (Nos. 18, 25). No synergism was obtained between citric acid and lecithin, or between butyl gallate and lecithin.

The failure of a-tocopherol is very difficult to explain, in view of the good reports about its inhibiting effect. The level at which it was used, was increased to 0.5%, without any beneficial effect. The failure to obtain synergism between tocopherol and citric acid or hydroquinone (Table 31 Nos. 14 15) is indeed disappointing. In order to make sure that the poor quality of the substrate did not provide too stringent a test for lecithin or tocopherol, they were tried out in a fresh and much more stable liver oil (Nos. 21 - 24), but no protective effect was obtained. In fact, it almost seemed as if a pro-oxidative effect is noticeable, although the information is too scanty to allow one to draw definite conclusions.

The synergistic effect of citric acid with hydroquinone and butyl gallate is very marked (Nos. 19, 20)

...../while 0.1%

FIG. 4.



PEROXIDE DEVELOPMENT IN THE PRESENCE OF SEVERAL ANTIOXIDANTS (SAME SUBSTRATE). (100°C.)

while 0.1% hydroquinone gives a protection factor of 4, and 0.1% citric acid gives 3 (Table 32), a combination of the two gives much better protection (P.F.15.4) than could result from an additive effect. Likewise the P.F. of a mixture of 0.1% butyl gallate (P.F.2.5) and 0.1% citric acid (P.F.3) is 6.4, more than the sum of the two. Here, however, the synergism is not so marked as with citric acid and hydroquinone.

From these results it appears that hydroquinone (0.25%) or a mixture of 0.1% hydroquinone and 0.1% citric acid, will probably be the most suitable antioxidants for fish liver oils. From Table 28 it is apparent that the same relationship between decrease in potency and peroxide development holds, whether antioxidant is present or not.

The position of hydroquinone has been rather obscure as far as preservation of foods is concerned. Although it has been proved non-toxic for rats[128], and in spite of the fact that it is a recognised anti-pyretic[129], it is not described in the British Pharmacopoeia, and appears to be prohibited by the pure food laws of various countries[130]. As a result of the author's findings on the excellent anti-oxidative properties of hydroquinone, representations have been made to the Union Health Department, which body has now consented to allow the addition of 0.1% hydroquinone as antioxidant to fish liver oils.

In figure 4 a graphical illustration is given of the prolongation of induction period due to citric acid, butyl gallate, hydroquinone and a mixture of hydroquinone and citric acid. Note the superiority of hydroquinone and the marked synergism between hydroquinone and citric acid.

(ii) Storage tests.

Some interesting observations can be made from tables

.....32 and 33

TABLE 32

Decrease in $E^{1\%}_{1cm}$ and induction period of liver oil stored with added antioxidants for one year.

Antioxidant	Conc.	Induction Period (Hours)					$E^{1\%}_{1cm}$					% Total Decrease	
	% of oil	0	3 months	6 months	9 months	12 months	0	3 months	6 months	9 months	12 months	Original I.P.	$E^{1\%}_{1cm}$
Hydroquinone	0.25	7.6	6.2	5.2	5.4	5	9.37	9.06	9.27	9.11	8.94	34	4.6
	0.10	4	3.1	2.9	3.2	2.6	9.37	9.12	9.14	9.16	8.92	35	4.8
	0.05	2.1	1.6	1.6	1.6	1.3	9.37	9.18	9.09	9.31	9.04	38	3.6
	0.01	1.4	1.1	1	0.9	0.7	9.37	9.13	8.99	9.12	8.74	50	6.7
Butyl Gallate	0.27	5.5	5.2	3.2	3.6	3.1	9.37	9.16	9.21	9.07	8.72	44	6.9
	0.10	2	1.4	1.3	1.5	1.2	9.37	9.20	9.30	9.39	8.50	40	9.3
	0.05	1.1	0.9	0.6	0.6	0.6	9.37	9.09	9.26	8.84	8.6	45	8.2
	0.01	0.9	0.6	0.3	0.3	< 0.25	9.37	9.10	9.30	9.14	8.58	> 72	8.4
Citric Acid	0.25	2.5	1.9	1.6	1.6	1.6	9.37	9.15	8.96	8.91	9.06	36	3.3
	0.10	2.3	1.8	1.5	1.6	1.6	9.37	9.32	9.26	9.31	8.83	30	5.8
	0.05	2.1	1.5	1.3	1.4	1.4	9.37	9.34	9.22	9.16	8.76	33	6.5
	0.01	1.2	1	0.5	0.4	< 0.25	9.37	9.03	9.31	9.2	8.71	> 79	7
Untreated Oil	-	0.8	0.5	0.25	< 0.25	< 0.25	9.37	9.21	8.90	8.75	8.42	> 69	10.2

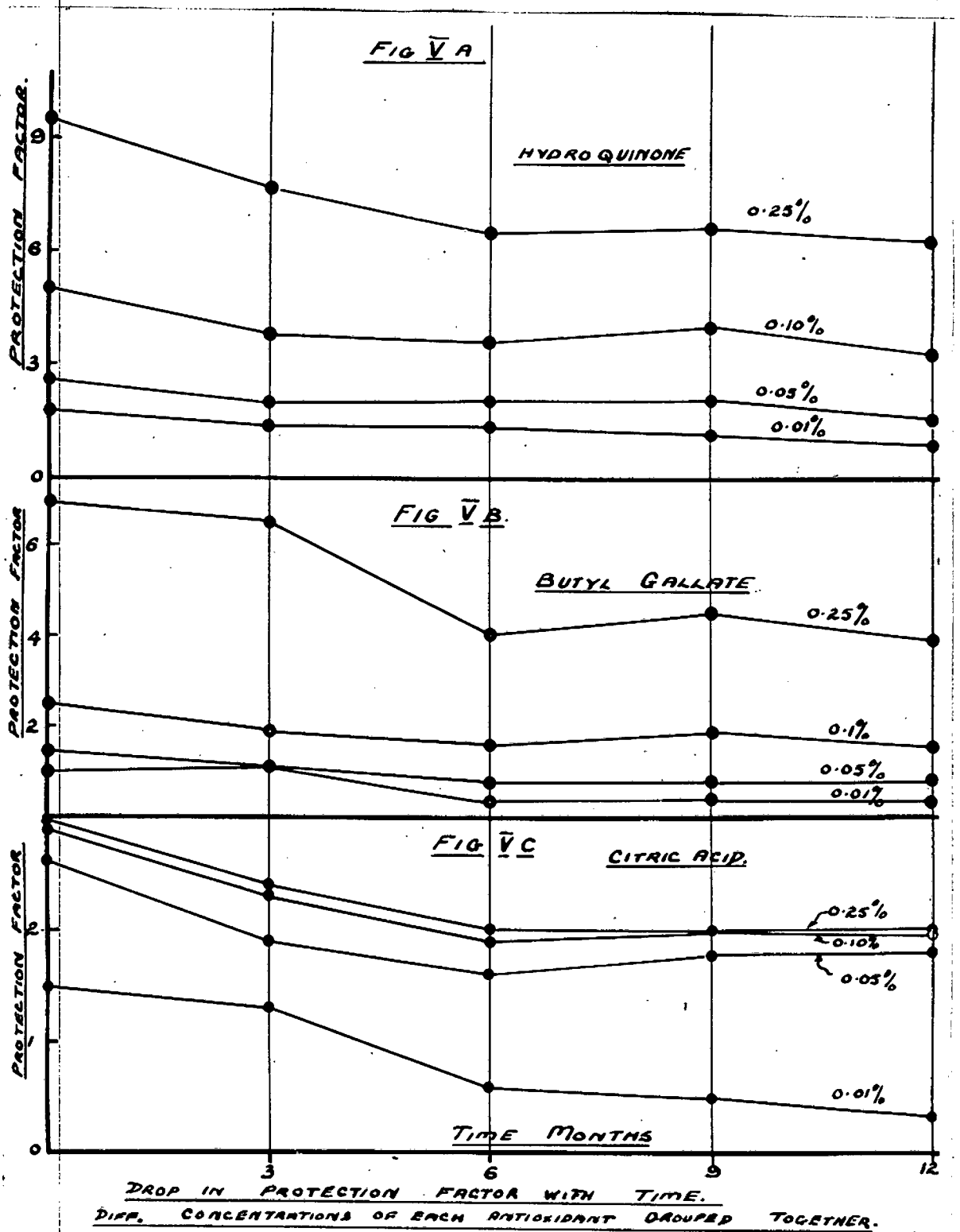
TABLE 33.

Storage decrease in protection factor (P.F.) of different antioxidants at different concentrations, where

$$P.F. = \frac{\text{Induction period of oil plus antioxidant}}{\text{Induction period of untreated oil.}}$$

(Induction periods from Table 32)

Antioxidant	Conc. % of oil	Protection Factor: Start	3 months	6 months	9 months	12 months	% decrease in P.F.
Hydroquinone	0.25	9.5	7.7	6.5	6.7	6.2	35
	0.10	5	3.9	3.6	4	3.2	36
	0.05	2.6	2	2	2	1.6	38
	0.01	1.8	1.4	1.3	1.1	0.9	50
Butyl Gallate	0.25	6.9	6.5	4	4.5	3.9	44
	0.10	2.5	1.8	1.6	1.9	1.5	40
	0.05	1.4	1.1	.75	.75	.75	46
	0.01	1.1	.75	0.4	0.4	0.3	73
Citric Acid	0.25	3.1	2.4	2	2	2	35
	0.10	2.9	2.3	1.9	2	2	31
	0.05	2.6	1.9	1.6	1.8	1.8	31
	0.01	1.5	1.3	.6	0.5	0.3	80



DROP IN PROTECTION FACTOR WITH TIME.

DIFF. CONCENTRATIONS OF EACH ANTIOXIDANT GROUPED TOGETHER.

FIG 6 A.

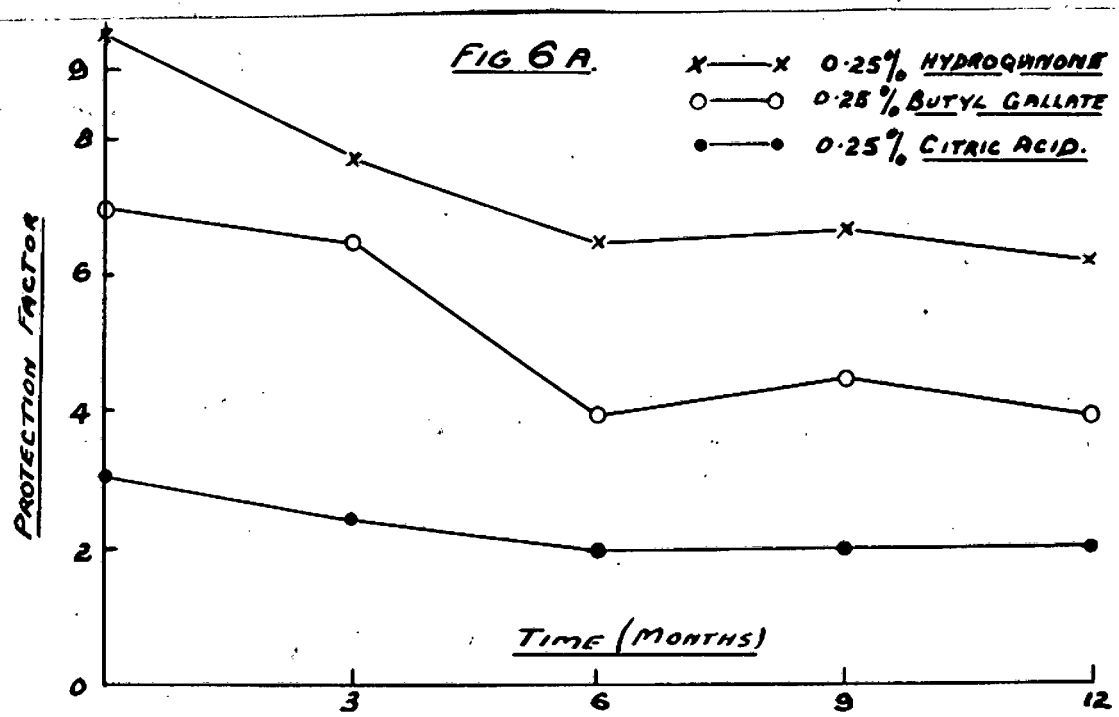


FIG 6 B.

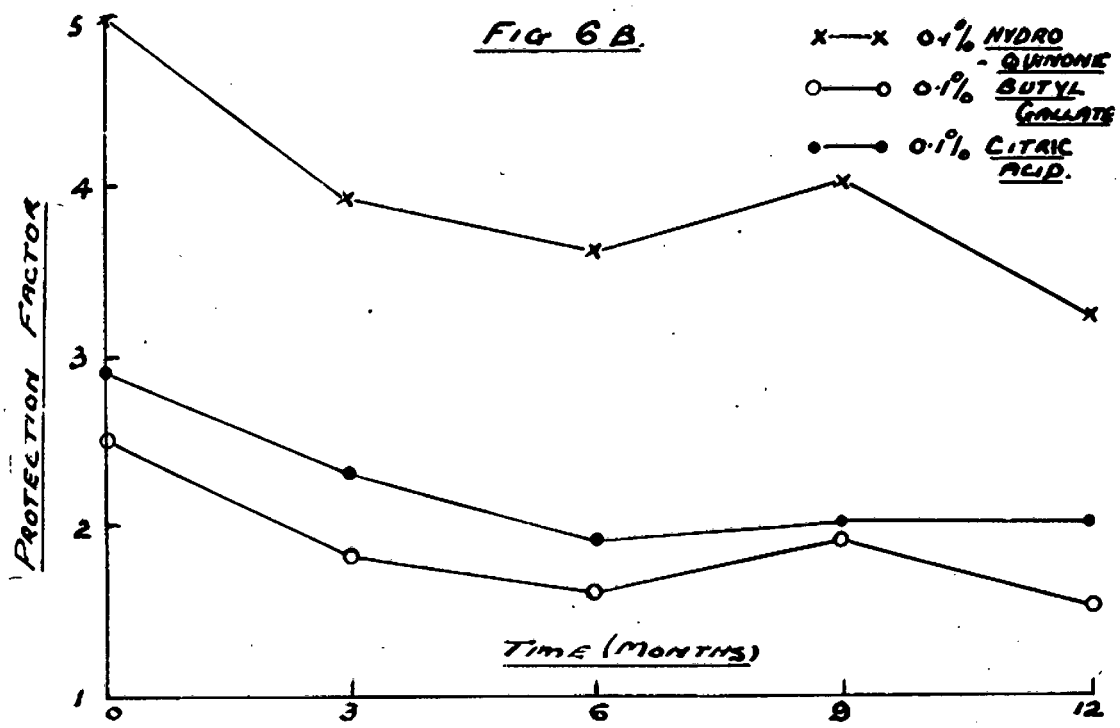
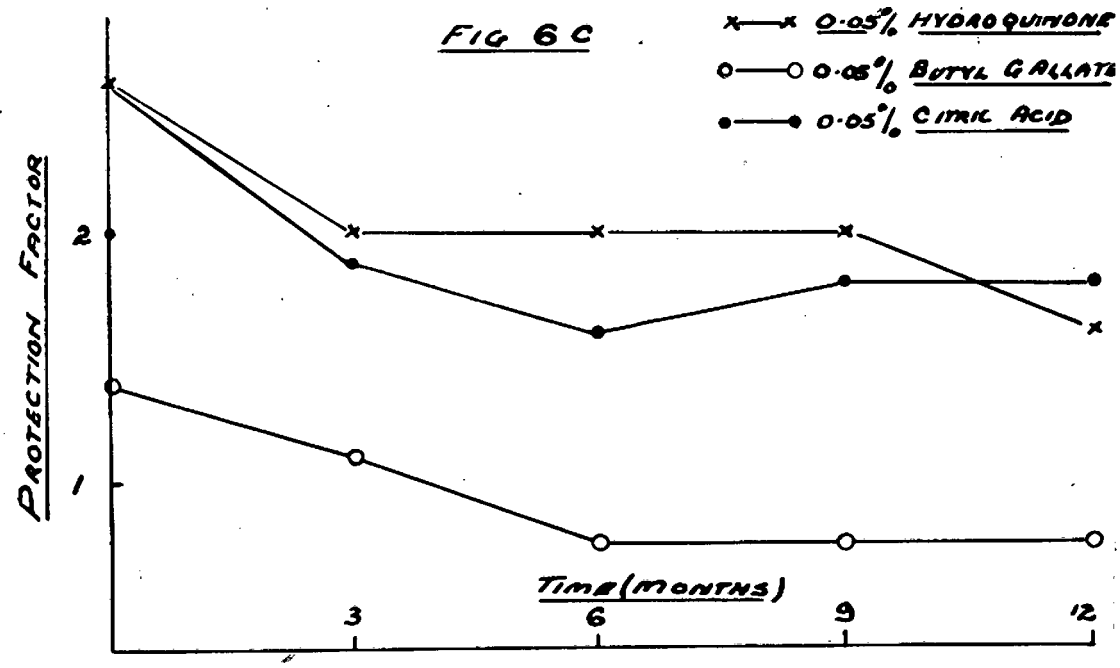


FIG 6 C



DROP IN PROTECTION FACTOR WITH TIME (DIFFERENT ANTI-OXIDENTS AT SAME CONCENTRATION GROUPED TOGETHER.).

32 and 33 and figures 5 and 6, in which the drops in potency and induction period, during storage of the oil with antioxidants, are illustrated.

As far as loss of vitamin A is concerned (Table 32) the results are not as spectacular as those for decrease of induction period. They do indicate, however, that in all protected samples the loss of vitamin A was smaller than in the untreated blank. The smallest loss occurred in the hydroquinone-protected samples, especially where the concentration of antioxidant was 0.05% or higher. Citric acid comes second as far as protection of vitamin A is concerned, while the loss in the gallate-protected samples is noticeably greater.

The observation that induction period decreases relatively much faster than $E^{1\%}_{1cm}$ value, confirms the suggestion[32] that **different mechanisms** of degradation occur during storage at room temperature and during accelerated oxidation, where it has been shown (figures 2 and 3) that a linear relationship exists between $E^{1\%}_{1cm}$ and peroxide value.

It should also be pointed out that induction period is a measure of susceptibility to oxidation and that the protected samples will be much more resistant to oxidation after one year, if the oils are exposed, and no longer stored under ideal conditions, like the way in which this test has been conducted.

In Table 32 the shortening of induction period during storage is indicated, while in Table 33, the calculated corresponding protection factors have been tabulated.

In figure 5, drop in protection factor during storage is illustrated, and the different concentration of each antioxidant have been grouped together, so that the protection offered by varying amounts of antioxidant can be observed at a glance, as well as the decrease during

...../storage

storage. Note the small difference between protection factors obtained with varying concentrations of citric acid above 0.01%.

In figure 6, different antioxidants at the same concentration have been groupedtogether, and this illustration is convenient for comparing different antioxidants used at the same concentration. Note the superiority of hydroquinone at all concentrations. Note too that butyl gallate offers better protection than citric acid at a concentration of 0.25%, but at lower concentrations it is less active than citric acid. The marked superiority of hydroquinone is most noticeable at concentrations higher than 0.05% - at 0.05% or lower it is no better than the same quantity of citric acid.

From figures 5 and 6 it is apparent that the protection factor does not remain constant, but drops during storage. The decrease is very serious during the first six months, after which the protection factor, or induction period, remains relatively constant. After nine months several samples showed a slight increase of induction period. This effect was most noticeable in the samples with 0.1% hydroquinone and 0.25% butyl gallate. While this is an interesting phenomenon and may be the result of a complex oxidation mechanism, it is not serious enough to affect the general trend of decrease in protection factor during storage.

With all three antioxidants, the protection obtained with 0.01% of the material was so slight (Protection factors less than 2), that this may be considered a lower useful limit of antioxidant concentration. Even at 0.05%, butyl gallate is markedly less active than 0.1% citric acid or hydroquinone (Table 33) and may be regarded as inactive, for all practical purposes. These results are surprising in view of earlier results[104] where good protection was obtained with 0.01% ethyl or butyl gallate

...../or tocopherol,

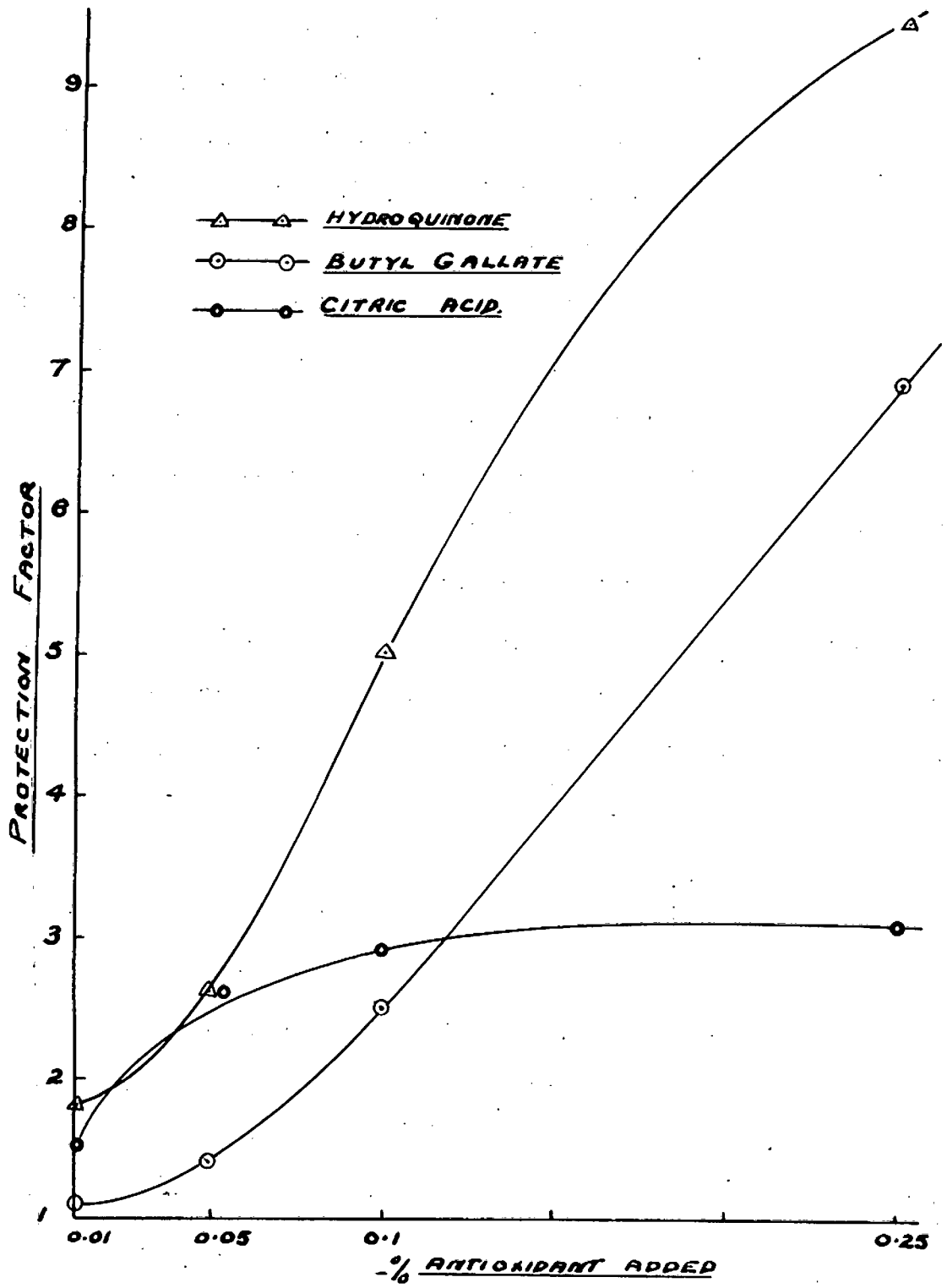


FIG 7

RELATIONSHIP BETWEEN ANTIOXIDANT CONCENTRATION AND PROTECTION OBTAINED.

or tocopherol, in halibut liver oil emulsions and margarine. The optimum concentration of antioxidant, as determined in the present series of tests, is more in accordance with the results of Holmes et al.[88], who used 0.1 - 1% hydroquinone.

On the whole the samples of oil, stabilised with citric acid, suffered the smallest decrease in protection factor during storage for one year (Table 33). The protection obtainable with citric acid is small, however, compared with that offered by hydroquinone.

It appears that there is a general drop in protection factors, amounting to anything from 30 - 100% of the original, during storage for one year, depending on type, and, to a lesser degree, on concentration of antioxidant. In the case of hydroquinone and citric acid, the decrease is less (35 to 38% and 31 to 35% respectively) than in the case of butyl gallate (40 to 46%). It is significant, however, that the total drop in protection factor is fairly constant, provided the initial protection is significant.

In figure 7 the variation of protection obtained, with concentration of antioxidant, is illustrated graphically. In the case of citric acid, constancy is reached at a concentration of 0.05%, or very nearly so. This phenomenon does not support the chain-theory of oxidation inhibition, discussed by Lovern[107], according to which one would expect a direct proportionality between % antioxidant added and protection factor obtained. The curves for hydroquinone and butyl gallate, on the other hand, show almost direct proportionality, and support the chain-theory.

The main results of this investigation can be summarised in the following points :-

(i) The antioxygenic activities of hydroquinone, butyl gallate and citric acid, at four different concen-

/centrations

trations each, have been determined, and expressed in terms of protection factors. The all round superiority of hydroquinone has been demonstrated, as well as the activity of butyl gallate at concentrations of 0.1% or higher, and citric acid at concentrations of 0.05% or higher. Useful comparisons between the relative efficiencies of the three antioxidants at different concentrations can be made by consulting Table 33.

(ii) As far as hydroquinone and butyl gallate are concerned, protection varies proportionately with concentration of antioxidant, but with citric acid, no very significant advantage is gained by using more than 0.05%.

(iii) All antioxidants should be used at a concentration greater than 0.01%, in order to obtain significant protection.

(iv) During storage for one year under ideal conditions, induction periods of protected oils decrease by anything from 30 - 80%. The most rapid decrease is noticed during the first three or six months, after which the induction period remains practically constant.

(iii) Antioxidants from milk powder.

An attempt has been made to investigate the possibility of extracting antioxidative principles from milk. Josephson and Dahle[131] found that heat treatment of whole butter (205°C for 10 minutes) considerably improved its keeping qualities. The same treatment in the case of separated butterfat was only successful if 1% of commercial skim milk powder was added to the butterfat.

Commercial whole milk powder has been used with liver oils, and the following tests were carried out :-

No.	Method	Induction Period (hours).
1	Substrate A	0.8
2	Substrate A plus 5% milk powder, heated at 205°C for 10 minutes, then filtered	1.1
3	Substrate B	2.25
4	Substrate B, treated in same way as No. 2	2.25
5	Substrate B plus 5% milk powder, heated at 100°C for 30 minutes, filtered	2.25
6	20 ml. Arachis oil plus 5 g. milk powder heated at 205°C for 10 minutes, filtered and added to 80 ml. substrate B	3
7	20 ml. Arachis plus 10 g. milk powder, then treated in same way as No. 6	3

From these results it appears that little, if any, antioxygenic constituents can be extracted from milk powder, in such a simple manner.

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2. Effect of materials of construction of processing plant and containers.

The influence of materials of construction of plant and containers, is usually adverse. All metals may be regarded as oxidation accelerators, reducing the stability of oils to varying degrees.

Royce[96] describes the pro-oxidative effect of copper. A review given by Lea[132] indicates the activity of one part copper in ten million, which increased the rate of oxidation in herring oil $2\frac{1}{2}$ times. Iron, present in ten times this quantity, was only half as active. Ziels and Schmidt[133] report that all metals tested had a marked pro-oxidative effect, except aluminium and nickel (on hydrogenated cottonseed oil). Lead, manganese, copper, cobalt and iron were the worst offenders. These authors suggest a mechanism of reaction whereby metallic soaps are formed

...../by interaction

by interaction of the metal with free fatty acid present.

The information on the effect of metals on rate of oxidation is widely scattered and not at all plentiful, and no evidence could be obtained that fish liver oils, and the rate of destruction of vitamin A have ever been fully investigated. It was therefore decided to determine the effect of various metals on induction period of a fresh shark liver oil, and to store the oil in contact with these metals, and determine the rate of decrease of induction period and vitamin A potency.

The oil was obtained by collecting fresh livers in a wooden box, and extracting the oil from these as quickly as possible, using glass containers only. In this way a good fresh oil, that had not been in contact with metals, was obtained.

Aliquots of this sample of oil were aerated in contact with strips of well-cleaned metal, and stored in contact with metal, while the induction period and $E^{1\%}_{1cm}$ were determined at regular intervals. A small head-space of air was left in each case, including the blank, in order to simulate practical storage conditions.

The following metals, normally used in plant or container construction, or used for making instruments that are used at some time or other during the production of liver oils, were tested:- Mild steel, two types of stainless steel commonly used viz. chrome steel and chrome-nickel steel, galvanised zinc, aluminium, lead, copper, brass and tin. At the same time the oil was aerated in contact with rubber, as rubber piping is invariably used, and with traces of old oxidised oil, to compare the effect of oxidised oil with that of metallic pro-oxidants, and to confirm earlier reports[134, 135] that oxidised oil catalyse the destruction of vitamin A in fresh oils.

In all cases the ratio oil to metal was 250 ml. in

...../contact

in contact with 8 sq. inches, as this corresponds more or less to the ratio encountered in storage. The metal strips were cleaned very well by scraping, pickling in acid and were then dried after rinsing in acetone. Aerations were done at 100°C and induction period measured to a point when the peroxide value exceeded 25.

In the first place, a preliminary aeration was carried out in the presence of copper and tin, which were expected to be the most active and least active metals respectively, in order to ascertain whether the same relationship exists between destruction of vitamin A and development of peroxide in the presence of pro-oxidants, as that previously established (fig. II). The results are given in Table 34.

Table 34.

Decrease of $E^{1\%}_{1cm}$, and development of peroxides (P.V.), in liver oil aerated at 100°C in presence of copper and tin.

Time (hours)	Samples.					
	Substrate A		A plus copper		A plus tin	
	P.V.	$E^{1\%}_{1cm}$	P.V.	$E^{1\%}_{1cm}$	P.V.	$E^{1\%}_{1cm}$
0		5.35		5.35		5.35
0.5			23.6	3.1	5.35	-
1	5.72	4.88	37.6	1.83	9.17	4.86
2	9.9	4.36			26	3.41
2.5					75.7	1.46
3	19.8	4.22				
3.5	38.7	2.39				

From these results it appears that the same relationship holds, and that vitamin A is rapidly destroyed after the end of the induction period (P.V. 25) is reached.

The storage test, originally planned to continue for one year, was stopped after nine months, as it was quite clear, even after six months' storage, that all natural inhibitors had been completely destroyed. In fact, the

...../induction

induction periods after three months are so low that the oils may be considered fully sysceptible to rapid oxidation. It should be pointed out that aeration of stored samples was not done in contact with the metal, so that all metals had only been in contact with the oil at room temperature. The rapid destruction of natural resistance to oxidation becomes even more significant if one considers that most of these samples showed induction periods of 1 - 2 hours when aerated in the presence of metals, and that these induction periods decreased to 0.7 hours or less as determined by aeration, in the absence of metals, after three months' storage.

If one considers the loss of vitamin A (Table 35) the position becomes even more serious. Over a period of nine months, there is a drop in $E^{1\%}_{1cm}$ ranging from 16 - 88%, while the blank, stored under the same conditions, but not in contact with metals, lost only 2.8%. Incidentally, this is the lowest storage loss recorded in the present investigations, and can probably be explained by the fact that this oil was a particularly fresh and pure oil, having been obtained from fresh liver and kept out of contact with all metals.

The spot and storage results are summarised in Table 35. Spot tests were carried out with the metals present, while the stored samples were aerated out of contact with metals.

From these figures it would appear that all metals cause a very serious increase in susceptibility to oxidation. In decreasing order of activity (considering the induction periods obtained), the metals are grouped as follows :-

Copper
Brass
rusted mild steel
lead
chrome nickel steel
chrome steel
galvanised zinc

...../aluminium

TABLE 35

Decrease in $E^{1\%}_{1cm}$ and induction period of liver oil stored in contact with various metals.

No.	Metal	Induction Period				$E^{1\%}_{1cm}$				% drop in $E^{1\%}_{1cm}$.
		1	2	3	4					
		0	3 months	6 months	9 months	0	3 months	6 months	9 months	
1	Blank	3.2	3.1	2	2	5.35	5.12	5.25	5.20	2.8
2	Copper	0.6	< 0.2	< 0.2	< 0.2	"	2.82	1.09	0.65	87.7
3	Lead	1.3	0.6	< 0.2	< 0.2	"	4.88	3.83	3.31	38.1
4	Galvanised Zinc	1.8	0.7	< 0.2	"	"	4.70	4.71	4.33	19
5	Aluminium	2	0.7	0.3	"	"	4.70	4.07	3.71	30.6
6	Mild Steel	2.2	0.7	0.3	"	"	4.81	4.13	3.85	28
7	Chrome Steel	1.8	0.5	0.3	"	"	4.70	3.64	3.32	37.9
8	Chrome Nickel Steel	1.7	0.5	0.3	"	"	5.06	4.23	4.13	22.8
9	Tin	2	0.6	0.4	"	"	4.60	4.57	4.48	16.2
10	(Rubber)	2.6								
11	Rusted Steel	1.1								
12	Brass	0.7								
13	Copper plus 0.1% Citric Acid in oil	1.4								
14	5 p.p.m. Copper (as Cucl) plus 0.1% Potassium Citrate plus 0.1% Citric Acid	0.4								
15	Copper plus 0.1% Cupron	1.1								
16	5 p.p.m. Copper (as Cucl) plus 0.1% Cupron	0.3								
17	Blank plus 0.1% Cupron	3.1								
18	Blank plus 1% old oxidised oil	1.7								

Note: In samples 2-13 and in No. 15, strips of metal were used, as described in text. In Nos. 14 and 16, Cuprous Chloride was added to the oil. Citric acid and potassium citrate were added as alcoholic solutions. Induction periods in column 1 only determined in contact with metals. All further samples stored in contact with metals, but I.P. determined on oil out of contact with metal.

aluminium
tin
polished mild steel.

Rubber comes after plished mild steel, but it should be noted that rubber did cause a reduction of induction period from 3.2 to 2.6 hours. Polished mild steel appears to exhibit less activity than tin or aluminium, but the old sample that had been rusted but cleaned well, was much more active. This phenomenon will probably be encountered with all metals once corrosion has started to destroy the smooth surface. The pro-oxidative activity of the stainless steels is surprising, as one would naturally expect them to be less active than mild steel. Yet the same experience has been reported by King et al.[136]

It should be noted furthermore, that 1% of old oil (No. 18) acted pro-catalytically, being superseded only by copper, brass, rusted steel and lead.

In samples 13 and 14 an attempt was made to counteract the effect of copper by adding citric acid as anti-oxidant. While this was moderately successful in sample 13 where the oil was aerated in contact with metallic copper, the attempt failed in experiment 14, probably due to the greater activity of copper when added as cuprous chloride.

The same phenomenon was noticed in samples 15 and 16, where cupron did seem capable of taking up the copper ions formed in the sample containing metallic copper, but could not prevent the pro-oxidative effect of copper when present as a salt. Note that the sample contained only 5 parts copper per million and a very large excess (0.1%) cupron. Cupron by itself did not materially affect the normal induction period.

The great activity of copper, whether present in metallic form or as a salt, agrees with the results obtained[136] for the pro-catalytic effect of copper on lard.

...../In figure 8,

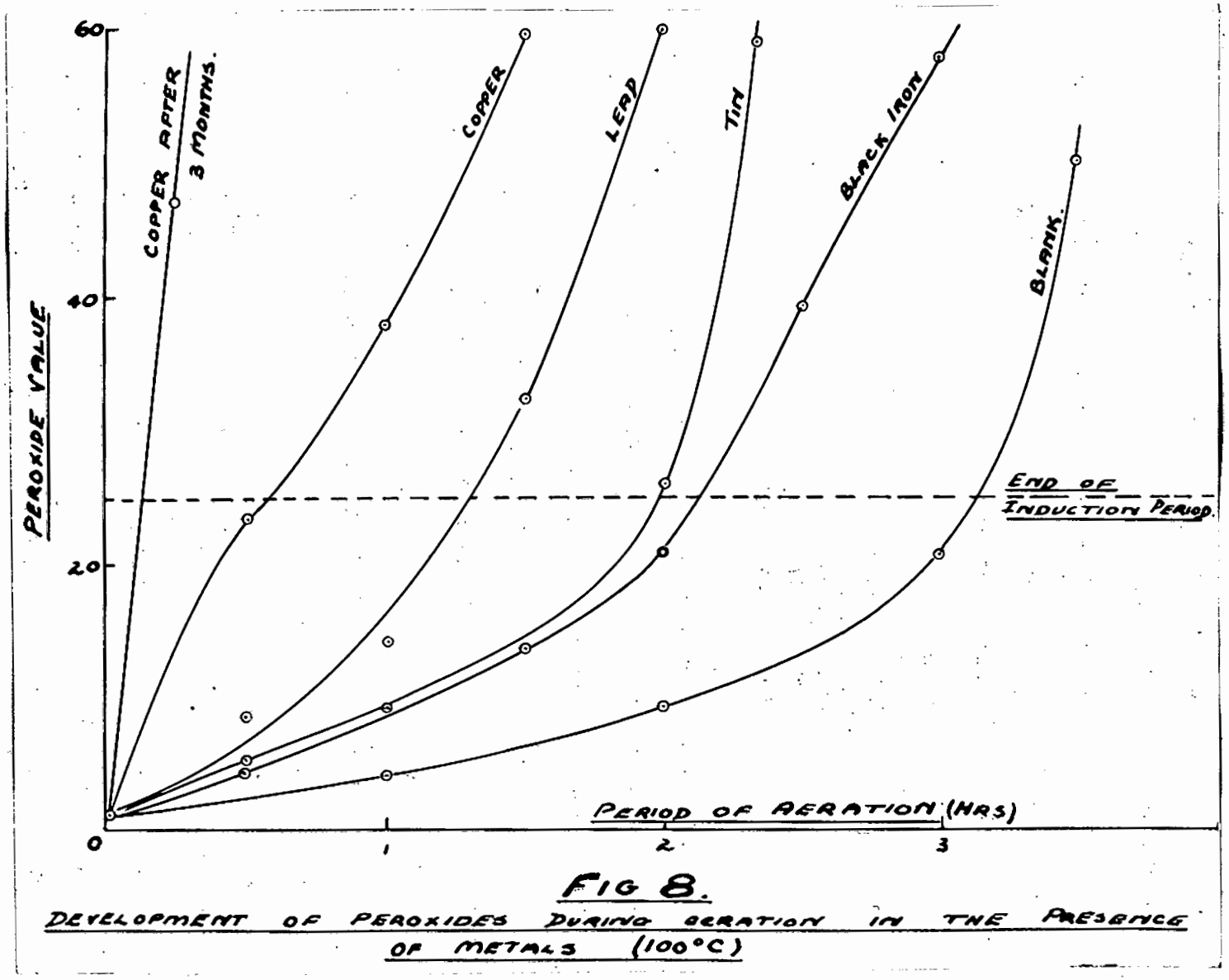

FIG 8.

DEVELOPMENT OF PEROXIDES DURING AERATION IN THE PRESENCE OF METALS (100°C)

In figure 8, a graphical illustration is given of the shortening of induction period when the oil is aerated in contact with copper, lead, tin, or new mild steel (black iron), as well as a curve to indicate the very rapid rate of peroxide development in an oil that had been stored in contact with copper for three months. It is quite obvious from the latter curve, that all resistance to oxidation had been destroyed.

If the metals are now arranged in decreasing order of activity (on the basis of % vitamin A destroyed during storage) the following grading is obtained :-

copper
lead
chrome steel
aluminium
mild steel
chrome nickel steel
galvanised zinc
tin.

While this arrangement differs from that obtained by a consideration of relative lowering of induction period, certain features are common to both. Copper, lead and chrome steel are the most active oxidation accelerators, while tin and galvanised zinc are least active. Aluminium, while not causing a very serious decrease of induction period during aeration, appears to be an active pro-oxidant for vitamin A.

On the whole, the serious losses of vitamin A appear to present an exaggerated picture of the influence of metals on the stability of the vitamin. These results do serve, however, to indicate that metals are powerful pro-oxidants; they serve also to give a comparison between the activities of various metals commonly used in plant construction, and the advantage to be had by using tin or tinned iron wherever possible.

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3. Effect of liver preservatives on stability of oils.

As far as could be ascertained, no attempt has ever been made to determine the storage stability of oils produced

...../from

from fish or fish livers which had been preserved in different ways.

Drummond and Hilditch[137] indicated the relationship between the time of storage of cod livers, and the quality of the resulting oil. With increased time of storage of liver, the oils become darker, and the free fatty acid content rises sharply. This action is probably directly due to action of the natural enzymes in the tissues. Bacterial contamination likewise can cause degradation due to the action of enzymes secreted by the bacteria. The production of lipases and lipoxidases by micro-organisms have been shown[138] to cause fatty acid formation and oxidative rancidity.

Modern methods of production of liver oils involve the use of alkalis, and temperatures of 100°C, and it can be expected that under these conditions the produced free fatty acids will be neutralised and all micro-organisms will be inactivated. However, we can never be sure that the resulting oil will not contain traces of degration products formed by oil-attacking organisms or by organisms that act on the protein material of the liver, and that these traces may not be just enough to catalyse further oxidative deterioration, as has been shown by Lea[139] in the case of pork fat.

The products of the metabolism of putrefactive bacteria, include such odorous materials as amines, skatole and indole[138], fatty alcohols etc., which are oil-soluble and will undoubtedly affect the quality of the oil, and may catalyse further decomposition.

A storage test has been carried out with the samples of oil obtained from livers preserved in different ways, as described earlier, in chapter 3, table 16. All samples were stored in well-filled glass containers, and put away in a dark cupboard, in order to rule out the catalytic

...../effects

effects of light and atmospheric oxygen. The samples of oil were not de-aerated or treated in any other way. At intervals of three months the induction period and vitamin potency of each sample were determined. The results are summarised in Table 36.

It will be remembered from Chapter 3, that the samples of liver were pickled in preserving solution, unless otherwise stated.

Discussion of results in Table 36.

(i) The variation in $E^{1\%}_{1cm}$ values of the samples is significant. The potency of any sample undoubtedly depends on degree of preservation. For the purpose of this test, however, more value is attached to further loss of vitamin A during storage than to loss during preservation of liver.

Note the smaller losses, during storage in oils from some of the well-preserved samples of liver (Nos. 7, 8,9) and on the other hand the bigger loss in the oil from unpreserved liver (No. 2).

On the whole, more significant results are obtained from a consideration of drop in induction periods, and these are more important, as induction period gives a direct measure of susceptibility to oxidation.

(ii) From a consideration of the initial induction periods, it appears that the efficiency of any preservative can be judged by the induction period of the recovered oil, and that the lack of preservative is clearly demonstrated by the low stability of unpreserved material viz. No. 2 which was further reduced to practically nothing after three months. The loss of potency over a period of one year is also big in this sample. The blank i.e. liver processed fresh, gave the highest induction period, and remained very stable during storage. Against this, the sample of liver stored at $0^{o}C$ for a week, yielded an

...../oil

TABLE 36.

Decrease in Induction Period (I.P.) and potency ($E^{1\%}_{1cm}$) during storage of oils from livers preserved in different ways.

No.	Preservative used with liver (Period of storage 7 days)	Induction Period of Oil					$E^{1\%}_{1cm}$					Total drop in I.P. (%)	Total drop in potency (%)
		0	3 mths.	6 mths.	9 mths.	12 mths.	0	3 mths.	6 mths.	9 mths.	12 mths.		
1	**Blank** i.e. processed immediately	2.8	2.6	2.5	2.2	2	28.5	27.3	26	26.7	26.5	29	7
2	No preservative - storage at room temp.	1	0.4	0	-	-	29.6	29.3	27.6	26.9	26.0	100	12.2
3	No preservative but stored at 0°C	2.1	2	1.9	1.8	1.7	28.1	27.5	27.6	27.1	26.5	19	5.7
4	1% Dry borax	2.3	2	1.8	1.8	1.7	29.6	28.1	27.6	27.6	27.1	26	8.5
5	1% Borax in sea water	2.5	2.1	2	1.9	1.7	29.8	28.5	27.7	27.5	27.2	32	8.7
6	0.1% do. do.	2.2	2.2	1.5	0.5	-	28.6	28	26	25.7	25.0	100	12.6
7	0.25% formalin (40%) in sea water	2.6	2.1	2.2	2	2	27.9	27.2	26.8	26.2	26.2	23	6.1
8	0.25% formalin in 10% salt water	2.7	2.2	2.2	2.3	2	29.2	27.9	27.9	28.2	28.1	26	3.8
9	0.1% Sodium nitrite in sea water	2.5	2.1	1.9	2	1.8	28	28.6	27.3	27.1	26.6	28	5
10	0.1% Sodium nitrite in 10% salt water	2.7	1.9	1.8	1.9	1.7	29.7	28.8	27.2	27.4	27.1	37	8.8
11	0.1% $NaNO_2$ plus 0.1% Hydroquinone in sea water	2.3	2	1.9	1.9	1.7	29.9	29.1	28.8	28.3	28.0	26	6.4
12	0.5% Sodium sulphite in sea water	1.4	1.4	0.5	0	-	28	27.7	26.1	25.8	25.3	100	9.6
13	0.5% Sodium sulphite in 10% salt water	2.1	2	1.6	1.7	1.5	28.8	28.4	28.5	28.2	27.9	29	3.1
14	0.5% Sulphite plus 0.1% Butyl gallate in sea water	1.4	1.2	0.5	0	-	27.7	26.7	26	26.1	25.8	100	6.9

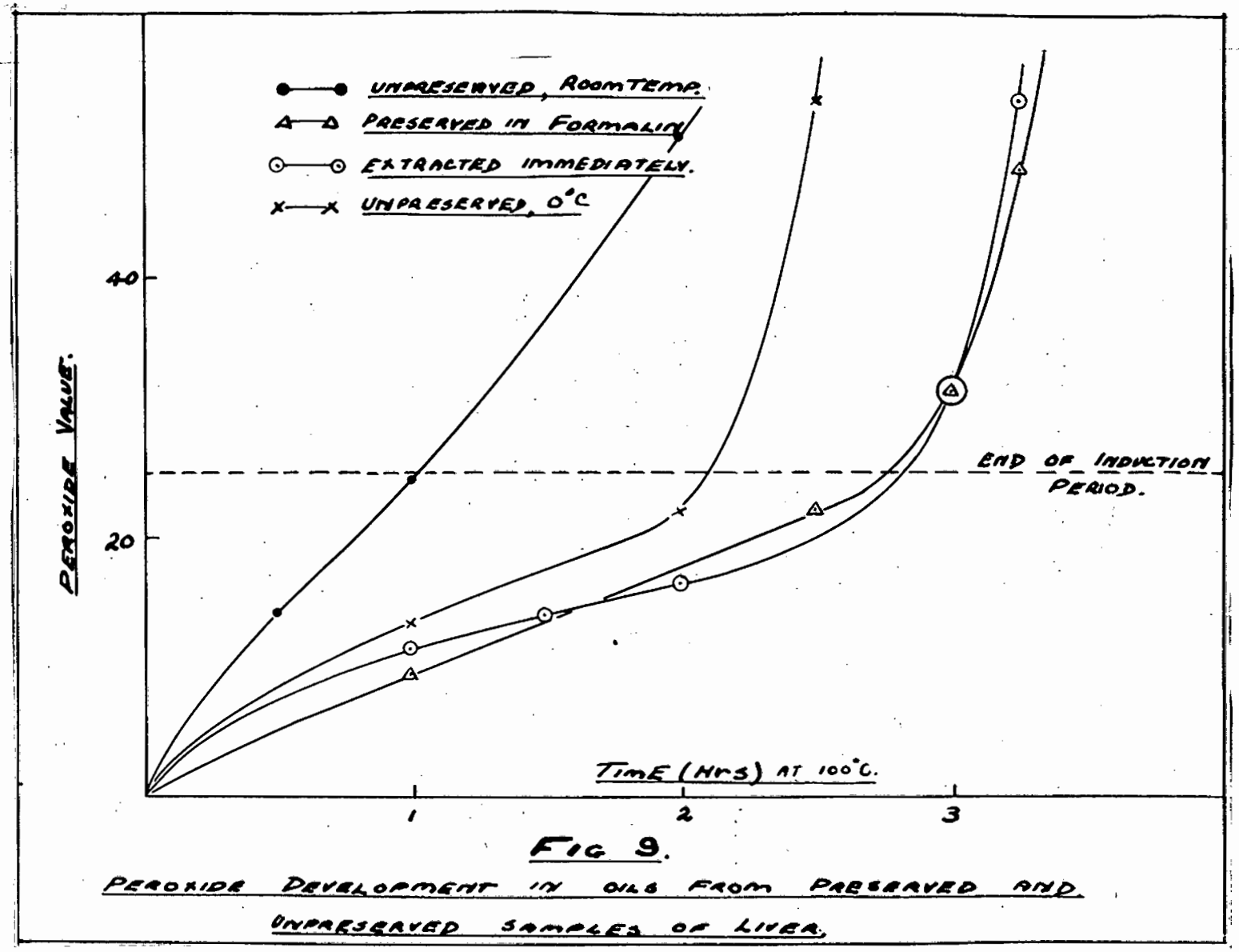

FIG 9.

PEROXIDE DEVELOPMENT IN OILS FROM PRESERVED AND UNPRESERVED SAMPLES OF LIVER.

oil with lower stability, judged by induction period, and did not lose more vitamin A during storage, in fact slightly less.

The well-preserved samples i.e. those preserved with borax, formalin and nitrite, all had induction periods of 2.2 - 2.7 hours, and these never dropped below 1.7 hours during storage. Lower, stabilites were obtained from the samples preserved with sulphite, and only sulphite in 10% salt water produced an oil of I.P. $\geq$ 2. Note too that the poorly preserved samples quickly lost whatever resistance to oxidation they had.

(iii) After one year the oils from liver preserved with formalin are the only ones with two-hour induction periods.

(iv) The oil from liver with 0.1% borax appeared to be quite stable. After 3 months however, there was a sharp decrease in induction period, and the total loss of vitamin amounted to over 12%, which is high under these favourable storage conditions.

(v) The most obvious deduction to be made from these results is that proper preservation of fish livers is an important matter, and should be attended to with care. Quite apart from the point of quality or physical appearance of the oil, proper preservation of liver has a marked bearing on storage stability of the oil.

In figure 9, a graphical illustration is given of peroxide development, during aeration at 100°C, of oils from preserved and unpreserved samples of liver. The prolongation of induction period caused by preserving the liver with formalin is significant, the rate of peroxide development being the same as in the freshly extracted oil. Preservation merely by storage of the liver at 0°C is less effective, and the much more rapid development of peroxides in oil from unpreserved liver is significant.

4. The effect of production methods and refining on stability of liver oils during storage.

In vegetable oils, which are as a rule much more stable than fish oils, and do not develop rancidity at the same rate, or to the same extent as fish oils, refining can be carried out to produce a palatable product without great concern about the effect that such a treatment may have on the suceptibility of the oil to oxidation.

In fish liver oils, however, where a decrease in induction period means increased danger of destruction of vitamin A, a careful investigation has to be made of the influence of refining methods on stability, before the relative merits of these processes can be assessed.

Mattill and Crawford[140] have shown that acid and alkali treatments, and decolourising processes, can shorten the induction period of crude maize oil considerably. With pilchard oil[141] the same effects were noticed, and a preliminary storage test indicated that such refined oils, after one month's storage, readily absorbed oxygen upon being placed in the oxygen absorption apparatus. Holm et al.[142] advanced the theory that such a reduction of induction period is due to destruction or removal of natural antioxidants. This explanation is now generally accepted, and is indeed easily understood in the case of alkali refining, which is bound to destroy phenolic inhibitors, which class represents most of the known antioxidants[98].

Brocklesby[143] points out that alkali or acid treatment increases the rate of peroxide formation in oils of low unsaturation, and completely removes the induction period in more highly unsaturated oils.

The effect of refining is noticed, not only by accelerated tests, but also at low temperatures, and it has been reported[144] that there is much more rapid development of peroxides in refined soybean oil during storage at 80°F

...../than in

than in crude oil.

It was decided then to compare the stabilities of primary and refined shark liver oils, and to determine further drops in induction period and vitamin potency in these oils during storage for one year.

For this experiment a sample of fresh primary liver oil was selected, and refined in several ways, as described in Chapter IV, i.e. alkali refined, bleached with terra silicea (diatomite), decolourised with Merck's clarocarbon, and steam-vacuum deodorised. Induction periods and vitamin A potencies of all the samples were determined, and the results were compared with those obtained from a few commercial samples of crude and refined vegetable oils. The vegetable oils had been subjected to alkali neutralisation, followed by steam-vacuum deodorisation and were not treated in any way before these tests were carried out. The results are tabulated in figure 37. Induction period was determined by aeration at 100°C and a peroxide value of 25 accepted as the end of the induction period.

Table 37.

Induction periods of crude and refined fish-liver and vegetable oils.

Raw material	Method of refining	Induction Period (hours)	Decrease in I.P. during processing
Shark liver oil	- (crude)	2.4	-
do.	steam-alkali	1.2	50
do.	decolourised - carbon	1.4	41.5
do.	bleached - diatomite	1.5	37.5
do.	steam-vacuum	1.4	41.5
Arachis 30/8/45)	- (crude)	2.7)	-
do.)	Alkali plus steam-vacuum	2.2)	18.5
Arachis 29/11/45)	- (crude)	3.1)	-
do.)	Alkali plus steam-vacuum	2.1)	32.2
Arachis 20/11/45)	- (crude)	6)	-
do.)	Alkali plus steam-vacuum	5)	16.7

...../Discussion

Discussion.

The results in table 37 illustrate :-

(i) A considerable variation in induction periods of crude arachis oils, even over a short period of three months (all samples from the same commercial firm) but

(ii) a consistent shortening of induction period caused by refining of these samples.

(iii) A considerable shortening of induction period in all the refined shark liver oil samples, indicating the drastic effect of all these refining processes. It appears that the natural induction period is reduced by about 35 - 50%.

The results in table 38 illustrate the decrease in potency and induction period during storage of these refined oils.

The unrefined blank lost about half of its natural resistance to oxidation and the vitamin potency decreased by 12% during storage for 12 months.

The alkali refined sample decreased in vitamin potency by only 16%, which is a relatively low figure if one remembers that refining caused no destruction of the vitamin. Upon storage for one year the induction period decreased by only 25%, but alkali refining caused a 50% decrease at the start, and the final sample, with an induction period of under one hour, can be regarded as very unstable and susceptible to oxidation.

The carbon-decolourised oil lost practically all resistance to oxidation after six months' storage, while the potency decreased by 18%, and this, in addition to a loss of 6% during processing, indicates that carbon decolourisation is perhaps the most harmful refining process to which a liver oil can be subjected.

There is little difference between the bleached and steam-vacuum deodorised samples. In both these experiments,

...../a loss of

TABLE 38.

Decrease of potency ($E^{1\%}_{1cm}$) and Induction Period in crude and refined samples of shark liver oil during storage at room temperature.

Samples	Induction Period					$E^{1\%}_{1cm}$					Total drop in I.P. (%)	Total drop in $E^{1\%}_{1cm}$ (%)
	0	3 mths.	6 mths.	9 mths.	12 mths.	0	3 mths.	6 mths.	9 mths.	12 mths.		
Original oil (crude)	2.4	1.9	1.5	1.3	1.3	9.95	9.64	9.38	8.97	8.73	46	12.3
Alkali refined	1.2	1.2	1.1	1.1	0.9	9.96	9.84	9.47	8.74	8.35	25	16.2
Decolourised - (Merck's clarocarbon)	1.4	1.2	1	0.4	0	9.32	9.32	9.12	7.98	7.63	100	18.1
Bleached - (Diatomite)	1.5	1.2	0.6	1	0.9	9.75	9.57	9.15	8.47	8.25	40	15.4
Steam-vacuum deodorised	1.4	1.1	1.2	1	0.9	9.71	9.61	9.33	8.68	8.08	36	16.8

a loss of 2% of the vitamin A occurred during refining, a further 15 - 17% during storage, while the induction period was shortened by about 40% during processing and again by 40% during storage.

These experiments seem to verify the observed decrease in stability caused by refining methods, and to indicate the danger of losing vitamin A during storage of refined liver oils, unless precautions are taken, such as the incorporation of suitable antioxidants, or storage in inert atmospheres, or at low temperatures.

As far as the use of alkalis in the production of liver oils are concerned, the destruction of natural resistance to oxidation is not so marked, as indicated previously (table 22, chapter III). In that series of experiments it was shown that liver oils with induction periods of 3 - 3.5 hours can be produced by alkali-digestion, and that the dessication-extraction method produced nothing better. These facts can probably be explained by considering that during alkali-digestion of livers, the alkali is present in much more dilute solution than is the case during alkali-refining, with less danger of destroying all the natural inhibitors.

---

5. Effect of dissolved air on storage stability and protection offered by inert gases.

Since oxidation is one of the most important causes of spoilage in fats and oils, many attempts have been made to pack or store fats or products containing fats, out of contact with air. The use and practicability of inert atmospheres have been described by several workers. The reports on the suitability of different inert gases have been rather conflicting. Emery et al.[146] found that an atmosphere of carbon dioxide does not prevent the development of rancidity in fats. Neither nitrogen nor carbon dioxide were found[137] to retard oxidation in cod liver oil, but the

...../latter experiment

latter experiment was only conducted by filling the head space above the oil with the gas under examination.

On the other hand, Callow[147] has found marked protection of bacon fat by storing in carbon dioxide. Fats stored in the gas did not develop rancidity during storage for twelve months while control samples, stored under atmospheric conditions, were quite rancid after four months.

An attempt has been made to compare the storage stability of an oil saturated with air i.e. as produced commercially, with the stabilities of oils in which the air had been displaced by nitrogen and carbon dioxide.

A good commercially produced liver oil was selected and one part stored as such in completely filled glass bottles. No head space was left, as the object was to determine the deterioration caused by dissolved air only.

A further portion of this oil was evacuated at 100°C, and then cooled to room temperature under an atmosphere of carbon dioxide, so that the oil could be considered saturated with the gas at ordinary temperature. The oil was stored in completely filled bottles, which were inverted so that an oil seal was obtained at the stopper. In the same way a further portion of the oil was saturated with nitrogen, and all samples were stored in a dark cupboard at room temperature.

At regular intervals (3 months) a sample of each oil was removed and the induction period and vitamin potency determined. The results are summarised in table 39.

Discussion.

Although this test has only been carried out on a single specimen of oil, the results are significant. There is very good agreement between the results for carbon dioxide and nitrogen, both stored samples being much better than the sample stored with dissolved air.

...../While the

Table 39.

Variations in induction period (I.P) and vitamin potency ($E^{1\%}_{1cm}$) of a liver oil stored in air, nitrogen and carbon dioxide.

Period of storage	Conditions of storage					
	Air		Nitrogen		Carbon dioxide	
	I.P.	$E^{1\%}_{1cm}$	I.P.	$E^{1\%}_{1cm}$	I.P	$E^{1\%}_{1cm}$
0	2.4	9.95	2.4	9.95	2.4	9.95
3 months	1.9	9.64	2.1	9.91	2.1	9.90
6 months	1.5	9.38	2.1	9.80	2.1	9.82
9 months	1.3	8.97	2.1	9.04	2.1	9.16
12 months	1.3	8.73	1.9	9.00	2	9.10
Total decrease	46	12.3	21	9.6	17	8.5

While the decrease in induction periods of samples stored in inert atmospheres is only 17 - 20% as against 46% for the control, the loss of vitamin A is also much smaller. It must be borne in mind that the raw material was a plant samples of liver oil which may have been produced from poorly preserved liver. This oil had also been in contact with iron digesters and storage tanks, and the degree to which further deterioration is retarded in such an oil, cannot but prove the beneficial effect of storage in inert atmospheres.

In plant practice this could be achieved by deaeration of freshly produced oil in a simple piece of equipment, and breaking the vacuum with carbon dioxide or nitrogen. In addition, the head-space in drums or storage tanks can be filled with the gas.

It was observed that the samples stored in nitrogen and carbon dioxide exhibited delayed stearin separation for the whole period. In the blank, stearin was quickly formed, and settled within two weeks to a thin and firmly

...../packed

packed layer at the bottom of all the containers. In the samples stored in inert atmospheres, there was no sign of "stearin" after a year, and the samples were sparkling clear right through.

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6. Effect of light on oxidative rancidity.

During the present investigation, no work has been done on the effect of light in promoting deterioration of fish oils, excepting the storage test recorded in table 30, Chapter V.

Normally, the commercial production of liver oils is organised in such a manner that the oil is very seldom exposed to light, and then only to diffused light.

However, a very brief review of the literature on this subject will be given in order to complete the review of factors influencing the stability of fish liver oils.

Ziels and Schmidt[133] show that strong light, mainly the portion of the spectrum between 325 and 460 millimicrons cause a marked deterioration in flavour and odour.

Coe[148] suggests that rancidity is due to photochemical activity of light. The pigments of animal or vegetable fats are photosensitive and may cause the formation of an unstable hydrogen peroxide which decomposes to form peroxides in the fat. The initiation of such a chain reaction can occur during the very short periods that the oil is exposed to light.

Lowen et al.[39] found that when oils were kept in stoppered bottles and exposed to diffuse daylight, no relation between peroxide formation and vitamin A destruction could be obtained, indicating that a different mode of autoxidation is catalysed in the presence of light.

The action of direct sunlight, diffused daylight, and electric light, on acceleration of oxidation in beef-kidney fat is strikingly illustrated by Lea[149].

...../From the foregoing

From the foregoing it is apparent that light can act as an oxidation accelerater, and that fish liver oils, or the livers, should not be exposed to light unnecessarily, and never to direct sunlight.

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7. Effect of moisture on storage stability of shark liver oils.

Although much work has been done on the influence of moisture on deterioration of products containing fats or oils as well as non-fat constituents, there is surprisingly little data available on the influence of moisture on the stability of pure fats.

While it has been found[150] that water increased the length of the induction period of butter-fat at 95°C, no influence could be detected on the induction period of lard[151]. Lea[152] found that water shortened the induction period of lard in glass.

King et al.[153] found in one test that the part taken by moisture, in the development of oxidative rancidity, as compared with other influences, was negligible.

It was decided to carry out a storage test in order to investigate the effect of moisture on stability.

Commercial liver oils normally contain 0.1 - 0.25% moisture and it would serve no useful purpose, from a practical point of view, to study the effect of higher moisture contents than this. The lower limit of moisture is determined by the moisture content of vacuum-dried oils, and this is usually 0.01 - 0.001%. So for the purpose of this test, two commercial oils A and B were selected, and their moisture contents determined. These oils were then dried under vacuum at 100°C for one hour, after which the moisture contents were again determined using the modified Smith, Bryant method as described in Appendix I.

The induction periods and vitamin A potencies of these oils were determined and they were then stored in

...../completely

completely filled and sealed bottles, so that the moisture contents could not change during storage. At three monthly intervals the same determinations were carried out. The results are recorded in table 40.

Moisture contents were as follows :-

Oil A dry 0.006%
Oil B moist ..... 0.21 %
Oil B dry ..... 0.01 %
Oil B moist ..... 0.23 %

Table 40.

Variations in Induction Period (I.P.) and vitamin Potency ($E^{1\%}_{1cm}$) during storage of dry and moist shark liver oils.

Period of storage	Description of Sample.			
	Oil A dry	Oil A moist	Oil B dry	Oil B moist
(a) Induction period.				
0	3.9	3.9	3.2	3.2
3 months	3.7	3.7	3.1	3
6 months	3.5	2.9	3.1	2.9
9 months	3.5	2.8	2.6	2.4
Total drop (%)	10.3	28.1	18.7	25
(b) Vitamin A potency ($E^{1\%}_{1cm}$)				
0	22.50	22.50	23.51	23.51
3 months	21.70	21.61	23.45	23.16
6 months	20.43	20.72	22.90	22.88
9 months	20.01	20.20	21.72	21.33
Total drop (%)	11.1	10.2	7.7	9.3

Discussion.

From the results in table 40 it is apparent that the total decrease in induction period is less in a "dry" oil than in a normal "moist" liver oil. In sample A the difference is very significant, but even in sample B, the vacuum-dried oil was more stable than the "moist" oil after nine months.

As far as vitamin potency is concerned, the total loss, as far as oil A is concerned, is slightly higher in the dried sample, but in oil B, the dried sample suffered

...../a slightly

a slightly smaller loss of vitamin A during storage.

On the whole, these results suggest a mechanism of degradation whereby the susceptibility to oxidation is increased by the presence of moisture, without a corresponding influence on the vitamin A being noticeable.

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8. Summary - Precautions to be taken in the production and storage of fish liver oils.

For the benefit of those concerned with the production of fish liver oils, a summary will be given of the points to be watched carefully in this industry. The author feels convinced that the commercial producer of fish oils can never exercise too much care in controlling the conditions of production and storage, and only by taking all possible precautions, at every stage, can a good and stable oil be produced and, loss reduced to a minimum.

The following precautions are necessary :-

(1) The gall bladder should be opened and the gall extruded immediately the liver is taken out of the fish. The liver should then be rinsed in clean sea water, and immediately placed, and weighted down, in a preservative solution of sea water containing 0.25% formalin (40%) on the weight of liver, and 1 part in 2,000 sodium nitrite. If the livers are being collected in a 45 gallon drum, the drum should contain about 4 - 6 gallons of water in which had been dissolved 1 - 2 lbs. commercial formalin, and ½ - 1 oz. sodium nitrite. The lid of this drum should never be left off, and full drums of liver should always be stored below deck, out of the heat and light of the sun.

Alternatively the washed liver can be rolled in powdered borax, containing no lumps, but this must

...../be done

be done carefully and not haphazardly, so that all parts of the liver can come in contact with preservative.

(2) While bearing in mind the practical difficulties, the necessity of landing and processing the livers with as little delay as possible, is stressed.

(3) Absolute cleanliness of equipment is essential. All run-ways and machinery should be hosed and "steamed down", while digestion and storage tanks, as well as containers for the liver, should be cleaned spotlessly and sprayed with a dilute solution of formalin. Stickwater contains dissolved protein that is an excellent medium for growth of bacteria, and should not be allowed to dry on the surfaces of containers. Care should be taken that a "ring" of oil never forms in settling or digestion tanks, as this will oxidise quickly and contaminate later batches.

(4) The use of tin or heavily tinned steel is recommended for construction of all containers, including drums for the collection of livers. On no account should copper or brass be used, and corroded iron surfaces should be avoided.

(5) If the livers were particularly old and the separated oil is dark and rancid, alkali-refining is necessary, and will result in considerable improvement in quality.

(6) The incorporation of suitable antioxidants should be regarded as a necessary step in production. There are so many factors that influence the stability of liver oils adversely, that artificial means of counteracting this must be resorted to .

(7) If oils are to be stored for periods exceeding three months, de-aeration and saturation with nitrogen or carbon dioxide will be beneficial.

VI. APPLICATION OF PRIMARY FISH LIVER OILS.

A. RAW MATERIAL FOR PREPARING CONCENTRATES.

The bulk of the South African production of fish liver oils is used for preparing concentrates which are used for food fortification, margarine manufacture and therapeutic purposes. This is perhaps the reason why more importance has always ben attached to quantity than to quality. This is logical in a way, but the producer of primary oils can never lose sight of the fact that oils of poor quality are always more susceptible to oxidation and more difficult to refine, and the quality and stability of the concentrates produced in this country will depend to a considerable degree on the quality of the raw material. Normally it will not be necessary to refine liver oils which are to be concentrated, provided all precautions against spoilage had been taken in their production.

B. MANUFACTURE OF MARGARINE.

It is not essential to use vitamin A concentrates for margarine fortification. A refined and sweet oil containing 50,000 I.U. vitamin A per gram is suitable, and approximately one pound of oil will then be required to fortify a ton of margarine[176], so that this represents a large possible outlet for refined liver oils of fairly high potency.

It was planned originally to investigate the suitability of refined shark liver oils for margarine fortification and to determine loss of vitamin potency during manufacture and storage. This has not been possible owing to the fact that no start has yet been made with the manufacture of margarine in South Africa.

C. ANIMAL FEEDING.

Unlike the position in America, practically no

...../fish liver

fish liver oil is used for animal feeding in this country. Manufacturers of balanced animal rations maintain that enough carotene is supplied by some of their raw materials, such as lucerne meal and maize.

D. THERAPEUTIC USES.

Refined fish liver oil is used in the manufacture of a large number of pharmaceutical preparations such as vitamin capsules, emulsions, syrups etc., or the oil is consumed as such.

Owing to the characteristic fishy odour and taste, children are usually very reluctant to take the undisguised oil. Many masking flavours have been suggested and tried out, but very few of them appeal to everybody. The most suitable flavouring material appears to be sweet orange oil or lemon oil, and 1 - 2% added to fish liver oil, produces a much more palatable product.

In many countries it has become customary to prepare vitaminised sweets and to include this in the diet of all school children.

A few experiments have been carried out to investigate the suitability of refined soupfin shark liver oils for this purpose and to determine losses of potency occurring during manufacture and storage for one month, which is regarded as the normal maximum period of storage of confectionery before it is consumed.

With the assistance of a confectionery manufacturer, several types of vitamin A-fortified sweets have been prepared viz. chocolates, toffees, soft gelatine sweets, acid drops, and soft-centred chocolate bars. Conditions of manufacture were varied so that temperatures of 80 - 230°F were employed. In all cases excessive stirring was avoided, to minimise the danger of oxidation. Normal storage conditions were simulated i.e. sweets were stored

...../in closed

in closed boxes at room temperature. Vitamin A was determined by the method described earlier[165]. All sweet were fortified to the extent of approximately 4000 I.U. vitamin A per ounce, using an oil containing 25,000 I.U/ gram. The results are summarised in table 42.

Table 42.

Destruction of vitamin A during manufacture, and storage for one month, of vitaminised sweets.

No.	Description of sample	Temperature of manufacture (°F)	Loss of vitamin A during manufacture (%)	Further loss of vitamin A after one month (%)
1.	Plain chocolate slab	80	9.1	12.8
2.	do. do.	120	15.5	13.6
3.	Chocolate with soft centre, all fish oil incorporated in centre	80	10	5.9
4.	Toffee	120	16	15.3
5.	Toffee	230	53	14.2
6.	Toffee	160	25	21.2
7.	Soft gelatine sweets	-	40	26.7
8.	Hard acid drops	120	60	31.1

Discussion:

Several interesting observations can be made from the results in table 42.

It is clear that serious losses of vitamin A occur during manufacture and storage. In samples 1 and 2 the influence of temperature is reflected in the initial losses, while further loss during storage remains practically constant. The same effect is noticed in three similar samples of toffee viz. 4, 5 and 6, where the influence of temperature is marked.

Note the relatively small loss of potency in sample 3, i.e. the soft-centred chocolate. During storage for one month the loss amounted to only 5.9% and this must

...../be due

be due to the protection against atmospheric oxidation obtained with the outer chocolate layer.

The vitamin A loss in acid drops, both during manufacture and storage, is considerable and must be ascribed to the high citric and tartaric acid contents of these particular sweets. Likewise, a serious loss was noticed in the soft gelatine sweets. Loss during storage can perhaps be explained by the fact that these sweets were translucent and unwrapped, and the catalytic effect of light may have accelerated oxidation.

In this experiment various flavouring materials were used, and the best results were obtained with orange and peppermint. They both mask the fishy flavour so well that no fishiness can be detected in properly prepared samples.

These results may assist to clear several points in regard to the manufacture of vitaminised sweets, and a brief summary of the most important points will be given :-

(a) Suitable flavourings, such as mint or orange should be used.

(b) Best protection of vitamin A obtained by incorporating the fish liver oil into the soft centre of chocolate-coated sweets.

(c) Low temperatures during manufacture is necessary, preferably below 100°F.

(d) Excessive acid ingredients should be avoided.

(e) Thorough mixing of all ingredients to ensure even distribution of vitamin A, but aeration caused by excessive stirring must be avoided.

(f) Sweets should be wrapped in dark wrapping materials to exclude light.

(g) Rapid distribution of vitaminised sweets.

VII. THE MANUFACTURE OF VITAMIN A CONCENTRATE.

(1) REVIEW.

The average vitamin A content of primary fish liver oils produced in this country is about 0.75%. Many attempts have been made to prepare concentrates, and so many modifications of these methods have been described and patented, that a considerable bibliography on the subject already exists.

The advantage of concentrates lies in the ease of administration to humans and animals, owing to the smaller doses required. The fortification of food is also greatly facilitated, and the danger of spoiling the flavour of such foods is ruled out.

The three most important concentration processes are the saponification-extraction method, the "short-path" or molecular distillation, and the differential solubility method.

(a) Concentration by saponification.

The oldest and best-known of these processes is the saponification-extraction method in which the oil is saponified, either partically or completely, and the unsaponified or unsaponifiable portion extracted with a solvent, and recovered by evaporation of the solvent. Caustic soda or caustic potash is usually used for saponification while ethyl ether and ethylene dichloried are the two most popular solvents.

The disadvantages of this process are:

(i) loss of vitamin A due to incomplete extraction by the solvent. Recovery of vitamin is seldom over 85% in plant operation.

(ii) fire hazard and/or formation of emulsions with most solvents;

(iii) hydrolysis of the vitamin to the alcohol form

...../which is

which is more susceptible to oxidation and claimed to have a lower biological activity;

(iv) limitation of degree of concentration, by content of unsaponifiables in primary oil. An appropriate example is dogfish liver oil containing up to 10% of unsaponifiable constituents and with a potency of about 10,000 I.U. vitamin per gram. By complete saponification the vitamin can only be concentrated ten times and with an average plant recovery of 85%, the concentrate will only have a potency, of 85,000 I.U./gram, which does not warrant the cost of processing.

A review[169] of the literature on concentration by saponification indicates the many patents that have been taken out for complete or partial saponification of liver oils. Quite recently, further modifications of the partial-saponification-solvent-extraction method have been described[170]. These refer chiefly to the use of new solvents such as isopropanol, and the mixing of oil and solvent prior to addition of alkali.

It is very doubtful, however, if these new developments will revive this old-fashioned process which appears to have been superseded by more modern discoveries.

The recovery of vitamin A in the unsaponifiable matter of South African fish liver oils has recently been studied by Black et al.[184] These workers found an average recovery of 93.3% of the vitamin A in the unsaponifiable matter of twenty samples of soupfin shark liver oil, covering a range of $E^{1\%}_{1cm}$ values from 6.86 - 98.5. In the case of dogfish, geelbek (Cape salmon) and kabeljou, the average recoveries were 94.3, 94.6 and 94.4% respectively, while a recovery of 99% is reported in seventeen samples of hake liver oil.

...../These results

These results show clearly that 93 - 94% must be regarded as the theoretical maximum recovery of vitamin A in saponification-extraction methods of concentration. In large-scale operations one can expect much lower recoveries as a result of inevitable losses.

(b) Concentration by molecular distillation.

The so-called "short-path" distillation is a new discovery, not fully ten years old, and which has only been applied commercially for a few years.

The process is gaining importance rapidly, and has several distinct advantages to recommend it. The process, which has been perfected and described by Hickman[171, 172, 173] and his associates, consists essentially of subjecting the vitamin containing oil to an elevated temperature (170°C - 240°C) for a short period under very high vacuum (0.001 mm. Hg. or less). This procedure causes some of the oil and proportionally more of the vitamin ester to vaporise. The vapours are condensed on adjoining ($\frac{1}{2}$" - 4") cool surfaces. The condensate is therefore a fraction of the primary oil greatly enriched in vitamin. The operation, recently described in detail by Olive[174], is difficult to control and calls for specialised equipment due to the very high vacuum required, and the necessity of subjecting the oil to the high temperature for a very short time only.

The great advantages of this process are firstly that it produces vitamin A in ester form, and secondly that the bulk of the oil is not lost or converted into less valuable fatty acids as in the saponification process.

The great disadvantage of this process is the high cost involved. It has not yet been possible to produce vitamin D concentrates at a competitive price,

...../and even

and even as far as vitamin A is concerned, the process may be ousted by cheaper methods.

(c) Concentration by selective solubility.

The latest method for concentrating vitamin A, or the so-called propane-process, is not yet in commercial use. Very shortly, however, the first commercial plant in the world will be erected in South Africa, and the author is privileged to give a brief description of the process, which has not yet appeared in public print.

The process employs liquid propane as a selective solvent to concentrate both vitamins A and D, and produces vitamin A in the ester form, while the stripped crude oil is not destroyed or changed, and is available for commercial purposes as in the molecular distillation process.

Briefly the process consits of dissolving the primary vitaminiferous oil in several volumes of propane, and neutralising free acidity, all under pressure. The neutral oil-propane mixture is passed into a tower and washed counter-current with a stream of fresh propane. By careful pressure and temperature control, and suitable temperature gradients, two immiscible phases separate continuously in the tower. The upper phase consists of propane with a small amount of oil, and contains almost all the vitamins, and passes continuously to a solvent recovery plant from which the vitamin concentrate is recovered. The lower phase is passed into a second tower, where it is stripped of colour bodies and other impurities again with a stream of fresh propane, and then passes to a solvent recovery unit from which the stripped and refined liver oil is obtained.

This process, unlike molecular distillation, pro-

...../duces

duces a concentrate containing vitamins A and D in the same ratio as present in the crude oil. A further consideration, in fact the most important feature of this process, is its inexpensiveness. The cost of concentration is estimated at about one-tenth of that of molecular distillation. These features, in addition to the advantages of molecular distillation which the process also embodies, may yet elevate the propane process, at the expense of all the others, to the position of No. 1 process for concentration of oil-soluble vitamins, and we can only hope that this will eventually result in a considerable decrease in price of vitamin A and D concentrates.

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(2) METHODS EXPLORED.

(a) Several attempts have been made to concentrate vitamin A by partial or complete saponification.

(i) In the first of these experiments the oil was "cold-saponified" i.e. heated to 65°C and the requisite amount of caustic soda in the form of a 20% aqueous solution added slowly, while the oil was being stirred continuously saponification proceeds rapidly under these conditions, and the reaction is complete in 15 - 30 minutes.
The concentrate was recovered from the saponified mixture in several ways. It was hoped that it might be possible to dissolve the soaps in hot water and recover the unsaponified fraction by centrifuge. This method was not feasible owing to the enormous volumes of water required to bring all the soap into solution, and persistent foaming during centrifugal separation.

(ii) A second alternative is to use solvents for recovery of the unsaponified or unsaponifiable

...../portion

portion of the oil. When the saponified mixture is added to the solvent great care has to be exercised to avoid excessive stirring, as very persistent emulsions are formed. When ethyl ether was used, emulsification could be avoided, but with ethylene dichloride, a very popular solvent[175], it was almost impossible to avoid emulsification. In all trials, the recovery of vitamin A was never more than 70%, and more often in the neighbourhood of 50% The low recoveries can undoubtedly be ascribed to absorption of the vitamin on the soap, more intimate contact between soap and solvent resulted in very stable emulsions, and the method was not pursued any further.

More successful attempts were made by partial or complete saponification of the oil with alcoholic alkali. The resultant alcoholic solution of soap and unsaponified oil was suitably diluted with water and extracted with ether or ethylene dichloride by counter-flow in a vertical packed tower, according to well-known and standard procedure. Care has to be exercised in adjusting the rates of flow of solvent and soap solution so that a clean break occurs in the middle of the tower.

Several trial saponifications were conducted to determine the optimum concentration alkali to be used, the proportion alcohol in the mixture, the quantity solvent for complete extraction, and the time required for complete conversion.

The quantity of caustic soda required depends of course on the saponification equivalent of the oil. Normally 15 grams per 100 grams of oil ensure complete saponification, and provide a suitable excess for

...../most shark

most shark liver oils. The alkali is dissolved in 70% ethyl alcohol solution to ensure best results during subsequent extraction. If the concentration of alcohol is much higher, it is very difficult to obtain separation in the extraction tower. Time of reaction is one hour - the reaction is probably complete after about 30 minutes in most cases, but certain oils do require longer than that.

The following example of a saponification mixture has been found suitable :-

100 g. liver oil
15 g. NaOH.
95 ml. ethyl alcohol (95%)
30 ml. water.

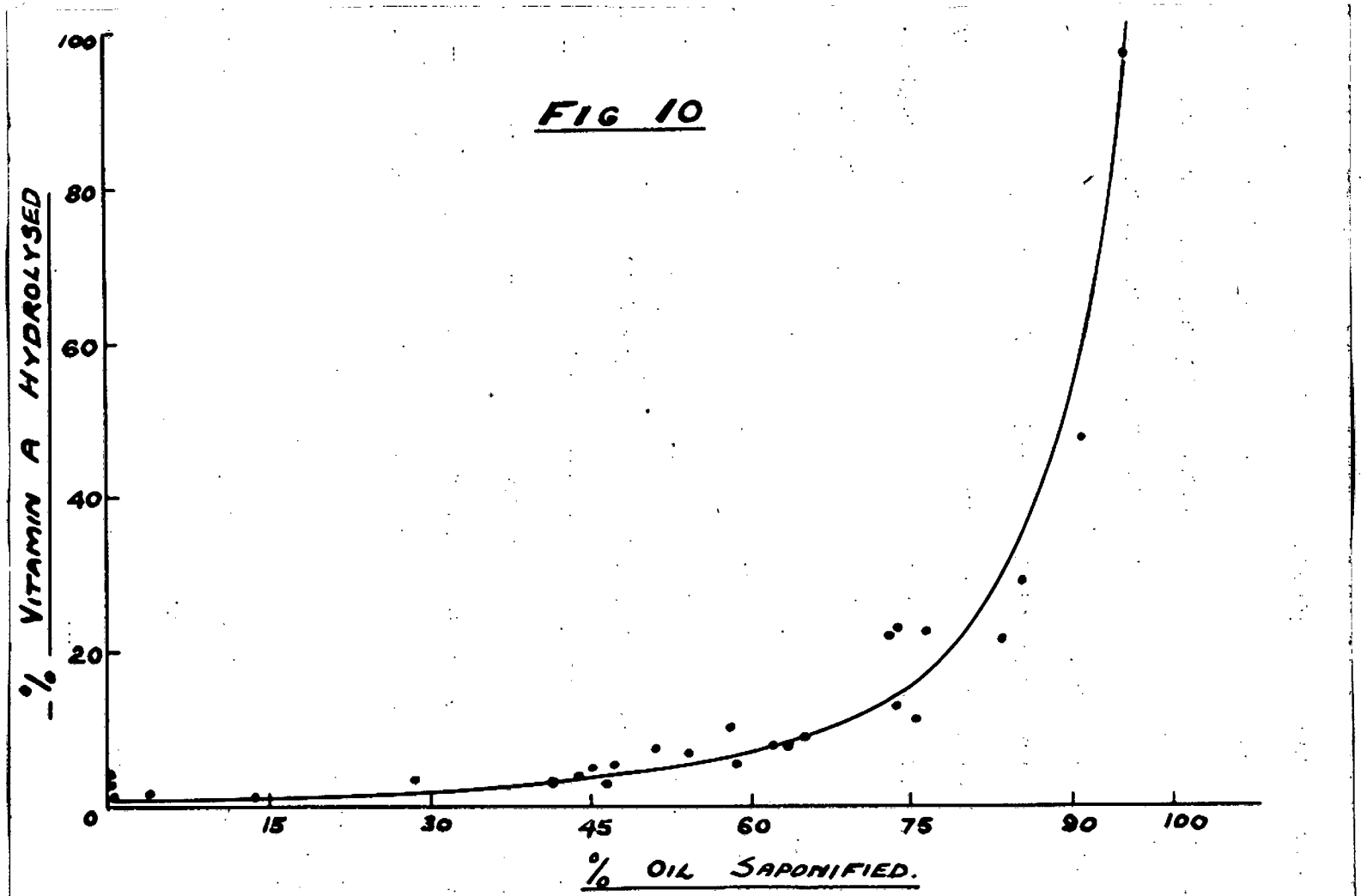
The mixture is refluxed for one hour, after which 450 ml. water are added. The solution is cooled to room temperature, and passed slowly into the bottom of a packed tower through which ethylenedichloride is passing downwards, the whole column being full of solvent at the start of operations. The rates of flow are adjusted in such a way that separation occurs about one-third of the way down the column.

After all the soap solution has passed through the column, the ethylene dichloride is re-circulated while water is passed through counter-current. After washing, the ethylene dichloride is evaporated, and the unsaponifiable fraction and/or unsaponified portion of the oil recovered.

In the first experiments recovery of vitamin A was only 50 - 60%. With improvements in the technique, recoveries of 88 - 92% were recorded. These figures appear to be low, but it is very doubtful whether better yields are ever obtained in commercial practice (See this chapter (1)(a)).

(b) Survival of vitamin A esters during partial saponification.

In the course of these experiments, the author



HYDROLYSIS OF VITAMIN A ESTERS DURING PARTIAL SAPONIFICATION OF LIVER OIL.

has evolved a new method of applying the partial saponification method for concentration. This new process has some very admirable features to recommend it, and it is hoped that the method will find commercial application in the processing of low-potency oils such as dogfish or stockfish liver oils. Patent considerations make it impossible to disclose any details at this stage, and the author's contribution in this respect cannot proceed beyond a summary of work done to study the survival of vitamin A esters during partial saponification.

It has been stated earlier, that every attempt should be made to preserve the vitamin A in ester form, and it is not known under what conditions the fatty glycerides will saponify preferentially, if at all. This will undoubtedly depend on such factors as temperature, concentration of alkali used, period of contact between alkali and oil, nature of oil etc.

The liver oils of a few species of fish were partially saponified, under varying conditions, the resultant oils were extracted with ether, recovered, and assayed for free and esterified vitamin A. The results are summarised in table 41 and illustrated graphically in figure 10.

Discussion:

From the data in table 41 and fig. 10, it appears that 50% of the oil can be saponified before the vitamin A esters are hydrolysed. The presence of approcimately 5% free vitamin A up to this point must be ascribed to limitations of the method of estimating free and esterified vitamin A (see Appendix II).

If more than 50% of the oil is saponified, the vitamin A appears to be attacked gradually, so that at 65% saponification about 9% of the vitamin appears to

...../be present

be present in the alcohol form. The actual content of free vitamin A will probably be less, if allowance is made for the inaccuracy of the method.

Table 41.

Survival of vitamin A esters during partial saponification.

Species	Degree of Saponification (%)	% Vitamin A present in esterified form	% Free or hydrolysed vitamin A
Stockfish liver oil	0	96	4
	45.5	95.1	4.9
	54.5	93.3	6.7
	51.5	92.5	7.5
	62	92	8
Cape Salmon (Geelbek) liver oil	0	99.1	0.9
	3.9	98.2	1.8
	28.7	96.2	3.8
	64	92	8
	73	77.5	22.5
	74	76.6	23.4
	76.2	77	23
	95	3.5	96.5
Soupfin shark liver oil 1	0	96.5	3.5
	41.2	96.6	3.4
	44	96.4	3.6
	46.3	96.8	3.2
Soupfin shark liver oil 2	13.7	99	1
	29.2	99.5	0.5
	47.5	95.2	4.8
	58	90.8	9.2
	65.2	91	9
	75.3	88.5	11.5
	90.9	52.1	47.9
Soupfin shark liver oil 3	41.5	96.9	3.1
	58.5	94.3	5.7
	73.8	87.8	12.2
	85.5	71	29
	83.3	78.5	21.5

At 75% saponification, about 22% of the vitamin was hydrolysed in the case of geelbek liver oil, but only 11 - 12% in the soupfin shark liver oil.

Even at 85 - 90% saponification, not more than 30% of the vitamin A appears to be hydrolysed, but beyond 90% saponification the conversion to the alcohol form is

...../probably rapid

probably rapid.

From these figures it definitely seems as if the glycerides are saponified preferentially and that vitamin A esters are much more resistant to attack by alkalis. This means that partial saponification can safely be carried to a point where about two-thirds of the glycerides will be saponified before the vitamin A is seriously affected.

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(3) **PROSPECTS.**

In view of the decision to erect a vitamin concentration plant employing the propane process in South Africa, further work on the new partial saponification process, discussed in the previous paragraph, has been suspended. The method depends in part on extraction of the concentrate with a suitable solvent, and as soon as the implications of the propane technique is fully understood, this work will be resumed. The partial saponification process will undoubtedly supplement the propane process in a useful manner. If all primary oils can be saponified to the extent of 50%, the capacity of the propane concentration unit will be doubled.

APPENDIX I.

A note on the estimation of water in fish oils.

When the influence of moisture on stability of shark liver oils was investigated, a rapid and accurate method for estimation of water was required to replace the usual but unsatisfactory method of drying the oil at 100°C under vacuum to a constant weight.

The Smith and Bryant method[156], for estimating small quantities of water in organic liquids, has been tried out, and modified suitably. It is a simple and rapid method, and gives results which are within the accuracy claimed for the method.

The modified method was used to estimate water in a number of fish liver oils. Known quantities of water were added to the samples, and total moisture content again determined. The results are given in table AI.

Table AI.

Moisture and added moisture estimation in oil.

Material	Moisture content (%)	% H2O added	Total % H2O estimated	Error (%)
Liver oil A	0.134	0.20	0.334	0
Liver oil B	0.166	0.2624	0.428	-0.10
do.	0.165	0.2624	0.424	-0.83
Liver oil C	0.10	0.246	0.340	-1.73
Liver oil D	0.175	0.486	0.655	-0.91
Arachis oil	0.077	0.673	0.728	-2.90
Liver oil E	0.163	0.213	0.372	-1.06
Liver oil F	0.181	0.305	0.482	-0.82
Average error				-1.04

The average error is 1%, whereas no greater accuracy than plus minus 1% is claimed for the original method.

A description of the modified method will be given, because it may be found useful, and even the original method

...../is not

the precision about 1%.

The absolute alcohol must not contain more than 0.15% water, and it is necessary to dry commerical absolute alcohol over sodium, and re-distil, before it will be suitable for this determination.

APPENDIX II.

Quantitative estimation of the alcohol and ester forms of vitamin A.

In the work on alkali-digestion of liver and alkali-refining of the oil, as well as on partial saponification of liver oil, it was necessary to determine the ratio of esterified to free vitamin A in the oil, since it has been claimed[154, 157] that the ester form has a higher biological activity and also that the alcohol form is more susceptible to oxidation[162]. Every attempt should therefore be made not to hydrolyse the vitamin, which occurs naturally as esters of fatty acids.

The analytical method of Reed et al.[155] has been tried out on the following oils:-

(i) Primary shark liver oil $E^{1\%}_{1cm} = 20.4$

(ii) Same oil, completely saponified and unsaponifiable fraction diluted to original weight with arachis oil.

(iii) Mixture consisting of 2 parts of (i) plus 1 part of (ii).

As sample (i) is a primary oil, it could be expected to contain mainly vitamin A ester. Sample (ii) on the other hand could be expected to contain only free vitamin, while (iii) should contain 65% esterified vitamin.

These samples were assayed and the results are summarised in table AII. In addition to these samples, the analytical figures for a few alkali-digested oils before and after alkali-refining, are given, and the analysis of an oil extracted by solvent, after dessication of the liver with anhydrous sodium sulphate.

Discussion:

From these results it would appear :-

(i) The indicated presence of 4.2% esterified vitamin A in the completely saponified oil (sample 2) must either be ascribed to limitations of the method of analysis or

...../to the

to the the fact that it is difficult to hydrolyse vitamin A completely under conditions that will easily saponify the fatty acid glycerides. Note that this same sample (No. 10, table AII) was analysed by chromatographic absorption of free vitamin A on alumina. By this method 5.3% of the vitamin was not absorbed and again this may be due to method error or to a genuine survival of approximately 5% of the vitamin A as ester in a "completely saponified" oil. The agreement between the two methods almost leads one to believe that normal saponification procedure will not hydrolyse vitamin A completely, a theory which is strengthened by the results in table 41 (fig 10) where it has been indicated that fatty acid glycerides are saponified preferentially. However, in the absence of definite proof, the presence of 5% of the vitamin A, as ester, in completely saponified oils, will be regarded as due to method error, and all figures for vitamin A ester content of oils must be regarded as about 5% too high.

Table AII.

Free and esterified vitamin A in fish liver oil.

No.	Sample.	% of vitamin A present as ester	% of vitamin A present as alcohol
1	Primary liver oil 1 (alkali-digested)	95.6	4.4
2	Non-sap. of 1 in arachis oil	4.2	95.8
3	2 parts 1 plus 1 part 2	63.5	36.4
4	do. do.	65.6	34.4
5	Primary oil 2	96.0	4.0
6	Primary oil 2 alkali-refined	95.5	4.5
7	Primary oil 3	96.3	3.7
8	Primary oil 3 alkali refined	96.6	3.4
9	Oil 4, solvent extracted	93.7	6.3
10	Sample 2 above, assayed by chromatographic method	5.3	78

...../(ii) Alkali-refining

(ii) Alkali-refining does not hydrolyse the vitamin A ester, and

(iii) Alkali-digestion of livers does not hydrolyse the vitamin A.

(v) The method is satisfactory considering how well the results of 3 and 4 agree with the calculated values.

APPENDIX III.

Analytical Methods.

1. Vitamin A.

(i) Vitamin A in oils or $E^{1\%}_{1cm}$ 328 mμ was determined by means of the Carr-Price reaction[166, 167], with antimony trichloride in chloroform, using a photo-electric colorimeter at wavelength 620 mμ.

(ii) Vitamin A in vitaminised chocolate and sweets was estimated by the Raynes and McLellan's application of the Carr-Price reaction[165].

2. Iodine value.

This was determined by the Wijs method, the time of contact being one hour[158].

3. Peroxide value.

This was determined by the Wheeler method[159].

4. Saponification Equivalent.

This was determined by the A.O.A.C. method[168], a period of 20 minutes being allowed for refluxing.

5. Laboratory extractions of oils.

Quantitative extractions were done by the methods described by Rapson et al.[182]

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TABLE 4.

FRACTIONAL DISTILLATION OF LIQUID ESTERS OF FAT FEMALE SHARK LIVER OIL (103.5 g. distilled.)

No.	G.	Temperature: Bath	Temperature: Column: Middle	Temperature: Column: Head	Calculation: S.E.	Calculation: I.V.	Saturated: C.14	Saturated: C.16	Saturated: C18	Saturated: C20	Saturated: C22	Saturated: C24	Composition of Ester Fractions: Unsaturated: C.14	C.16	C.18	C.20	C.22	C.24	Total
L. 1.	4.970	229 °C	176 °C	120 °C	261.7	87.2	0.6023	1.601					0.460	2.307					4.970
L. 2.	6.564	221	206	141	273.4	116.7		1.721						3.266	1.577				6.564
L. 3.	6.354	225	222	143	281.2	141.4		2.274						1.489	2.591				6.354
L. 4.	5.718	229	226	147	285.	155.4		0.424						1.396	3.898				5.718
L. 5.	6.187	232	227	150	288.	169.								1.260	4.927				6.187
L. 6.	6.191	234	228	152	294.6	185.3									5.976	0.215			6.191
L. 7.	7.438	236	228	153	297.9	202.9									6.112	1.326			7.438
L. 8.	6.832	239	239	157	303.2	227.6									4.107	2.725			6.832
L. 9.	8.029	245	244	162	309.9	263.2									2.637	5.392			8.029
L.10.	7.327	247	244	165	315.6	301.1									0.712	6.615			7.327
L.11.	7.241	249	245	169	320.2	324.7										6.574	0.667		7.241
L.12.	5.531	250	244	165	325.7	340.4										3.766	1.765		5.531
L.13.	6.739	252	244	168	330.7	354.3										3.255	3.484		6.739
L.14.	4.686	255	246	165	334.5	369.8										1.601	3.085		4.686
L.15.	7.088	257	248	168	337.2	356.3										1.690	5.398		7.088
L.16.	5.709	Residue	Residue	Residue	344.4												5.556	0.153	5.709
							0.602	6.020					0.460	9.718	32.537	33.159	19.955	0.153	102.604.
Total	102.604																		
% Esters recovered	99.1																		99.1
Corresp. Acids (wt.)	97.88						0.57	5.71					0.43	9.21	30.98	31.69	19.14	0.15	97.88
% Acid (wt.)	100.						0.58	5.83					0.44	9.41	31.65	32.38	19.55	0.15	100.
% of Total Acids	71.2						0.41	4.15					0.31	6.70	22.53	23.05	13.92	0.11	71.2

Original whole oil S.E. = 301.2

I.V. = 195.

C.14 Esters calculated as mono-ethenoid, while mean unsaturation for the others were determined as:-

	S.E.	Av. Unsat.	I.V.
(C.16	266.46	- 3.5 H.	166.7
(C.18	294.1	- 3.9 H.	168.3
(C.20	318.0	- 8. H.	319.2
(C.22	343.7	- 10.3 H.	380.

TABLE 5.

FRACTIONAL DISTILLATION OF SOLID ESTERS OF FAT FEMALE SHARK LIVER OIL (105.0 g. distilled.)

No.	G.	Temperature Bath	Temperature Column Middle	Temperature Column Head	S.E.	I.V.	Calculation Composition of Ester Fractions: Saturated C.14	Saturated C.16	Saturated C.18	Saturated C.20	Saturated C22	Saturated C24	Unsaturated C.14	Unsaturated C.16	Unsaturated C.18	Unsaturated C.20	Unsaturated C.22	Unsaturated C.24	Total
S. 1.	4.726	225 °C	178 °C	127 °C	253.5	8.45	2.426	1.902					0.195	0.203					4.726
S. 2.	5.797	223	181	130	257.8	9.	2.178	3.180					0.149	0.290					5.797
S. 3.	6.255	228	182	124	260.3	9.55	1.806	3.837					0.155	0.457					6.255
S. 4.	6.535	219	176	128	260.5	9.5	1.860	4.038					0.157	0.480					6.535
S. 5.	5.849	220	170	121	262.9	9.45	1.233	4.044					0.095	0.477					5.849
S. 6.	6.444	227	178	124	266.4	10.	0.674	5.094					0.036	0.640					6.444
S. 7.	7.648	221	185	125	268.7	13.35	0.252	6.318						1.078					7.648
S. 8.	6.279	219	182	128	272.2	19.25		4.551	0.428					1.088	0.212				6.279
S. 9.	6.602	217	186	127	271.7	18.35		4.954	0.350					1.111	0.187				6.602
S.10.	6.810	217	197	124	272.	19.6		4.946	0.430					1.211	0.223				6.810
S.11.	6.477	223	196	126	274.7	23.9		3.918	0.884					1.243	0.432				6.477
S.12.	6.215	241	191	132	281.2	34.9		2.191	1.620					1.211	1.193				6.215
S.13.	6.613	229	205	139	294.4	76.65		0.074	0.654					0.306	5.579				6.613
S.14.	5.613	234	227	134	305.2	126.5			1.318	0.508					2.136	1.651			5.613
S.15.	6.407	254	238	149	325.4	230.				2.036						3.091	1.280		6.407
S.16.	4.553	262	270	171	344.9	215.											4.345	0.208	4.553
S.17.	5.086	Residue	Residue	Residue.	363.7	-											1.410	3.676	5.086
Total	103.91						10.29	49.047	5.684	2.544			0.787	9.795	9.962	4.742	7.035	3.884	103.91.
% Esters recovered	99.0																		99.0
Corresp. Acids (wt.)	98.72						9.83	46.50	5.42	2.43			0.74	9.28	9.50	4.53	6.75	3.74	98.72
% Acid (wt.)	100.						9.96	47.10	5.49	2.46			0.75	9.40	9.62	4.59	6.84	3.79	100.
% of Total Acids.	28.8						2.87	13.56	1.58	0.71			0.22	2.71	2.77	1.32	1.97	1.09	28.8

Original whole oil S.E. 301.2

I.V. 195

All C.14, C.16 and C18 unsaturated esters taken as mono-ethenoid.

C.20 unsaturated taken as - 8 H
C.22 " " " - 10.3 H
C.24 " " " - 10. H

TABLE 6.

CALCULATED COMPOSITION OF FAT
FEMALE SHARK LIVER OIL.

Acid	"Solid" Acids (28.8%)	"Liquid" Acids (71.2%)	Total (Weight %)
SATURATED:			
Myristic (C_{14})	2.87	0.41	3.28
Palmitic (C_{16})	13.56	4.15	17.71
Stearic (C_{18})	1.58	-	1.58
Arachidic (C_{20})	0.71	-	0.71
Behenic (C_{22})	-	-	
UNSATURATED:			
Tetradecenoic (C_{14} - 2H)	0.22	0.31	0.53
Hexadecenoic (C_{16} - 2H)	2.71	-	2.71
C_{16} - 3.5H	-	6.70	6.70
Oleic (C_{18} - 2H)	2.77	-	2.77
C_{18} - 3.9H	-	22.53	22.53
C_{20} - 8H	1.32	23.05	24.37
C_{22} - 10.3H	1.97	13.92	15.89
C_{24} - 10H	1.09	0.11	1.20
TOTAL	28.80	71.2	99.9

TABLE 7.

FRACTIONAL DISTILLATION OF LIQUID ESTERS OF THIN FEMALE SHARK LIVER OIL (107.65 g. distilled.)

		Temperature					Calculated Composition of Ester Fractions												
		Bath	Column				Saturated						Unsaturated						
No.	G.		Middle	Head	S.E.	I.V.	C.14	C.16	C.18	C.20	C.22	C.24	C.14	C.16	C.18	C.20	C.22	C.24	C26	Total.
L. 1.	6.799	264.°C	164 °C	124 °C	258.8	66.33	0.824	1.380					1.400	3.195						6.799.
L. 2.	5.945	276.	178	133	269.3	80.62		0.946						4.764	0.235					5.945.
L. 3.	6.714	274.	187	145	281.9	90.38		1.251						1.969	3.494					6.714.
L. 4.	5.694	276.	187	145	287.6	96.25		1.026						0.538	4.130					5.694.
L. 5.	5.478	277.	191	146	291.5	102.9		0.089						0.059	4.730					5.478
L. 6.	5.718	277.	194	146	292.1	111.95		0.353						0.290	5.095					5.718
L. 7.	5.460	278.	195	148	296.8	127.2									5.083	0.377				5.460.
L. 8.	5.908	280.	196	143	299.2	144.7									4.863	1.045				5.908.
L. 9.	6.352	296.	204	156	304.9	175.4									3.714	2.638				6.352.
L.10.	8.850	293.	230	175	318.	243.3									0.570	8.280				8.850
L.11.	7.157	280.	228	177	330.7	307.7										3.780	3.377			7.157
L.12.	5.802	282.	218	169	331.2	311.4										2.921	2.881			5.802
L.13.	6.933	288.	225	174	331.8	318.2										3.313	3.620			6.933
L.14.	6.873	298.	233	177	336.3	332.9										2.083	4.790			6.873
L.15.	5.900	312.	222	171	339.8	335.9										1.034	4.866			5.900
L.16.	5.602	316.	221	172	344.4	298.8											5.558	0.044		5.602
L.17.	5.589	Residue.	Residue	Residue	373.6													5.247	.342	5.589
Total	106.77						0.824	5.625					1.400	10.815	31.914	25.471	25.092	5.291	.342	106.774.
% Esters recovered	99.2																			99.2
Corresp. Acids (wt.)	101.92						0.78	5.33					1.32	10.25	30.40	24.35	24.07	5.09	0.33	101.92
% Acid (wt.)	100.						0.77	5.23					1.30	10.06	29.83	23.89	23.62	4.99	0.32	100.
% of Total acids	62.5						0.48	3.26					0.81	6.28	18.61	14.91	14.74	3.11	0.20	62.4

Original whole oil

S.E. 327.95

E 1% ~~cm.~~ 1c.m. 86.04

I.V. 167.75

I.V. of non-vit. A fraction = 157.0

C.14 and C16 unsaturated calculated as mono-ethenoids while average unsaturation in other "liquid" fractions:-

	S.E.	Av. Unsat.	I.V.
(C.16			
(C.18	295.2	- 2.8 H.	120.4
(C.20	319.5	- 6.5 H.	258.2
(C.22	344.2	- 9.8 H.	361.4
(C.24		Assume - 10 H.	
(C.26		" - "	

TABLE 8.

FRACTIONAL DISTILLATION OF SOLID ESTERS OF THIN FEMALE SHARK LIVER OIL (103.9 g. distilled.)

No.	G.	Temperature: Bath	Temperature: Column: Middle	Temperature: Column: Head	S.E.	I.V.	Calculated Composition of Ester Fractions: Saturated: C.14	C.16	C.18	C20	C22	C.24	Unsaturated: C.14	C.16	C.18	C.20	C22	C24	Total.
S. 1.	4.757	234 °C	168 °C	120 °C	249.3	6.87	3.181	1.255					0.206	0.115					4.757
S. 2.	5.980	229	171	128	259.8	7.8	1.866	3.637					0.129	0.348					5.980
S. 3.	6.122	235	176	132	261.9	8.18	1.267	4.339					0.103	0.413					6.122
S. 4.	6.839	236	182	135	266.3	8.5	0.729	5.501					0.033	0.576					6.839
S. 5.	6.663	235	176	132	267.2	9.85	0.544	5.428					0.018	0.673					6.663
S. 6.	6.375	234	165	125	267.5	10.1	0.453	5.246					0.011.	0.665					6.375
S. 7.	6.583	236	170	126	268.6	10.52	0.250	5.602						0.731					6.583
S. 8.	6.027	249	173	128	273.	17.19		4.333	0.580					0.898	0.216				6.027
S. 9.	5.514	261	182	142	278.1	33.32		2.415	1.086					1.240	0.773				5.514
S.10.	6.578	266	194	149	292.5	48.66		0.524	2.367					0.428	3.259				6.578
S.11.	7.230	268	195	160	293.1	50.1		0.490	2.557					0.396	3.787				7.230
S.12.	5.515	276	200	152	298.	63.61			2.084	0.021					3.068	0.342			5.515
S.13.	5.653	281	208	154	311.	126.2			1.062	1.012					1.232	2.347			5.653
S.14.	5.455	306	230	178	325.3	200.7				1.581						2.958	0.916		5.455
S.15.	8.30	309	232	179	341.7	214.5				0.715	0.983					0.627	5.975		8.300
S.16.	9.563	Residue	Residue	Residue	369.9	149.7											0.673	8.890	9.563
Total	103.152						8.290	38.770	9.736	3.329	0.983		0.500	6.483	12.335	6.274	7.564	8.890	103.152.
% Esters recovered	99.3																		99.3
Corresp. Acids (wt.)	98.16						7.81	36.76	9.28	3.19	0.94		0.47	6.14	11.75	6.00	7.26	8.56	98.16.
% Acid (wt.)	100.						7.96	37.45	9.45	3.25	0.96		0.48	6.26	11.97	6.11	7.40	8.71	100.
% of Total Acids	37.6						2.99	14.08	3.55	1.22	0.36		0.18	2.35	4.50	2.30	2.78	3.27	37.6

Original whole oil S.E. 327.95

I.V. 167.75

I.V. of non vit. A portion = 157.0

All C.14, C.16, C.18 Unsaturateds taken as Mono-ethenoid.

C.20 Unsaturated as - 6.5 H

C.22 Unsaturated as - 9.8 H

TABLE 9.

CALCULATED COMPOSITION OF THIN FEMALE SHARK LIVER OIL.

Acid	"Solid" Acids (37.6%)	"Liquid" Acids (62.4%)	Total (Weight %)
SATURATED:			
Myristic (C_{14})	2.99	0.48	3.47
Palmitic (C_{16})	14.08	3.26	17.34
Stearic (C_{18})	3.55	-	3.55
Arachidic (C_{20})	1.22	-	1.22
Behenic (C_{22})	0.36	-	0.36
UNSATURATED:			
Tetradecenoic (C_{14} - 2H)	0.18	0.81	0.99
Hexadecenoic (C_{16} - 2H)	2.35	6.28	8.63
Oleic (C_{18} - 2H)	4.50	-	4.50
C_{18} - 2.8H	-	18.61	18.61
C_{20} - 6.5H	2.30	14.91	17.21
C_{22} - 9.8H	2.78	14.74	17.52
C_{24} - 10H	3.27	3.11	6.38
C_{26} - 10H	-	0.20	0.20
TOTAL	37.6	62.4	99.99

TABLE 10.

FRACTIONAL DISTILLATION OF LIQUID ESTERS OF SHARK EMBRYO LIVER OIL (distilled 102.2 g.)

		Temperature					Calculation Composition of Ester Fractions											
		Bath	Column				Saturated						Unsaturated					
No.	G.		Middle	Head	S.E.	I.V.	C.14	C.16	C.18	C20	C.22	C.24	C.14	C16	C.18	C.20	C.22	C.24	Total
L. 1.	5.015.	270 °C	158 °C	120 °C	262.4	65.9	0.600	1.199					0.625	2.791					5.015
L. 2.	6.608	271	162	121	276.7	81.15		2.205						2.275	2.128				6.608
L. 3.	5.942	270	173	132	289.8	98.33		2.157							3.785				5.942
L. 4.	6.987	275	182	136	292.1	116.6		1.672							5.315				6.987
L. 5.	6.105	279	195	142	297.8	162.8									5.264	0.841			6.105
L. 6.	6.622	283	200	147	303.3	218.2									4.013	2.609			6.622
L. 7.	7.040	284	200	149	312.6	277.6									1.554	5.486			7.040
L. 8.	7.159	284	214	159	322.3	343										5.808	1.351		7.159
L. 9.	6.980	287	216	164	331.1	368.9										3.132	3.758		6.890
L.10.	6.906	290	217	167	329.	377										3.619	3.287		6.906
L.11.	6.822	291	218	170	331.7	384.6										2.856	3.966		6.822
L.12.	7.964	291	220	169	337	391.1										1.759	6.205		7.964
L.13.	6.384	300	218	172	338.2	386.4										1.181	5.203		6.384
L.14.	5.782	305	230	177	340.9	367.7										0.524	5.258		5.782
L.15.	3.075	311	233	171	351.9	307.9											2.109	0.966	3.075
L.16.	5.957	Residue	Residue	Residue	369												0.582	5.375	5.957
Total	101.26						0.600	7.233					0.625	5.066	22.059	27.815	31.719	6.341	101.258
% Esters recovered	99.1																		99.1
Corresp. Acids (wt)	96.74						0.58	6.86					0.59	4.80	21.01	26.58	30.42	6.10	96.74
% Acid (wt.)	100.						0.60	7.09					0.61	4.96	21.72	27.48	31.44	6.31	100.
% of total Acids.	67.7						0.40	4.80					0.41	3.36	14.70	18.60	21.28	4.27	67.7

Original whole oil S.E. 329.8
I.V. 215.6.

C.14, C.16 calculated as monoethenoids, while average unsaturation in other "liquid" fractions:-

	S.E.	Av. Unsat.	I.V.
C.16			
C.18	294.5	- 3.5 H	150.8
C.20	317.7	- 8.3 H	331.5
C.22	343.4	- 10.6 H	391.7
C.24		assume - 10 H	

TABLE 11.

FRACTIONAL DISTILLATION OF SOLID ESTERS OF SHARK EMBRYO LIVER OIL (distilled 102.8 g.)

							Calculated composition of Ester fractions.											
		Bath	Column				Saturated.						Unsaturated					
No.	G.		Middle	Head	S.E.	I.V.	C.14	C.16	C.18	C.20	C22	C.24	C.14	C.16.	C18	C.20	C.22	C.24	Total.
S. 1.	6.043	250 °C	203 °C	126°C	256.7	9.9	2.439	2.999					0.228	0.377					6.043
S. 2.	7.159	258	209	131	260.8	10.2	1.947	4.463					0.177	0.572					7.159
S. 3.	7.260	261	175	136	264.9	13.5	1.035	5.203					0.103	0.919					7.260
S. 4.	7.565	262	178	138	267.9	27.45	0.364	5.007					0.007	2.187					7.565
S. 5.	5.775	263	170	133	264.9	13.26	0.825	4.149					0.080	0.721					5.775
S. 6.	6.529	268	173	136	262.7	7.0	1.457	4.599					0.082	0.391					6.529
S. 7.	5.753	268	178		265.	8.1	0.861	4.406					0.047	0.439					5.753
S. 8.	6.600	270	180		266.6	10.61	0.644	5.222					0.033	0.701					6.600
S. 9.	6.296	273	183		274.9	22.5		3.857	0.905					1.127	0.407				6.296
S.10.	6.924	274	184		280.9	35.4		2.468	1.743					1.396	1.317				6.924
S.11.	6.250	290	172		291.5	47.1		0.620	2.251					0.498	2.881				6.250
S.12.	6.518	298	214		293.7	97.2		0.320	1.953					0.117	4.128				6.518
S.13.	6.418	305	230		321.2	274.2				1.196						4.697	0.525		6.418
S.14.	8.848	308	240		335.2	344.9				0.444	0.236					2.470	5.698		8.848
S.15.	3.744	309	241		345.	222.7											3.518	0.226	3.744
S.16.	4.570	Residue	Residue	Residue	348.9												3.633	0.937	4.570
Total	102.25						9.572	43.313	6.852	1.640	0.236		0.757	9.445	8.733	7.167	13.374	1.163	102.25
% Ester recovered	99.5.																		99.5
Corresp. Acids (wt)							9.02	41.07	6.55	1.57	0.23		0.71	8.95	8.32	6.85	12.83	1.12	97.20
% Acid (wt)							9.28	42.25	6.72	1.62	0.24		0.73	9.21	8.56	7.05	13.20	1.15	100.
% of Total Acids	32.3						3.00	13.65	2.17	0.52	0.08		0.24	0.97	2.76	2.28	4.26	0.37	32.3

Original whole oil S.E. 329.8

I.V. 215.6

All C.14, C.16, C.18 unsaturateds taken as mono-ethenoid, exepting S.12 where C.18 was taken as - 3.5 H.

C.20 unsat. taken as - 8.3 H.
C.22 " " " -10.6 H.
C.24 " " " -10 H.

TABLE 12.

CALCULATED COMPOSITION OF SHARK EMBRYO LIVER OIL.

Acid	"Solid" Acids (32.3%)	"Liquid" Acids (67.7%)	Total (weight %)
SATURATED:			
Myristic (C_{14})	3.00	0.26	3.26
Palmitic (C_{16})	13.65	4.80	18.45
Stearic (C_{18})	2.17	-	2.17
Arachidic (C_{20})	0.52	-	0.52
Behemic (C_{22})	0.08	-	0.08
UNSATURATED:			
Tetradecenoic (C_{14} - 2H)	0.24	0.41	0.65
Hexadecenoic (C_{16} - 2H)	2.97	3.36	6.33
Oleic (C_{18} - 2H)	1.46	-	1.46
C_{18} - 3.5H	1.30	14.70	16.00
C_{20} - 8.3H	2.28	18.60	20.88
C_{22} - 10.6H	4.26	21.28	25.54
C_{24} - 10H	0.37	4.27	4.64
TOTAL	32.30	67.7	100.00

TOEWYDING.

Hierdie werk word in dankbaarheid gewy aan die nagedagtenis van my ouers, wyle

A.W. en M.Lategan .