Studies on Solvent Fractions From Fish Liver Oils*

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A GREAT deal of work has been reported on the stability of crude and refined oils and fats and the effect of a variety of treatments and antioxidants on the stability of such products. Much less data are in the literature on the stability of solvent fractions from various oils and fats and the literature is particularly lacking in data on the stability characteristics of solvent fractions prepared from the more common vitamin-containing fish liver oils. It is generally accepted that crude oils are more resistant to atmospheric oxidation than are the same oils partially or exhaustively refined and the decrease in stability is largely proportional to the decrease in antioxidant content of the oils. Even though crude fish liver oils are comparatively unstable on exposure to air, they contain highly active antioxidants since various refining treatments make the oils less stable to oxidative changes.

In previous work from this laboratory it was shown first that the naturally occurring antioxidants associated with fish liver oils are completely removed by treating the said oils with activated carbon in the presence of a solvent for the oil (1); secondly that highly active antioxidant extract concentrates can be prepared from the usual type crude vegetable oils by extraction of the oils with solvents, i.e., isopropanol, methanol and the like, under various temperature conditions (2); and, thirdly that the pure tocopherols—α, β, and γ, alone or when associated with added phosphatides in fish liver oils—exhibit a different type of oxidation behavior than that displayed by the fish liver oils alone (3). Robeson and Baxter (4) isolated α-tocopherol from mangona shark liver oil by distillation means and concluded that the α-tocopherol found was the major antioxidant present in the oil. Bird (5), using halibut liver oil, showed that exhaustive extraction with 80% aqueous methanol removed only part of the antioxidants while with water or dilute aqueous alkali complete extraction or destruction of the antioxidant principles occurred. Zucker (6) extracted vitamin D from cod liver oil with ethyl alcohol; however, no data were reported on the relative stability of the various fractions. Freeman (7) prepared high iodine value oils exhibiting drying qualities from vegetable oils, fish oils, and the like by dissolving the oils in solvents such as isopropanol, 97% aqueous acetone, etc., followed by cooling the mass to about —20°C. and recovering the solvent extract fraction containing the drying oils. Dombrow (8) prepared high potency A and D fractions from fish liver oils by extraction with polar solvents like isopropanol at temperatures ranging from room temperature to —70°C. and states that the high potency extract fractions contain the bulk of the naturally occurring antioxidants present in the starting oils.

This investigation reports partial analysis data on various solvent fractions from numerous types of crude fish liver oils and also stability data on the various products as determined by the rate of peroxidation and vitamin A destruction on exposure of the oils to air. It was postulated at the onset of this work that if any of the oils to be examined contained α-tocopherol as their major antioxidant as reported for mangona and soupfin shark liver oils by Robeson and Baxter (4), the solvent extract fractions should exhibit generally the same type of peroxidation as that displayed by the same oils containing added α-tocopherol. It was assumed that any antioxidants present in the crude oils of the tocopherol-type would be concentrated in the solvent extract fractions. The samples of soupfin shark liver oil B and halibut liver oil A used were identical with those used in another investigation (3) relating to the effectiveness of α-tocopherol for inhibiting peroxidation and vitamin A destruction.

Materials and Methods

Fish Liver Oils. All of the fish liver oils used in this work were fresh, good quality oils, of known origin, and representative of the specie of fish. The oils were dry and were filtered just prior to being used. Because of the large number of oils examined in this investigation only a partial analysis of some of the oils and fractions thereof studied are included in this report. A partial analysis of some of the oils and fractions thereof studied for peroxide formation and vitamin A stability, as well as that of some other oils fractionated, is given in Table II.

Extraction Method. Broad aspects of the general methods used for carrying out the extraction of the oils studied are reported elsewhere (8, 10). In this work the solvents used were (1) 99% isopropanol, (2) 95% isopropanol, (3) 91% isopropanol, (4) 95% acetone, and (5) 100% methanol. All of the extractions, except those reported in Table II on soupfin shark liver oil B with 91% and 99% isopropanol at —25°C. ± 1°C., were conducted at —18°C. ± 1°C. Many other solvents, solvent mixtures, and temperatures were investigated; however, the differences were a matter of degree and have no bearing on the present study. Briefly, the fish liver oils studied were extracted by the following method:

When 99% isopropanol and methanol were used for the extractions, 2,000 ml. were added to a 3,000-ml. Erlenmeyer flask containing 500 gms. of oil. The flask was equipped with a nitrogen gas bubbling tube and a thermometer. The mass was then warmed to 50°C. in a water bath while shaking by hand and bubbling nitrogen gas through the oil-solvent mass. When 91% and 95% isopropanol and 95% acetone were used for the extractions, the calculated amount of the commercial grades, namely 99% isopropanol and 100% acetone, were added to 500 gms. of oil as above and the temperature of the mass raised to 50°C. The calculated amount of water required to dilute the solvents to 91% or 95% was then added and the extraction continued. The solvent-oil solutions or mixtures, depending upon the solvent used at 50°C., were then cooled first to +3°C. by placing the stoppered flask in a refrigerator and then to —18°C. or —25°C. in another refrigerator. The

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TABLE I

Description of Fish Liver Oils and Fractions Thereof

Oil	See Figure	See Curve	Fraction	Solvent	Vitamin A units/gm.	Peroxide Value	Iodine Value
Soupfin Shark Liver A	1, 1A, 1B	1	Original	None	67,000	1.9	162.8
Soupfin Shark Liver A	1, 1A, 1B	2	1st Extract	99% Isopropanol	153,000	17.3	165.0
Soupfin Shark Liver A	1, 1A, 1B	3	2nd Extract	99% Isopropanol	142,000	17.2	180.0
Soupfin Shark Liver A	1, 1A, 1B	4	3rd Extract	99% Isopropanol	128,000	14.4	178.7
Soupfin Shark Liver A	1, 1A, 1B	5	Residue	99% Isopropanol	44,800	6.5	159.5
Soupfin Shark Liver B	2, 2A, 2B	1	Original	None	105,000	5.3	175.9
Soupfin Shark Liver B	2, 2A, 2B	2	1st Extract	99% Isopropanol	232,000	2.4	177.7
Soupfin Shark Liver B	2, 2A, 2B	3	2nd Extract	99% Isopropanol	220,000	4.1	178.9
Soupfin Shark Liver B	2, 2A, 2B	4	3rd Extract	99% Isopropanol	209,000	7.5	175.0
Soupfin Shark Liver B	2, 2A, 2B	5	Residue	99% Isopropanol	74,700	3.4	176.3
Soupfin Shark Liver B	3, 3A, 3B	1	Original	None	105,000	5.3	175.9
Soupfin Shark Liver B	3, 3A, 3B	2	1st & 2nd Extr.	95% Isopropanol	261,000	6.8	178.5
Soupfin Shark Liver B	3, 3A, 3B	3	3rd & 4th Extr.	95% Isopropanol	258,600	11.2	175.9
Soupfin Shark Liver B	3, 3A, 3B	4	Residue	95% Isopropanol	74,700	7.0	172.1
Soupfin Shark Liver B	4, 4A, 4B	1	Original	None	105,000	5.3	175.9
Soupfin Shark Liver B	4, 4A, 4B	2	1st & 2nd Extr.	95% Acetone	165,000	4.1	215.5
Soupfin Shark Liver B	4, 4A, 4B	3	3rd & 4th Extr.	95% Acetone	146,500	12.4	217.6
Soupfin Shark Liver B	4, 4A, 4B	4	Residue	95% Acetone	80,600	4.8	165.6
Dogfish Shark Liver C	5, 5A, 5B	1	Original	None	12,500	2.0	111.6
Dogfish Shark Liver C	5, 5A, 5B	2	1st Extract	99% Isopropanol	26,300	1.1	117.1
Dogfish Shark Liver C	5, 5A, 5B	3	2nd Extract	99% Isopropanol	24,200	3.4	111.1
Dogfish Shark Liver C	5, 5A, 5B	4	3rd Extract	99% Isopropanol	22,600	2.3	114.8
Dogfish Shark Liver C	5, 5A, 5B	5	Residue	99% Isopropanol	8,380	2.1	110.9
Halibut Liver A	6, 6A, 6B	1	Original	None	94,600	2.0	140.8
Halibut Liver A	6, 6A, 6B	2	1st Extract	99% Isopropanol	157,000	1.5	156.5
Halibut Liver A	6, 6A, 6B	3	2nd Extract	99% Isopropanol	167,000	2.1	154.1
Halibut Liver A	6, 6A, 6B	4	3rd Extract	99% Isopropanol	158,000	4.4	142.5
Halibut Liver A	6, 6A, 6B	5	Residue	99% Isopropanol	64,300	3.5	139.5
Bluefin Tuna Liver	7, 7A, 7B	1	Original	None	42,000	2.7	165.5
Bluefin Tuna Liver	7, 7A, 7B	2	1st Extract	99% Isopropanol	102,000	2.0	169.2
Bluefin Tuna Liver	7, 7A, 7B	3	2nd Extract	99% Isopropanol	82,500	2.8	168.0
Bluefin Tuna Liver	7, 7A, 7B	4	3rd Extract	99% Isopropanol	78,000	8.7	165.8
Bluefin Tuna Liver	7, 7A, 7B	5	Residue	99% Isopropanol	27,900	3.6	164.3

flasks were then allowed to remain at —18°C. or —25°C. for approximately 12 hours. The supernatant solvent layer was then decanted and gravity filtered at the low temperatures. The residue on the filter paper was combined with the residue remaining in the flask with the solvent, being used for the extraction and two or more additional extractions conducted in a similar manner. The individual extractions were either freed of solvent separately or combined and freed of solvent by distillation under reduced pressure at less than 50°C. The solvent insoluble residues were freed of solvent traces in a like manner. The oil fractions produced were studied immediately or stored in full, tightly sealed, amber bottles at —25°C. until they could be examined.

Carbon Treatment. In a previous study (1) it was shown that treatment of a fish liver oil with activated carbon (Nuchar XXX) in the presence of a solvent for the oil resulted in the complete removal of antioxidants from the oil. In the present investigation (cf. Table III) it was decided advisable to carbon-treat some of the methanol residue fractions in order to determine whether or not antioxidants were still present. In each instance in Table III when the oil is said to be carbon-treated, the treatment was conducted using 20% by weight of Nuchar XXX based

TABLE II

Typical Partial Analysis Data on Fish Liver Oils and Fractions Thereof

Oil	Treatment	Vitamin A units/gm.	Peroxide No.	% F.F.A.	Color Red	Color Yellow	Iodine Value	Sap. Value	% Unsap.
Soupfin Shark B	None	105,000	5.0	0.22	4	36	184.5	181.6	4.42
Soupfin Shark B	99% isop. exts. (4), —25°C.	262,000	1.5	1.3	11.6	35	192.0	167.5	9.6
Soupfin Shark B	99% isop. residue, —25°C.	73,800	6.0	0.1	5.2	35	183.0	183.5	4.13
Soupfin Shark B	91% isop. exts. (6), —25°C.	276,000	2.0	5.7	79	35	177.0	147.5	13.7
Soupfin Shark B	91% isop. residue, —25°C.	92,600	5.0	0.1	4.4	35	184.0	182.0	3.14
Soupfin Shark C	None	103,000	2.5	1.5	5.3	35	175.9	178.0	5.75
Soupfin Shark C	Methanol exts. (4), —18°C.	208,000	1.5	27.4	65	35	184.5	163.5	9.42
Soupfin Shark C	Methanol residue, —18°C.	98,500	3.0	0.1	8.6	35	172.5	179.0	4.97
Mexican Shark	None	59,300	1.0	4.9	16.8	30	117.5	179.5	5.34
Mexican Shark	Methanol exts. (3), —18°C.	85,000	0	45.3	70+	too dark	139.4	168.7	7.2
Mexican Shark	Methanol residue, —18°C.	53,500	2.0	0.56	20	65	117.0	183.0	5.21
Halibut A	None	94,600	0.6	0.3	2.1	30	139.0	171.5	11.75
Halibut A	1st 99% isop. ext., —18°C.	157,000	1.5	4.6	9.8	18	153.0	162.5	12.9
Halibut A	2nd 99% isop. ext., —18°C.	167,000	2.1	1.6	4.4	20	153.0	169.0	11.5
Halibut A	3rd 99% isop. ext., —18°C.	158,000	4.4	0.69	4.9	30	145.8	163.5	10.8
Halibut A	99% isop. residue, —18°C.	64,300	3.5	0.12	3.1	35	135.8	172.0	9.9
Sole	None	73,300	1.5	0.85	9.3	25	123.3	186.0	10.4
Sole	99% isop. exts. (3), —18°C.	123,000	1.0	3.4	17.1	35	150.0	190.5	13.7
Sole	99% isop. residue, —18°C.	51,700	13.3	0.6	14	35	113.7	185.0	8.25
Swordfish	None	85,000	1.5	1.4	85	35	147.0	170.0	11.1
Swordfish	99% isop. exts. (3), —18°C.	141,000	1.0	6.5	28	35	150.0	181.5	15.2
Swordfish	99% isop. residue, —18°C.	63,300	6.4	1.7	59	35	136.0	167.5	8.79
Black Cod Visceral	None	85,700	2.4	3.1	25	30	130.3	175.9	9.42
Black Cod Visceral	99% isop. exts. (3), —18°C.	130,000	0	8.3	30	35	144.0	182.0	11.5
Black Cod Visceral	99% isop. residue, —18°C.	58,000	6.7	0.6	3.6	35	123.0	173.5	6.33

on the weight of oil in a solution (1 part by weight of oil to 3 parts by volume of solvent) of the oil in cyclohexane by the method already described (1). The same procedure was used for treating the extract fraction in Table V; however, it was necessary to use 40% Nuchar XXX in order to remove substantially all of the antioxidants from the extract fraction.

Oil Analysis. The vitamin A contents of the oil products were determined by dissolving the oils in a spectrophotometric grade of isopropanol and measuring the absorption coefficients in a Beckman Spectrophotometer at 3280 Å. The oil constants were obtained by official A.O.A.C. accepted methods. The peroxide numbers were ascertained by the method previously reported (9).

Storage Method. The oils and fractions thereof examined for peroxidation and vitamin A destruction were stored in the same general manner as in a previous study (9), namely, 2-ml. samples of the oils were accurately measured into standard size, thoroughly cleansed vials and the vials stored in a dark oven. Most of the results reported were obtained at 34.5° C. ± 0.5° C. In Table IV some data are included on the peroxide values and vitamin A destruction percentages of oil samples stored at higher temperatures, i.e., 50°C. and 65°C. These data are included to point out the inadvisability of carrying out work of the type reported herein and elsewhere (1, 2, 3, 9) on peroxidation and vitamin A destruction in fish liver oils at higher temperatures. At the various intervals indicated in the Figures and Tables individual peroxide values and vitamin A contents were determined on three vials of the same sample and the average values used in computing the data for the Figures and Tables.

The data in Tables I, II, III, and IV on the vitamin A potencies of the various oils and fractions thereof show that the solvent extract fractions are considerably higher in vitamin A unitage than the original oils and the residue fractions. This is particularly true in the case of the 99%, 95%, and 91% isopropanol extract fractions. The general extraction technique used is therefore a convenient method for preparing concentrated vitamin A extracts directly from crude fish liver oils (8, 10). The crude fish liver oils used in this investigation contained largely the ester form of vitamin A. From a vast accumulation of unpublished data on solvent fractionation work using the same type of oils and solvents employed in this work it can be said that solvent extract fractions prepared using 99% and 95% isopropanol contain chiefly the ester form of vitamin A. Extract fractions prepared with methanol at —18°C. contain mostly vitamin A alcohol while 95% acetone and 91% isopropanol extract fractions contain varying percentage mixtures of the two vitamin A forms, depending upon the fractionation temperature employed. Another factor of interest to note in the Tables is that some of the solvent extract fractions and the residue fractions exhibit slightly higher peroxide numbers than the original oils. This is believed to be due to the depletion of antioxidants from the oils being extracted by the first extract or two.

The analytical data on the solvent fractions in Tables I and II bring out the following interesting facts. The solvent extract fractions exhibit higher iodine values, free fatty acid contents, and unsaponified values than the crude oils. With methanol, particularly, the extract fractions possess very high free fatty acid values. The only extract fractions exhibiting iodine values very much higher than the starting oils were those produced using 95% acetone (cf. Table I). All of the solvent insoluble fractions displayed lower iodine values, free fatty acid contents, and unsaponified values than the original oils. Generally the solvent extractions concentrated more of the color components in the extracts than in the residues. This was not the case with swordfish liver oil. The 99% isopropanol extract fractions exhibited much less red color than the original oil or the residue fraction. In all instances when the crude oils were solvent- extracted, the extract fractions possessed the bulk of the odor, taste, and flavor constituents associated with the crude oils. The residue fractions were essentially bland in taste and nearly odorless.

The stability data on the crude fish liver oils and solvent extract and residue fractions studied are reported in the various Figures and in Tables III, IV, and V. A description of the curves and material represented in the Figures is summarized in Table I. All the results in the Figures and in Tables III and V were obtained by storage of the oils and fractions thereof at 34.5°C. The effect of using a higher storage temperature, namely 50°C. and 65°C., on certain of the oil materials is covered in Table IV. It was of interest to determine whether or not the peculiar type of peroxide behavior exhibited by the isopropanol extract fractions from the different shark liver oils—namely an increase in peroxide number until a relatively low maximum was reached — followed by a gradual decrease in peroxide number, would still show up at the higher storage temperatures. No data are included on the solvent insoluble fractions at the higher temperatures because of the relatively low degree of stability displayed by the materials at the lower temperature, 34.5°C. In the Figures 1 to 7B, inclusive, the peroxidation and vitamin A destruction data are plotted three ways, namely (1) peroxide number versus % vitamin A destruction at 34.5°C.; (2) peroxide number versus hours' storage at 34.5° C.; (3) % vitamin A destruction versus hours' storage at 34.5°C. From previous published work (1, 9) on stability data of the general type, it is believed that this method of presentation is the clearest and most comprehensive.

Peroxidation Versus Vitamin A Destruction

Previous work (1, 2, 3, 9) on the stability of crude fish liver oils showed that during the time required to destroy the vitamin A content of the oils on storage of the oils at 34.5°C., there was an uninterrupted increase in peroxide number of the oxidizing oils. In the carbon-treated oils (1) and other oils of poor stability the increase in peroxidation was very rapid and directly proportional to the per cent of vitamin A destroyed. In crude fish liver oils or carbon-treated fish liver oils, containing added α-tocopherol or α-tocopherol plus lecithin, the increase in peroxide value at a given per cent of vitamin A loss was even higher than in the blank oils at the same value of vitamin A destruction (3). It was, therefore, logical to believe in this present investigation that if any of the many different types of crude fish liver oils extracted contained an α-tocopherol-type antioxidant, the concentrated solvent extracts should exhibit a type of peroxidation versus vitamin A destruction relation-

TABLE III

Stability of Fish Liver Oils and Fractions Thereof at 34.5°C.

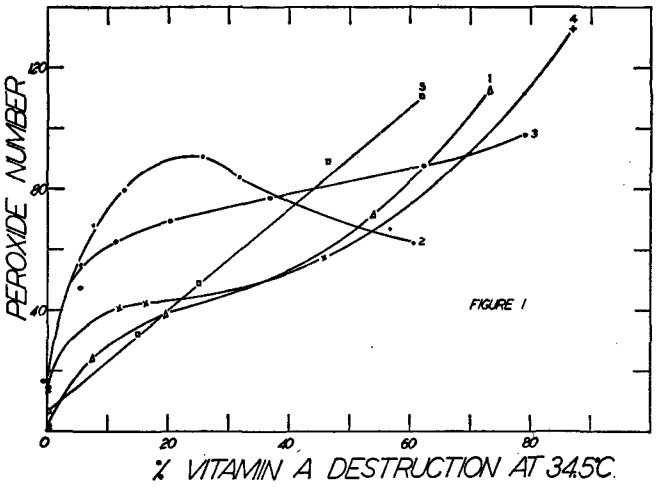
Oil	Treatment	Vitamin A units/ gm.		Days at 34.5° C.																				
				0	1	2	3	4	5	6	7	8	9	10	11	12	13	15	16	17	18	19	21	23	25	32
Halibut B	None	72,000	Peroxide No.	2.5			16.6		24.5	26.9	32.9	41.2	51.4													
			% Vit. A Destr.	0			5.1		13.8	20.6	34.3	47.5	68.4													
Halibut B	Methanol Extract	145,000	Peroxide No.	2.0			3.0				7.9			12.4		13.3		20.3								
			% Vit. A Destr.	0			18.4				49.3			64.3		68.5		97.8								
Halibut B	Methanol Residue	64,500	Peroxide No.	2.5	6.5	10.0	14.6	28.9	54.5	66.4																
			% Vit. A Destr.	0	1.4	1.9	7.9	38.8	51.7	69.2																
Halibut B	Methanol Residue Carbon-Treated	62,300	Peroxide No.	3.0	22.4	37.5	55.9																			
			% Vit. A Destr.	0	23.3	46.5	67,6																			
Swordfish	None	92,800	Peroxide No.	1.5			14.4		19.3	33.0	42.8	58.8														
			% Vit. A Destr.	0			8.8		20.2	44.5	48.5	65.3														
Swordfish	Methanol Extract	114,000	Peroxide No.	1.0			2.9				5.7			8.2		9.5										
			% Vit. A Destr.	0			14.7				45.4			61.4		75.7										
Swordfish	Methanol Residue	88,000	Peroxide No.	3.2	5.5	11.7	23.2	39.1	49.9	73.2																
			% Vit. A Destr.	0	4.6	7.2	23.1	46.6	52.8	68.3																
Swordfish	Methanol Residue Carbon-Treated	84,000	Peroxide No.	4.1	22.2	37.5	57.0	80.2																		
			% Vit. A Destr.	0	16.7	35.2	53.4	65.8																		
Swordfish	Isopropanol Extract*	141,000	Peroxide No.	1.0			10.4		11.4		13.8			19.8	25.0	31.1										
			% Vit. A Destr.	0			7.8		15.6		38.4			49.6	59.0	67.7										
Swordfish	Isopropanol Residue	63,300	Peroxide No.	6.4		29.9	41.3	54.3	69.3																	
			% Vit. A Destr.	0		26.1	42.2	57.4	66.9																	
Black Cod Visceral	None	85,700	Peroxide No.	2.4			9.2		17.0		25.5	27.0	28.7	37.1												
			% Vit. A Destr.	0			10.7		15.5		32.8	48.2	59.1	70.2												
Black Cod Visceral	Methanol Extract	104,000	Peroxide No.	0			2.1				3.6			5.2		4.5		5.4								
			% Vit. A Destr.	0			9.8				29.1			43.6		50.3		58.3								
Black Cod Visceral	Methanol Residue	81,200	Peroxide No.	2.5		8.7	11.9	21.5	25.3	35.6	48.4	54.1														
			% Vit. A Destr.	0		4.2	8.9	14.2	36.4	46.7	63.5	73.5														
Black Cod Visceral	Methanol Residue Carbon-Treated	80,500	Peroxide No.	6.4	18.3	32.9	48.6	61.1																		
			% Vit. A Destr.	0	21.3	44.5	58.2	73.9																		
Black Cod Visceral	Isopropanol Extract	130,000	Peroxide No.	0			12.8		12.3		12.9			15.1	14.9	19.5	19.7									
			% Vit. A Destr.	0			9.2		15.4		24.8			38.9	43.8	58.4	61.7									
Black Cod Visceral	Isopropanol Residue	58,000	Peroxide No.	6.7		18.7	27.9	38.7																		
			% Vit. A Destr.	0		25.0	43.5	60.3																		
Sole	None	73,300	Peroxide No.	1.5			13.6		17.9		28.2	39.4														
			% Vit. A Destr.	0			13.5		18.3		46.5	69.3														
Sole	Methanol Extract	137,000	Peroxide No.	0			2.4				3.5			6.0		6.4		6.9								
			% Vit. A Destr.	0			14.7				36.5			51.3		55.1		64.4								
Sole	Methanol Residue	67,000	Peroxide No.	3.2		8.7	13.9	24.0	33.1	51.0	54.5															
			% Vit. A Destr.	0		4.2	11.5	23.9	42.3	61.2	70.5															
Sole	Methanol Residue Carbon-Treated	64,500	Peroxide No.	2.0	16.3	28.9	40.2	52.9																		
			% Vit. A Destr.	0	20.6	40.2	54.8	62.5																		
Sole	Isopropanol Extract	123,000	Peroxide No.	1.0			7.5	8.7	9.9	13.1	17.8	21.7	22.2	26.1												
			% Vit. A Destr.	0			15.5	21.1	36.8	37.0	50.7	61.6	67.5	73.5												
Sole	Isopropanol Residue	51,700	Peroxide No.	13.3		31.3	41.5	51.7																		
			% Vit. A Destr.	0		42.5	56.5	69.9																		
Ling Cod	None	236,000	Peroxide No.	2.4			14.0	19.4	26.0	28.7	37.6	51.9														
			% Vit. A Destr.	0			11.5	24.2	34.0	43.0	60.5	73.8														
Ling Cod	Isopropanol Extract	376,000	Peroxide No.	1.5			10.7	12.3	9.9	7.2	19.2			27.1												
			% Vit. A Destr.	0			12.2	17.0	19.1	31.4	50.2			73.7												
Ling Cod	Isopropanol Residue	172,000	Peroxide No.	10.1	19.3	34.9	48.3	56.9																		
			% Vit. A Destr.	0	15.7	38.4	59.9	70.6																		
Dogfish A	None	17,000	Peroxide No.	3.5		7.5		14.3			22.7		30.6		33.5		39.1		42.0	46.3	53.5					
			% Vit. A Destr.	0		7.7		10.0			12.3		17.7		20.0		24.7		37.0	49.8	64.3					
Dogfish A	Isopropanol Extract	30,600	Peroxide No.	2.3			25.4		35.0		38.9		38.1		36.3		36.6	31.3		32.4		31.0	28.4		25.1	23.8
			% Vit. A Destr.	0			6.5		10.8		17.3		21.6		25.7		30.5	34.7		39.3		44.4	48.7		53.0	67.0
Dogfish A	Isopropanol Residue	11,700	Peroxide No.	1.8		5.5	5.9		11.9		31.3		63.4													
			% Vit. A Destr.	0		4.3	6.4		12.8		41.0		71.8													
Dogfish B	None	10,400	Peroxide No.	2.5			24.0			34.4		40.2		43.2			64.6									
			% Vit. A Destr.	0			5.5			11.1		16.1		25.0			60.8									
Dogfish B	Isopropanol Extract	21,000	Peroxide No.	4.8		20.4	27.1		43.0		49.3		56.7			63.2			69.0		70.6		67.3	69.3		
			% Vit. A Destr.	0		3.3	4.8		6.6		8.1		11.4			14.8			23.8		33.3		44.3	67.4		
Dogfish B	Isopropanol Residue	7,500	Peroxide No.	4.0		35.2	51.0	97.1																		
			% Vit. A Destr.	0		28.4	45.6	63.1																		

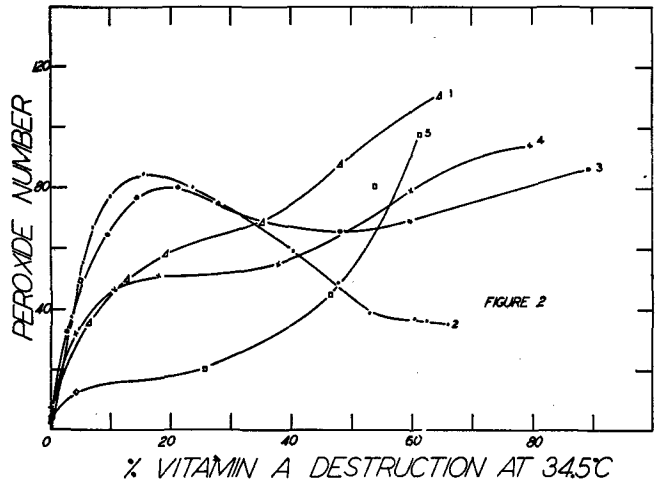
* When methanol extract and isopropanol extract are mentioned above, it means that the oil was extracted three times with the particular solvent at —18°C. and the extracts combined. The isopropanol used was 99%.

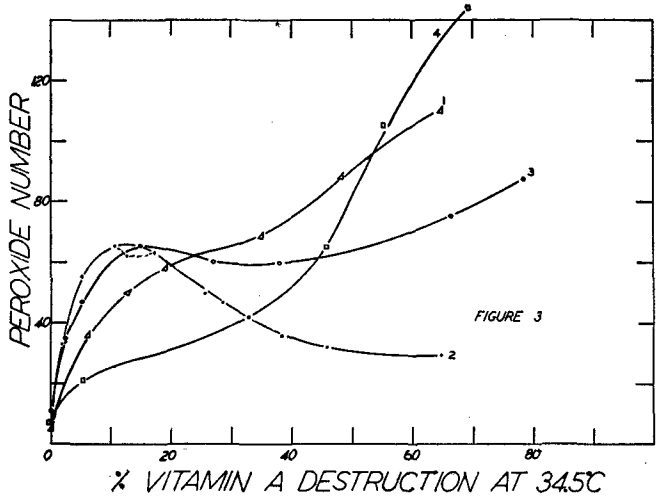
ship similar to that exhibited by analogous oils containing added α-tocopherol under like storage conditions.

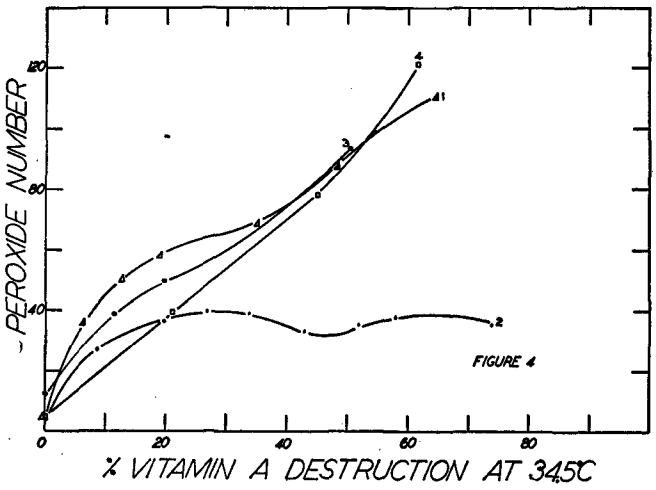
A close examination of the large amount of data contained in Figures 1 to 7, inclusive, and in Tables III, IV, and V fails to show any similarity in the relationship of peroxidation to vitamin A destruction as against that displayed by fish liver oils containing added α-tocopherol, or α-tocopherol plus lecithin (3). In all cases during the oxidation of the crude fish liver oils the solvent residue fractions and the methanol residue fractions carbon-treated, the peroxidation, and the destruction of vitamin A increased as the time increased. In fact, the methanol residue fractions carbon-treated (cf. Table III) and the solvent residue fractions (cf. Figures 1, 2, 3, 4, 5, 6, and 7), except the methanol residue fractions in Table III, displayed a fast rate of peroxide formation which was substantially proportional to the percentage of vitamin A destruction.

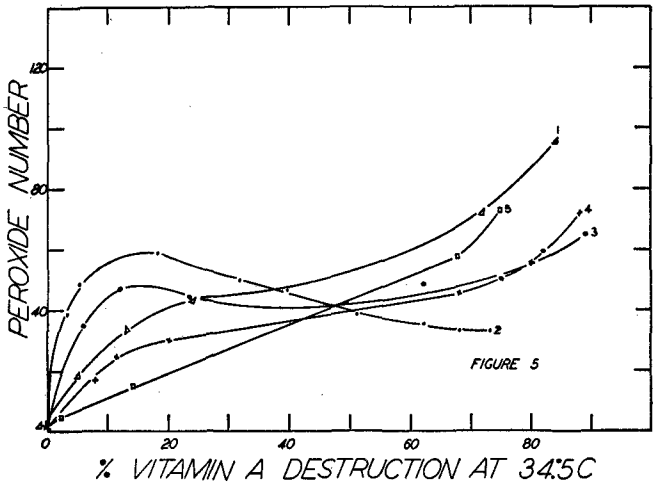
The remarkable phenomenon observed in this investigation is the type of peroxidation and the relation-











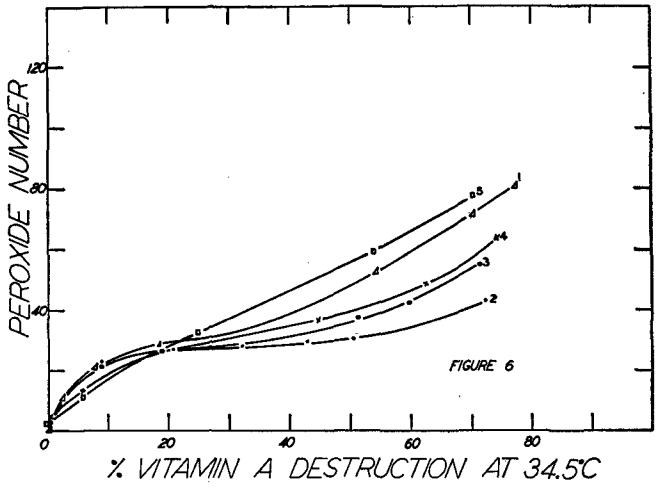


TABLE IV

Effect of Temperature on Stability of Fish Liver Oils and Fractions Thereof

Oil	Treatment	Units A per gm.	Temp. °C.		Time in Days																														
					0	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	28	30	32	33	38
Soupfin Shark B.....	None	105,000	34.5	Peroxide No.....	5.3		35.8		50.3		58.4		68.8	88.0	111.0																				
				% Vit. A Destr...	—		6.2		12.7		19.0		34.8	47.8	64.5																				
Soupfin Shark B.....	Isopropanol Ext.*.....	254,000	34.5	Peroxide No.....	1.5	15.2			21.0		53.6			66.7		69.3		72.9			71.8			62.0		53.6		49.8		47.2	46.3	45.1		39.6	34.4
				% Vit. A Destr...	—	1.2			5.9		6.3			13.0		17.7		18.9			25.6			30.3		31.9		35.0		37.0	42.2	44.0		47.2	54.0
Soupfin Shark B.....	Isopropanol Ext.*.....	254,000	50	Peroxide No.....	1.5	10.9		14.1		28.3	25.9	25.1	22.0	19.3		17.3		16.0			15.3			14.4											
				% Vit. A Destr...	—	5.5		16.2		23.6	27.9	30.7	34.2	37.8		44.9		50.0			56.7			64.8											
Soupfin Shark B.....	Isopropanol Ext.*.....	254,000	65	Peroxide No.....	1.5		7.4		8.9		7.7			6.9		7.5		6.2			6.0		6.2											
				% Vit. A Destr...	—		24.3		34.9		43.5			53.8		59.6		62.0			69.3		72.4											
Dogfish A.....	None	17,000	34.5	Peroxide No.....	3.5	7.5		14.3			22.7		30.6		20.0		24.7			37.0		49.8	64.3											
				% Vit. A Destr...	—	7.7		10.0			12.3		17.7		33.5		39.1			42.0		46.3	53.5											
Dogfish A.....	None	17,000	50	Peroxide No.....	3.5	10.9		12.4		17.4	17.3	17.5	19.8	21.8	39.1																			
				% Vit. A Destr...	—	5.7		15.2		27.8	32.3	38.9	50.2	58.5	79.3																			
Dogfish A.....	Isopropanol Ext.*.....	30,600	34.5	Peroxide No.....	2.3		25.4		35.0		38.9		38.1		36.3		36.6		31.3		32.4		31.0		28.4			29.0	25.1				23.8		
				% Vit. A Destr...	—		6.5		10.8		17.3		21.6		25.7		30.5		34.7		39.3		44.4		48.7			50.8	53.0				67.0		
Dogfish A.....	Isopropanol Ext.*.....	30,600	50	Peroxide No.....	2.3	10.2		11.4		12.5	12.1	11.4		10.1																				
				% Vit. A Destr...	—	15.7		27.4		35.0	39.2	42.5		46.8																				
Mex. Shark.....	None	16,900	34.5	Peroxide No.....	3.5		3.2				10.4		13.9		21.4				28.0			31.0			32.0		41.8			55.7					
				% Vit. A Destr...	—		1.8				2.4		6.0		8.4				15.6			22.2			30.5		49.1			61.4					
Mex. Shark.....	None	16,900	50	Peroxide No.....	3.5		7.6		14.4				21.7		22.2				26.1			31.3													
				% Vit. A Destr...	—		4.8		9.0				25.1		31.7				57.5			67.9													
Mex. Shark.....	Isopropanol Ext.*.....	32,400	34.5	Peroxide No.....	3.2		19.9		27.4		31.3		32.0		31.8		31.5				31.0			29.9			27.9								
				% Vit. A Destr...	—		3.7		9.3		11.7		17.6		19.7		22.5				27.4			32.1			—									
Mex. Shark.....	Isopropanol Ext.*.....	32,400	50	Peroxide No.....	3.2		12.9		13.1		13.4		13.6		11.1		10.8		10.0		9.7			8.9											
				% Vit. A Destr...	—		14.5		20.4		26.8		30.8		37.6		40.5		45.7		48.2			52.1											

* A total of three extractions were made at —18°C., using 99% isopropanol.

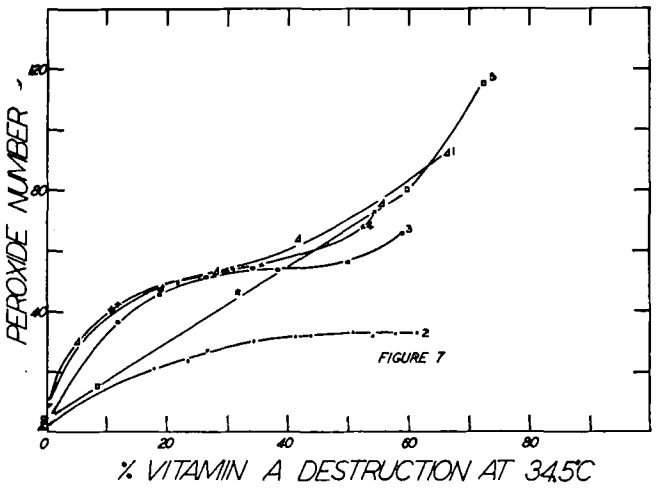
TABLE V

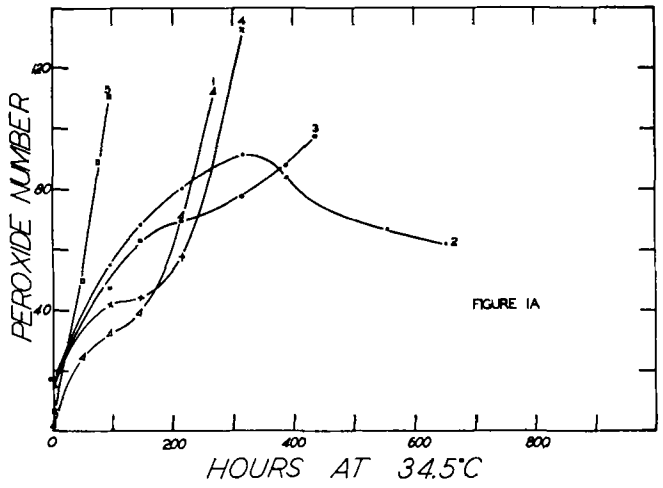
Effect of Carbon and Alpha-tocopherol on Stability of Soupfin Shark Liver Oils and the Soluble Fraction Thereof

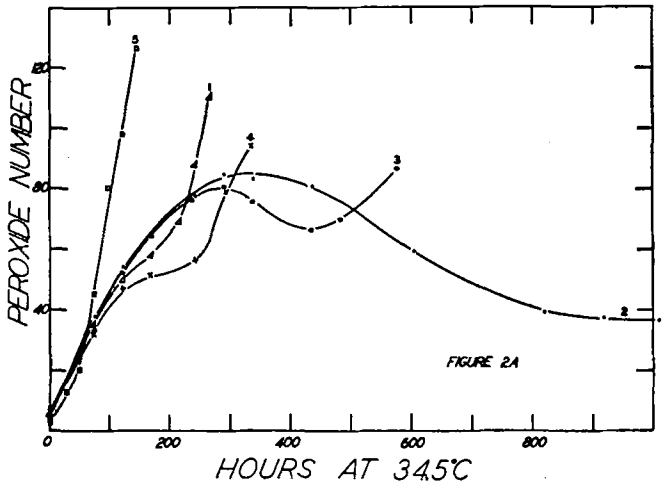
Oil	Treatment	Units A per gm.		Time in Days at 34.5°C.																												
				0	1	2	3	4	5	6	7	9	10	11	12	13	14	15	17	20	22	24	26	28	30	33	38
Soupfin Shark B.....	None	105,000	Peroxide No.....	5.3			35.8		58.4		58.4	68.8	88.0	111.0													
			% Vit. A Destr.	—			6.2		12.7		19.0	34.8	47.8	64.5													
Soupfin Shark B.....	+0.1% α-tocopherol	101,000	Peroxide No.....	5.3			48.9	62.4			87.9		99.3	102.0		120.0	151.0										
			% Vit. A Destr.				5.3	5.9			10.9		25.0	31.9		50.3	73.0										
Soupfin Shark B.....	Carbon-treated*	105,300	Peroxide No.....	4.0	28.2	54.9	89.1	119.0																		
			% Vit. A Destr.	—	18.7	36.8	59.1	72.0																		
Soupfin Shark B.....	Carbon-treated* +0.5% α-tocopherol	101,000	Peroxide No.....	4.0		45.7		90.0	115.0	124.0		180.0		200.0		214.0		251.0									
			% Vit. A Destr.	—		1.8		3.5	4.9	6.4		13.0		22.3		40.5		75.0									
Soupfin Shark B.....	Isopropanol Ext.‡	254,000	Peroxide No.....	1.5		15.2		21.0			53.6		66.7		69.3		72.9		71.8	62.0	53.6	49.8	47.2	46.3	45.1	39.6	34.4
			% Vit. A Destr.	—		1.2		5.9			6.3		13.0		17.7		18.9		25.6	30.3	31.9	35.0	37.0	42.2	44.0	47.2	54.0
Soupfin Shark B.....	Isopropanol Ext.‡ Carbon-treated*	255,000	Peroxide No.....	5.5			26.9	47.6		62.0	69.8	72.2	75.8	77.5	90.0											
			% Vit. A Destr.	—			0	5.4		10.1	20.1	28.3	35.6	35.6	61.5											
Soupfin Shark B.....	Isopropanol Ext.‡ Carbon-treated†	255,000	Peroxide No.....	3.0	10.2	18.0		40.7	49.9	58.9																
			% Vit. A Destr.	—	3.0	9.3		35.4	47.4	59.0																
Soupfin Shark B.....	Carbon-treated† +0.5% α-tocopherol	254,000	Peroxide No.....	3.9			53.5				90.1		121.6				147.5		152.5								
			% Vit. A Destr.	—			4.6				11.4		27.0				79.5		—								

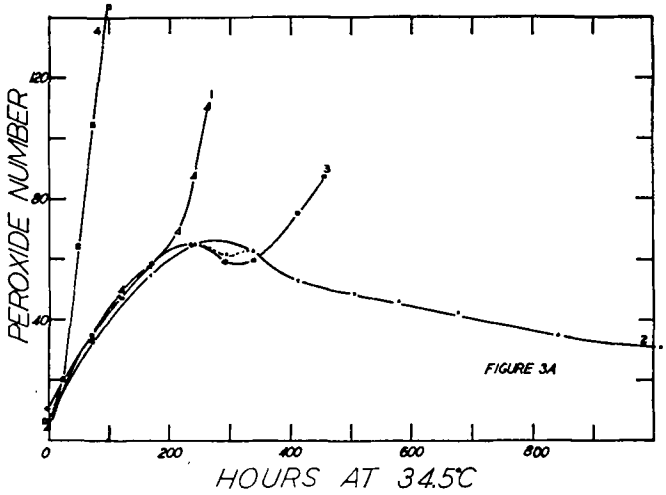
* 20% Carbon (Nuchar XXX) based on the weight of oil was used. ‡ A total of four extractions were made at —18°C. using 99% isopropanol.
† 40% Carbon (Nuchar XXX) based on the weight of oil was used.

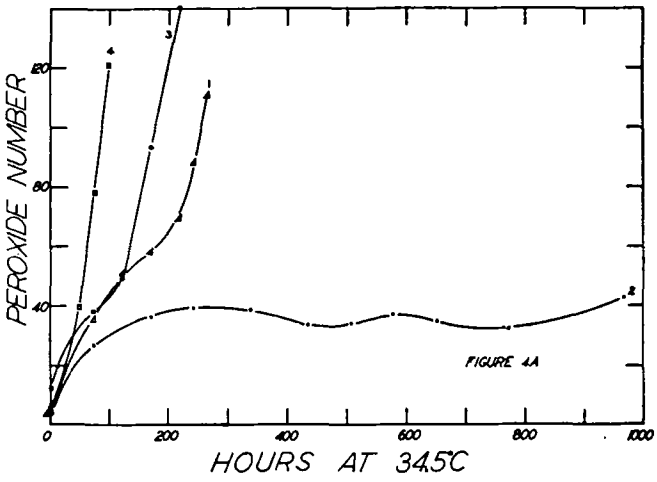
ship between peroxidation and vitamin A destruction of the solvent extract fractions. None of the solvent extract fractions, especially the first extract or two of the oils extracted and the extracts segregated, which obviously contained the naturally occurring antioxidants concentrated several fold therein, exhibited the type of peroxidation versus vitamin A destruction relationship observed for the crude oils examined and crude or carbon-treated oils containing added α-tocopherol or α-tocopherol plus lecithin. The extracts (first one or two or all the extracts combined) produced with 99% and 95% isopropanol from the soupfin shark, Mexican shark, and dogfish shark liver oils (cf. Figures 1, 2, 3, and 5 and Tables III, IV, and V) on oxidation at 34.5°, 50°, and 65°C. exhibited a gradual increase in peroxide number and a decrease in vitamin A content until the peroxide number reaches a relatively low maximum and after that point the peroxide number, as well as the vitamin A content, gradually decreases. For example, in Table IV the 99% isopropanol extract (total of 3 at —18°C.) from soupfin shark liver oil B after storage at 34.5°C. for 14 days exhibited a maximum per-

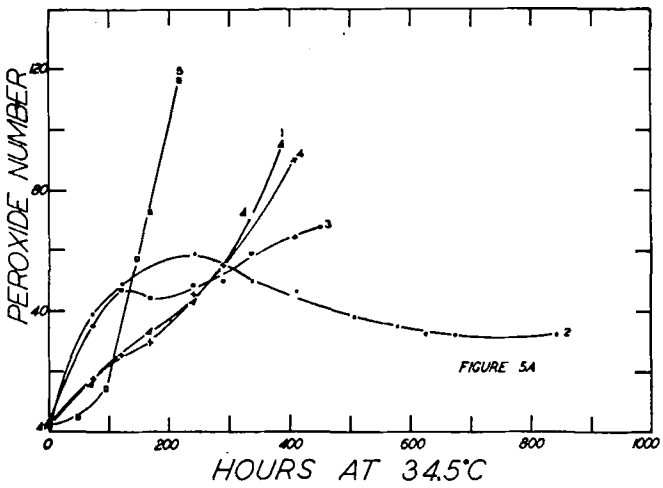


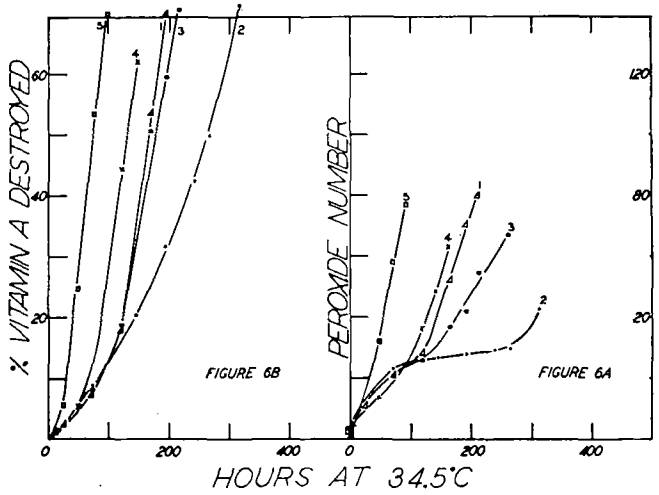


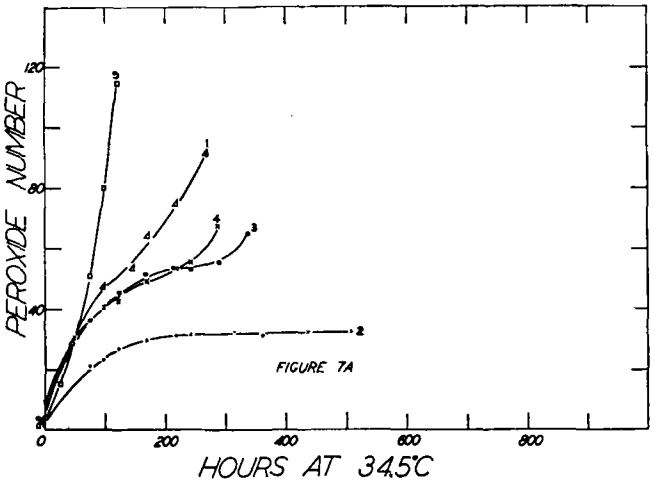










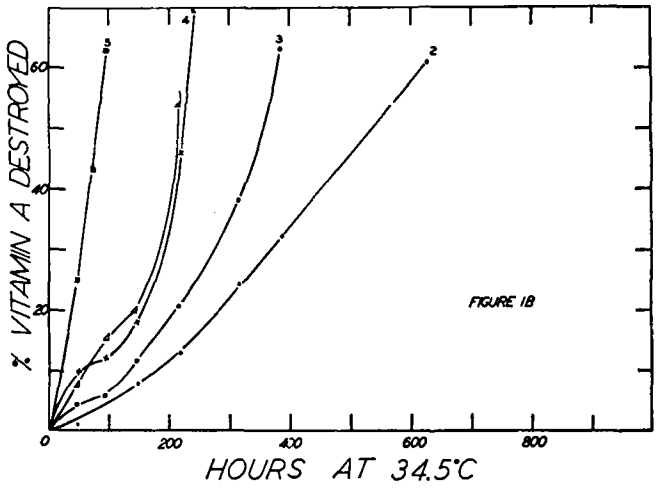


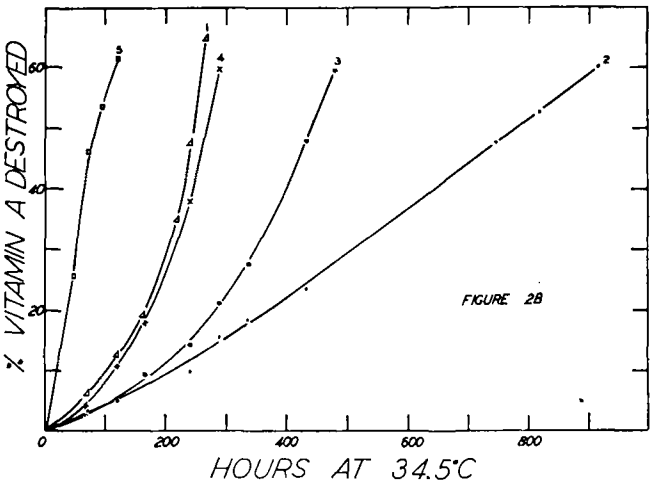
oxide number of 72.9 and a vitamin A loss of 18.9, while after 38 days the peroxide number gradually decreased to 34.4 and the loss of vitamin A increased to 54.0%. The extract when tested at 50°C. showed a maximum peroxide number of 28.3 and a vitamin A loss of 23.6 after 6 days, while after 20 days the peroxide number had decreased to 14.4 and the percentage of vitamin A destruction increased to 64.8. At 65°C. the same extract fraction reached a maximum peroxide number of 8.9 and a vitamin A loss of 34.9% after 5 days, while after 19 days the peroxide number was 6.2 and the per cent of vitamin A destroyed was 72.4. The extract fractions produced using 95% acetone (cf. Figure 4) and methanol (cf. Table III) from the different type oils after various percentage losses of vitamin A possessed considerably lower peroxide values than the original crude oils or the residue fractions. At the higher temperatures (50° and 65°C.) the peroxide numbers were much lower at a given loss of vitamin A than at 34.5°C. In a previous study (9) it was shown that oils stored at room temperature displayed higher peroxide numbers after comparable losses of vitamin A than at 34.5°C.

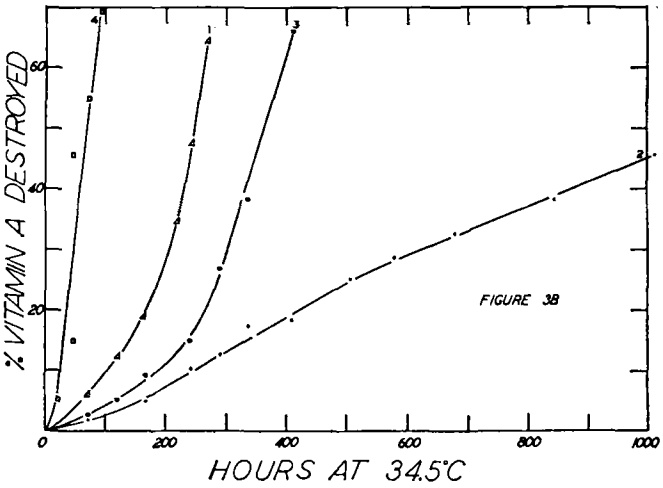
Vitamin A Destruction Versus Time

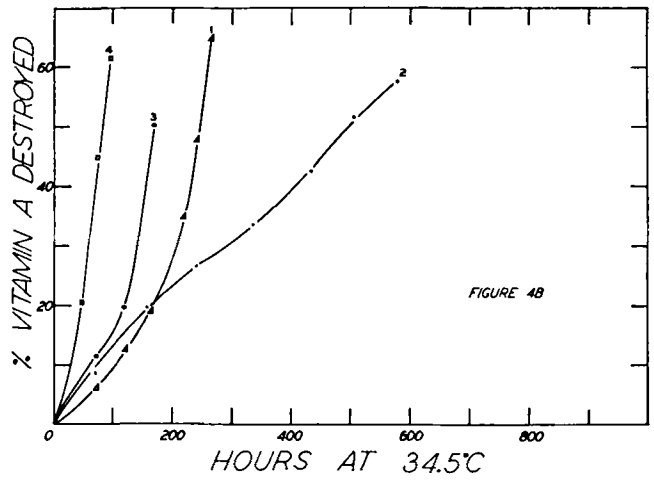
In vitamin A oils and products containing vitamin A the main goal strived for is always to maintain the loss of vitamin A on exposure of the products to air or during storage and usage at the bare minimum.

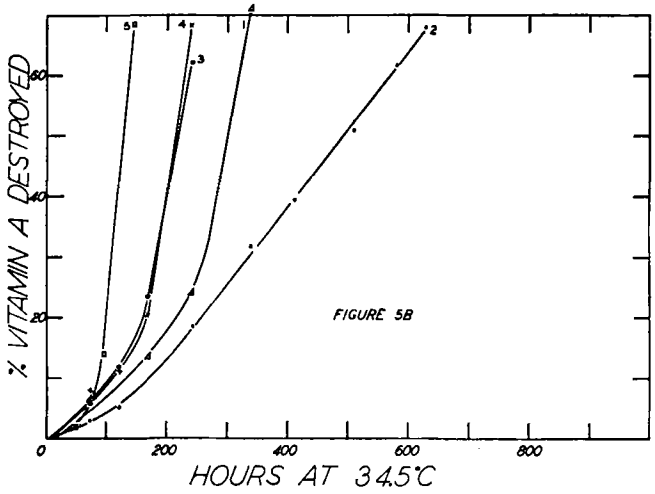
The stability data on vitamin A is recorded graphically in Figures 1B, 2B, 3B, 4B, 5B, 6B, and 7B and in Tables III, IV, and V. It is clear from these data that all of the solvent treatments given the numerous oils under study resulted in the concentration of the naturally occurring antioxidants in an active state in the extract fractions. All of the residue fractions exhibited much lower vitamin A stability than the crude oils. The residue fractions resulting after extracting the oil three or more times with 99% and 95% isopropanol and 95% acetone displayed a rate

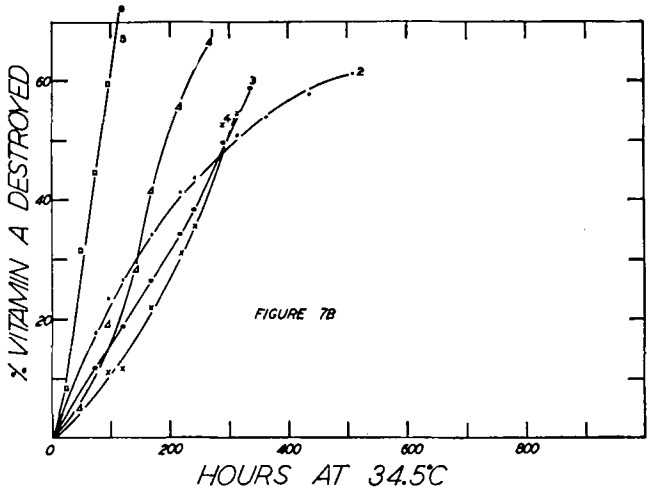












of vitamin A destruction indicative of the substantial absence of active antioxidants. The residue fractions produced, after extracting the oils with methanol (cf. Table III), still possess a small quantity of active antioxidants. However, when these materials were carbon-treated, the antioxidants were completely removed. When the various oils were extracted three times with 99% and 95% isopropanol and 95% acetone, the first extract or first two extract fractions possessed the highest concentration of antioxidants as evidenced by the greatest vitamin A stability. Subsequent extract fractions contained a lower concentration of antioxidants. The methanol extracts, which are known to contain largely the alcohol form of vitamin A, from the various oils studied also exhibited better vitamin A stability than the crude oils. When the oils and fractions thereof were studied for vitamin A stability at the higher temperatures—namely 50° and 65°C., as compared with 34.5°C.—the vitamin A stability decreased rapidly with an increase in temperature (cf. Table IV). For example, the isopropanol extract fraction (total of 3 extractions at —18°C.) after storage at 34.5°, 50°, and 65°C. showed the following percentage losses of vitamin A, respectively: in 7 days, 6.3, 27.9, and 43.5; in 10 days, 13.0, 37.8, and 53.8; in 14 days, 18.9, 50.0, and 62.0; and in 17 days, 25.6, 56.7, and 69.3.

Of the different types of crude fish liver oil examined, the lots of shark liver oil (soupfin, Mexican, and dogfish) were more stable than the oils from the other species of fish—namely halibut, swordfish, sole, lingcod, and blackcod visceral oil. Likewise, the solvent extract fractions prepared from the shark liver oils were more stable in vitamin A potency than comparable extracts from the oils of the other species of fish. In each instance, it can be said that the crude fish liver oils examined contained active, naturally-occurring antioxidants and that extraction of the oils with any one of the four solvents—namely, 99% and 95%isopropanol, 95% acetone, and 100% methanol—resulted in the removal and concentration of the antioxidants in the extract fractions.

Peroxide Number Versus Time

When fish liver oils containing active naturally-occurring antioxidants are exposed to air at 34.5°C., there is at first a rather slow rate of peroxidation during the active life of the antioxidants followed by a more rapid peroxidation after the exhaustion of the antioxidant activity. Fish liver oils depleted of their active antioxidants exhibit no initial period of retarded peroxidation, and peroxides accumulate rapidly and to a value directly proportional to the hours stored at 34.5°C. When α-tocopherol is added to a crude fish liver oil, carbon-treated fish liver oil, or to an extract of a fish liver oil carbon treated the rate that peroxides accumulate increases instead of decreases (Table V). The data collected in this investigation on peroxidation of the oils and fractions thereof examined at 34.5°C. are reported graphically in Figures 1A, 2A, 3A, 4A, 5A, 6A, and 7A and in Tables III, IV, and V and at 50° and 65°C. in Table IV. A quick glance at the peroxidation rates of the first extract fractions or the combined extract fractions of the many oils extracted and studied is sufficient evidence to support the statement that the antioxidants present in the oils examined are highly efficient for inhibiting peroxidation and possess none of the qualities displayed by α-tocopherol. For example, the data plotted as Curve 2 in Figure 4A shows that after approximately 1,000 hours of storage the peroxide value for the combined first two 95% acetone extracts from soupfin shark liver oil B was still around 40. In Curve 2, Figure 5A, the peroxide number on the first 99% isopropanol extract of dogfish shark liver oil rose slowly to a maximum of about 58 after 250 hours and then decreased gradually to a value of about 35 after 850 hours. The 2nd 99% isopropanol extract from the same dog-

fish liver oil (Curve 3, Figure 5A), the 2nd and 3rd 99% isopropanol extracts from soupfin shark liver oil B (Curves 3 and 4, respectively in Figure 2A), and the combined 3rd and 4th 95% isopropanol extracts from the same soupfin shark liver oil (Curve 3, Figure 3A) show clearly the characteristic peculiar behavior of the antioxidants present in these fish liver oil extracts. The fact that the peroxide number increases to a relatively low maximum and then gradually decreases, as shown by Curves 2 in Figures 1A, 2A, and 5A for the 99% isopropanol extracts from the shark liver oils (cf. data in Tables III and IV), or increases to a low maximum followed by a decrease and then a more rapid increase is believed to be due to the concentration and chemical nature of the antioxidants present. This characteristic type of peroxidation shows up not only at 34.5°C., but also at 50°C. and 65°C. (cf. Table IV). The rate of peroxide accumulation at the higher temperatures is much slower. Certainly it is logical to assume that peroxides form more rapidly at the higher temperatures but because of their instability decompose. The rapid decomposition of the peroxides at the higher temperatures explains why vitamin A is destroyed faster at these temperatures. The extract fractions from the oils prepared using methanol exhibit very slow peroxide accumulation rates at 34.5°C.—much slower than the crude oils themselves or the extracts prepared using 99% isopropanol (cf. Table III) from analogous oils.

Summary

Several different species of fish liver oil were fractionated using 91%, 95%, and 99% isopropanol, 95% acetone, and 100% methanol. All of the solvent extract fractions possessed higher vitamin A values than the oils themselves. Analytical data obtained on several of the oils and their fractions show that the solvent extracts have a higher content of unsaponifiable matter and of free fatty acids, as well as a higher iodine value, than the original oils or their residual fractions.

Studies involving peroxidation and vitamin A destruction rates established that the antioxidants associated with the crude oils are concentrated in the solvent extract fractions. It was shown that methanol removed only part of the antioxidants from the crude oils, while 99% and 95% isopropanol and 95% acetone removed substantially all of the antioxidants. The methanol extract fractions exhibited a very slow rate of peroxidation and better vitamin A stability than the crude oils. All of the solvent extract fractions showed increased vitamin A stability and a slower peroxidation rate than the crude oils or their residual fractions. The extracts prepared from three species of shark liver oil—namely soupfin, Mexican, and dogfish—using 99 and 95% isopropanol and 95% acetone displayed a type of peroxidation, on storage of the materials at 34.5°C., entirely different from that given by the crude oils or their fractions and by oils of analogous type containing added α-tocopherol, with and without added lecithin. The same characteristic type of peroxidation was observed when the storage tests were conducted at 50° and 65°C. The peroxide number of the samples stored at the higher temperatures increased very slowly, suggesting that the peroxides were decomposing about as rapidly as they were forming. This was borne out by the fact that vitamin A destruction was much more rapid at the higher temperatures.

On the basis of the type of peroxidation displayed by the various solvent extract fractions studied, it is felt justified to conclude that the crude fish liver oils studied—namely two lots of soupfin shark liver oil, three lots of dogfish liver oil, two lots of halibut liver oil, and one lot each of Mexican shark liver oil, bluefin tuna liver oil, swordfish liver oil, sole liver oil, lingcod liver oil, and blackcod visceral oil—do not derive their major antioxidant activity from a compound of the α-tocopherol type.

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