The Effect of Some Pro-oxidants and Antioxidants on the Stability of Natural Vitamin A

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A study was made on the influence of some pro-oxidants (metal ions) and antioxidants on the stability of vitamin A in fish liver oils and vitamin A concentrates from fish liver oils. The destruction of vitamin A and the change in peroxide number were determined in oil samples stored at 34.5°C.

Under the conditions used, the effects of the pro-oxidants and antioxidants were small. Iron and copper had the greatest pro-oxidative effect of the metal ions tested. Among the antioxidants tested, NN'-diphenyl-*p*-phenylene diamine was the best stabilizer.

Fish liver oils are the best natural sources of the vitamins A and D and are extensively used to supply these essential compounds. When the oils turn rancid, their vitamin contents are destroyed. Rancidity in fish liver oils is mainly due to atmospheric oxidation. As vitamin D has been found to be more stable to oxidation than vitamin A, most of the work in this field has been devoted to studies of the stability of vitamin A.

In this investigation, the catalytic effect of various concentrations of the metal ions Al^{3+} , Cu^{2+} , Fe^{3+} , Ni^{2+} , Sn^{4+} and Zn^{2+} on the destruction of vitamin A was studied. The pro-oxidative effect observed with the metal ions was weak compared to those reported by other investigators.

The stabilizing effect of the following antioxidants and synergists was also investigated: ascorbic acid, ascorbyl palmitate, citraconic acid, citric acid, BHA,** BHT, lecithin, NN'-diphenyl-*p*-phenylene diamine, NDGA, tiodiphenylamine and some commercial antioxidant mixtures. NN'-diphenyl-*p*-phenylene diamine was the best stabilizer and on the whole the effects of the antioxidants were comparatively small.

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** The abbreviations BHA, BHT and NDGA denote butylated hydroxy anisole, butylated hydroxy toluene and nordihydroguaiaretic acid respectively.

EXPERIMENTAL

The properties of the vitamin oils used in these experiments are summarized in Table 1.

Investigations were made on fish liver oils and on vitamin A concentrates obtained by molecular distillation of low potency fish liver oils. In the oils A, C, F and M the contents of the trace metals Cu, Mn, Ni and Fe were estimated. The amount of iron was in the range 0.1–0.5 p.p.m. and the amounts of the other metals were in the range 0.00–0.05 p.p.m. After the oils had been percolated through an ion exchange resin column, the amounts of Cu, Mn and Ni were in the range 0.00–0.01 p.p.m. and the amount of iron was less than 0.1 p.p.m.

Analysis

Vitamin A analysis. Vitamin A was determined using a method which closely resembles that given by Dowler and Laughland¹.

Adsorbent. Commercial bone meal was extracted in a Soxhlet apparatus with hexane and then with acetone in order to remove fatty material soluble in these solvents. The bone meal was dried in an oven at 110°C and the fraction which passed a 100 mesh but was retained by a 200 mesh sieve was used. To remove the finest particles the sieving on the 200 mesh sieve was made with the bone meal suspended in water. After this treatment, the adsorbent was again dried at 110°C.

Procedure. The chromatographic tube used had a length of 120 mm and an internal diameter of 8 mm. At the lower end it had a constriction. A plug of glass wool was placed in the tube and the bone meal adsorbent poured into the tube in the form of a suspension in hexane. After settling the height of the adsorbent column was 60 mm. Without further arrangements the rate of flow of the solvent through this column was 40–60 drops per min.

A 2 ml solution of oil in hexane which contained approximately 100 IU of vitamin A per ml was placed on the top of the column. The vitamin A esters were eluted by three successive portions of 2, 5, and 10 ml of hexane. As no vitamin A alcohol could be detected in any of the batches of oil used in these experiments, no measures were taken to elute this substance in the routine analyses.

The vitamin A in the eluate was determined by measuring the absorbancies at 312, 325 and 335 m μ in a Beckman spectrophotometer model DU. A corrected value for the absorbancy at 325 m μ was calculated by using the formula

$$A_{\text{corr}} = 7 A_{325} - 3.045 A_{312} - 3.955 A_{335}.$$

Using this corrected value, the $E_{1\text{ cm}}^{1\%}$ -value was calculated and the vitamin A contents were determined by using a conversion factor of 1 900. This method has certain limitations especially when the oil is oxidized. Components initially present in the oil or developing during its oxidation and having a relatively high absorbancy in the interval 290–325 m μ are not quantitatively removed by the simple purification on the bone meal column. On the other hand, it is not possible to use a method involving saponification when the oil has a high content of peroxides.

Peroxide value. The peroxide value was determined essentially as suggested by Wheeler². Samples (1 g) of oil were dissolved in 25 ml of a mixture of 60 % glacial acetic acid and 40 % chloroform in 200 ml conical flasks. A saturated solution of potassium iodide (0.5 ml) was added, and the flask gently rotated for 1 min. Distilled water (50 ml) was then added and the liberated iodine titrated immediately with 0.02 N sodium thio-sulphate. Starch was used as an indicator. The results are expressed in millimoles of peroxide per kg of oil.

Estimation of trace metals. The metals Cu, Fe, Mn and Ni were detected and semi-quantitatively estimated by a method given by Täufel³. Oil (20 g) was weighed in a thoroughly cleaned quartz crucible with a diameter of about 50 mm and a height of about 50 mm. The crucible was placed on an electric heating plate and when the oil had reached its ignition temperature, the vapours were ignited. The energy input to the electric plate was regulated to keep the flame of the burning oil as small as possible.

Table 1. Properties of the different oils tested

Oil	Vitamin A contents IU/g $\times 10^3$	Initial peroxide value	Time for 50 % destruction of vitamin A, hours	Peroxide value in the oil when 50 % of the vitamin A initially present is destroyed
A	92.6	22.1	440	80
B	94.2	2.8	578	41
C	44.4	1.9	965	17
D	84.3	2.2	276	52
E	94.4	7.4	212	67
F	45.7	9.6	276	81
G	84.0	1.8	216	71
H	81.0	5.3	209	57
J	97.7	2.9	750	38
K	101.7	2.2	632	34
L	81.8	3.1	247	55
M	38.0	0.5	205	48
N	77.4		272	58
O	72.4	2.3	264	70
P	39.8	0.5	204	59

The 20 g of oil were usually burnt to a coke-like residue in about 1–2 h. The crucible was then placed in an electric oven, where the combustion was completed at 500°C in 3–4 h.

The ash was dissolved in 0.5 ml of 6 N HCl. The resulting solution was evaporated to dryness by placing the quartz crucible in a slanting position on a sand bath in order to keep the resulting dry chloride in as small an area as possible. The dry chloride was dissolved in 0.2 ml of 6 N HCl. Sheets of Whatman No. 4 filter paper (230 $\times$ 420 mm) were washed to remove metal ions which might be present, by passing a solvent system of *n*-butanol (83 %) and 6 N HCl (17 %) through them by using a descending technique. The paper sheets were dried in a stream of warm air.

The hydrochloric acid solution of ash from the oil was placed in two spots on the starting line of two of the above mentioned washed paper sheets. The total amount of the solution was equally divided between the two spots. On each sheet four more spots with known amounts of metal chlorides were placed for comparison. On one sheet the chlorides of copper and nickel were placed and on the other the chlorides of iron and manganese. The paper sheets were chromatographed with the above mentioned solvent system for 5–7 h. During this time the solvent front had moved 200–300 mm. The paper sheets were dried in a stream of warm air. The sheet with the control spots of copper and nickel was sprayed with a solution of rubeanic acid (0.05 %) in 99 % ethanol and then treated with ammonia vapour. Copper gave a grey to green spot with an R_F -value of 0.1–0.2 and nickel a violet spot with an R_F -value of 0.02–0.08. Amounts of 0.05 μ g of copper and nickel were easily detected. The sheet with the control spots of iron and manganese was sprayed near the starting line (to $R_F = 0.3$) with a water solution of sodium cyanide (15 %) in order to bind the nickel ion in a complex that would not interfere with the determination of manganese. This metal was detected as a brown spot (R_F -value 0.05–0.15) after spraying the upper part of the sheet with a solution of formaldoxim according to Hecht and Gottlieb^{4*}. The sheet was subsequently treated with ammonia vapour. The method readily permits the detection of 0.05 μ g of manganese.

* The formaldoxim solution according to Hecht and Gottlieb is prepared by dissolving hydroxylamine hydrochloride (19.95 g) and paraformaldehyde (10 g) in distilled water (100 ml).

Iron present in amounts of approximately 5 μg could easily be seen as a yellow spot. Even 1 μg quantities were easily detected if the sheets were sprayed with a methanolic solution of ammonium rhodanide (5 %) and subsequently treated with hydrochloric acid vapours. The R_F -value of iron was 0.5–0.7.

Recovery experiments in which Cu, Mn and Ni were added to the oil in amounts of 0.1 p.p.m. showed that complete recovery was obtained with Mn and Ni, whereas a negligible loss of Cu occurred during the analytical process.

Procedure

The testing of the oil was performed according to a method used by Simons *et al.*⁵ Oil (2 ml) was carefully measured into several beakers, 23 mm in diameter and 27 mm in height, which had been thoroughly cleaned just prior to use. Care was taken to avoid smearing the oil on the walls of the beakers. The samples were placed in a constant temperature oven at $34.5 \pm 0.5^\circ\text{C}$. At recorded intervals, three (in some experiments two) beakers of each sample of oil were removed and individual determinations of the peroxide values and vitamin A contents were immediately performed.

Metallic ions were removed from the oils in the following way: A chromatographic tube, approximately 400 mm in length and 22 mm in diameter, was used. At its lower end there was a glass filter plate and a stopcock. The tube was filled with a slurry of an ion exchange resin (Amberlite IR 100, analytical quality) in distilled water to give a bed approximately 320 mm deep. The level of the liquid was not allowed at any time to fall below that of the resin. Percolation of 5 % HCl (1 800 ml) through the column over a period of at least 15 min ensured that the resin was in the hydrogen form. The column was washed with successive portions of distilled water (10 ml) until the effluent had a pH of about 4. The column was then prepared for the oil by percolating 100 ml of 2-propanol through it in order to remove water from the resin, followed by 100 ml of a mixture of 85 % 2-propanol and 15 % benzene.

Fifty ml of oil was mixed with 15 ml of benzene and 35 ml of 2-propanol and the mixture percolated through the column. Fifty ml of 30 % benzene in 2-propanol and then pure 2-propanol were added until all the oil had passed through the column. The solvents were removed from the oil-2-propanol-benzene mixture by evaporation under vacuum.

Metal ions were usually incorporated in the oil samples as chlorides dissolved in absolute ethanol. These solutions were added to the oil to give a final concentration of 2 % of the total amount of oil. In two experiments the ions of copper and iron were incorporated in the oils as stearates dissolved in paraffin oil. The solutions were added to give a final concentration of 2 % of the vitamin A oil. Antioxidants were added as solutions in propylene glycol or ground-nut oil and these solutions were always added to give a concentration of 2 % of the total amount of the vitamin A oil. Citric acid was added as a 30 % solution in absolute ethanol. As citric acid was usually added to a total concentration of 0.01 %, the amount of ethanol added in this way seldom exceeded 0.05 %.

RESULTS

The experiments are summarized in Tables 2–20.

In the tables the term "relative stability" means ratio of the time for 50 % destruction of vitamin A in oil containing metal ion or antioxidant, to the time for the same destruction of vitamin A in the same oil containing no metal ion or antioxidant.

Figs. 1–4 are typical diagrams showing peroxide value relative to time, percentage destruction of vitamin A relative to time, and percentage destruction of vitamin A relative to peroxide value.

Fig. 1. Increase in peroxide value in oil from which metal ions had been removed and to which various amounts of Cu had been added (as CuCl_2). The samples were kept in the dark and exposed to air at 34.5°C for different lengths of time.

○ Oil, (metal ions removed)
 △ » » » » + 1 ppm Cu
 ○ » » » » + 10 ppm Cu
 × » » » » + 100 ppm Cu

Fig. 2. Destruction of vitamin A in oil from which metal ions had been removed and to which various amounts of Cu had been added (as CuCl_2). The samples were kept in the dark and exposed to air at 34.5°C for different lengths of time.

○ Oil, (metal ions removed)
 △ » » » » + 1 ppm Cu
 ○ » » » » + 10 ppm Cu
 × » » » » + 100 ppm Cu

Fig. 3. Destruction of vitamin A relative to peroxide value in oil from which metal ions had been removed and to which various amounts of Cu had been added (as CuCl_2). The samples were kept in the dark and exposed to air at 34.5°C for different lengths of time.

○ Oil (metal ions removed)
 △ » » » » + 1 ppm Cu
 ○ » » » » + 10 ppm Cu
 × » » » » + 100 ppm Cu

Fig. 4. Destruction of vitamin A in oil with various antioxidants added and with no addition of antioxidant. The samples were kept in the dark and exposed to air at 34.5°C for different lengths of time.

○ No antioxidant
 △ 0.01 % citric acid
 ○ 0.01 % DPPD
 × 0.01 % citric acid + 0.1 % BHT + 0.02 % lecithin

Not only did the metal ions increase the destruction rate of vitamin A, they also decreased the peroxide value for the oil at a certain percentage of vitamin A destruction. When the metal ions were added as stearates, as in the two experiments with iron and copper, their catalytic effect was not so pronounced as when they were added as chlorides.

Table 2. Effect of Al^{3+}

Oil	Concentration of Al^{3+} p.p.m.	Time for 50 % destruction of vitamin A, hours	Peroxide number in the oil when 50 % of the vitamin A initially present is destroyed	Relative stability
A	0	440	80	1
A	10	268	61	0.61
A	100	216	30	0.48
B	0	578	41	1
B	1	526	35	0.91
B	10	380	22	0.66
B	100	298	13	0.52
C	0	965	17	1
C	100	379	12	0.38

Aluminium ions markedly increased the rate of vitamin A destruction and this effect was noticeable even at concentrations of 1 p.p.m. (Table 2).

At concentrations of 1 p.p.m. and 10 p.p.m., copper was the strongest accelerator of the destruction of vitamin A of all the metals tested. The addition of 10 p.p.m. and 100 p.p.m. of copper ions caused the same rate of destruction, but the peroxide value for the same percentage of vitamin A destruction was much lower in the oil with 100 p.p.m. of copper (Table 3).

Table 3. Effect of Cu^{2+} .

Oil	Concentration of Cu^{2+} p.p.m.	Time for 50 % destruction of vitamin A, hours	Peroxide number in the oil when 50 % of the vitamin A initially present is destroyed	Relative stability
P	0	204	59	1
P	1	173	43	0.85
P	10	96	27	0.47
P	100	96	5	0.47
E	0	212	67	1
E	1 *	219	66	1.03
E	10 *	127	50	0.60
F	0	276	81	1
F	10	89	23	0.28
F	100	89	11	0.28
C	0	965	17	1
C	100	145	5	0.15

* The copper ions were added as stearates dissolved in paraffin oil.

Table 4. Effect of Fe^{3+}

Oil	Concentration of Fe^{3+} p.p.m.	Time for 50 % destruction of vitamin A, hours	Peroxide number in the oil when 50 % of the vitamin A initially present is destroyed	Relative stability
P	0	204	59	1
P	1	238	60	1.17
P	10	144	43	0.71
P	100	44	23	0.22
G	0	216	71	1
G	6*	195	65	0.90
G	60*	122	54	0.56
F	0	276	81	1
F	10	126	46	0.46
C	0	965	17	1
C	100	87	8	0.09

* The ferric ions were added as stearates dissolved in paraffin oil.

Ferric ions at a concentration of 100 p.p.m. caused more rapid destruction of vitamin A than any concentration of the other metals tested. Iron did not accelerate the breakdown of vitamin A at 1 p.p.m. but increased its destruction at 6 p.p.m. The decrease in peroxide value at 50 % destruction of vitamin A was less pronounced when iron was added to the oil than when copper was added (Table 4).

Nickel ions had a moderately accelerating effect on the destruction of vitamin A. The decrease in peroxide value for 50 % destruction of vitamin A with nickel ions was less than for ions of copper and iron. It was confusing to notice that in oil C, which was found to be extremely sensitive to the

Table 5. Effect of Ni^{2+}

Oil	Concentration of Ni^{2+} p.p.m.	Time for 50 % destruction of vitamin A, hours	Peroxide number in the oil when 50 % of the vitamin A initially present is destroyed	Relative stability
H	0	209	57	1
H	1	191	57	0.91
H	10	138	54	0.66
H	100	76	38	0.36
C	0	965	17	1
C	100	610	14	0.63

Table 6. Effect of Sn^{4+}

Oil	Concentration of Sn^{4+} p.p.m.	Time for 50 % destruction of vitamin A, hours	Peroxide number in the oil when 50 % of the vitamin A initially present is destroyed	Relative stability
B	0	578	41	1
B	1	546	38	0.95
B	10	526	40	0.91
B	100	386	28	0.66
C	0	965	17	1
C	100	385	9	0.40

presence of all the other metal ions tested, the effect of nickel was comparatively small (Table 5).

The pro-oxidative effects of tin and zinc were weak. The increase in vitamin A destruction and the decrease in peroxide value were hardly detectable when these metal ions were added at concentrations of 1 p.p.m. At 10 p.p.m. these metals had a noticeable effect (Tables 6 and 7).

Table 8 shows that citric acid definitely stabilized vitamin A. In the range 0.1—0.01 %, the effect was practically independent of the concentration. Citric acid was effective in "trapping" copper ions which is shown in Table 12. Ascorbic acid had good antioxidant properties. The addition of 0.01 % of ascorbic acid to one of the tested oils increased the time for 50 % destruction of vitamin A by about 20 %. Ascorbyl palmitate had no effect (Tables 9 and 10).

Citraconic acid is considered to be a powerful antioxidant for margarine ⁶. In these experiments, however, citraconic acid had no antioxidant properties

Table 7. Effect of Zn^{2+}

Oil	Concentration of Zn^{2+} p.p.m.	Time for 50 % destruction of vitamin A, hours	Peroxide number in the oil when 50 % of the vitamin A initially present is destroyed	Relative stability
A	0	440	80	1
A	100	268	41	0.61
B	0	578	41	1
B	1	532	38	0.92
B	10	552	39	0.96
B	100	476	23	0.82
C	0	965	17	1
C	100	490	13	0.51

Table 8. Effect of citric acid on the stability of vitamin A oil alone and together with other antioxidants

Oil	Citric acid %	Other antioxidants	Time for 50 % destruction of vitamin A, hours	Peroxide number in the oil when 50 % of the vitamin A initially present is destroyed	Relative stability
N	0	—	272	58	1
N	0.01	—	309	60	1.14
M	0	—	205	59	1
M	0.01	—	215	63	1.05
M	0	Lecithin 0.1 %	268	53	1.30
M	0.01	Lecithin 0.1 %	271	48	1.32
K	0	—	632	34	1
K	0.05	—	695	33	1.10
K	0.1	—	695	33	1.10
K	0	Ascorbyl palm. 0.05 %	620	36	0.98
K	0.05	Ascorbyl palm. 0.05 %	680	34	1.08
C	0	—	965	17	1
C	0.01	—	1 064	16	1.10

(Tables 11 and 12). Table 12 shows the effects of citric, ascorbic and citraconic acids in an oil in which 10 p.p.m. of copper was incorporated.

Lecithin was found to be a very good antioxidant for stabilizing vitamin A. In the concentration range 0.01—0.1 % the stabilizing effect increased approximately linearly with the concentration. The effect of lecithin was added to the effect of phenolic antioxidants present but, as could be expected, the combined effect of citric acid and lecithin was less than the sum of the effects for each of them (Table 13).

DPPD (NN'-diphenyl-*p*-phenylene diamine) was the most powerful stabilizer of vitamin A of all the antioxidants tested. In the range 0.001—0.01 %,

Table 9. Effect of ascorbic acid on the stability of vitamin A oil alone and together with citric acid

Oil	Ascorbic acid %	Citric acid %	Time for 50 % destruction of vitamin A, hours	Peroxide number in the oil when 50 % of the vitamin A initially present is destroyed	Relative stability
O	0	0	264	70	1
O	0.01	0	321	73	1.22
L	0	0	247	55	1
L	0.01	0.01	285	54	1.15

Table 10. Effect of ascorbyl palmitate on the stability of vitamin A oil alone and together with citric acid

Oil	Ascorbyl palmitate %	Citric acid %	Time for 50 % destruction of vitamin A, hours	Peroxide number in the oil when 50 % of the vitamin A initially present is destroyed	Relative stability
K	0	0	632	34	1
K	0.01	0	613	36	0.97
K	0.05	0	620	36	0.98
K	0.05	0.05	680	34	1.08
J	0	0	750	38	1
J	0.05	0.05	790	37	1.05

Table 11. Effect of citraconic acid on the stability of vitamin A oil alone and together with citric acid

Oil	Citraconic acid %	Citric acid %	Time for 50 % destruction of vitamin A, hours	Peroxide number in the oil when 50 % of the vitamin A initially present is destroyed	Relative stability
O	0	0	264	70	1
O	0.01	0	254	73	0.99
L	0	0	247	55	1
L	0.1	0.01	268	48	1.09

Table 12. Effect of some acids together with Cu²⁺

Oil	Antioxidant	Cu ²⁺ p.p.m. as stearate in paraffin oil	Time for 50 % destruction of vitamin A, hours	Peroxide number in the oil when 50 % of the vitamin A initially present is destroyed	Relative stability
O	—	—	264	70	1
O	Ascorbic acid	0.01 %	321	73	1.18
O	»	0.01 %	100	34	0.38
O	Citric acid	0.01 %	288	68	1.09
O	»	0.01 %	211	72	0.80
O	Citracon. acid	0.01 %	254	73	0.96
O	»	0.01 %	70	17	0.27

Table 13. Effect of different concentrations of lecithin on the stability of vitamin A oil alone and together with other antioxidants

Oil	Lecithin %	Other antioxidant	Time for 50 % destruction of vitamin A, hours	Peroxide number in the oil when 50 % of the vitamin A initially present is destroyed	Relative stability
N	0	—	272	58	1
N	0.1	Citric acid 0.01 %	355	65	1.30
P	0	—	204	59	1
P	0.01	—	241	58	1.18
P	0.03	—	244	58	1.20
P	0.1	—	268	53	1.31
P	0.1	Citric acid 0.01 %	271	48	1.33
M	0	—	205	48	1
M	0.1	—	254	50	1.24
M	0.1	BHA 0.02 %	275	55	1.34
M	0.1	BHA 0.02 %	288	54	1.40
M	0.1	Tiodiph.amine 0.01 %	236	46	1.15
M	0.1	DPPD 0.01 %	370	50	1.80

its effect increased approximately linearly with the logarithm of the concentration (Table 14).

The results from the experiments made with BHA and BHT are summarized in Tables 15, 16, and 17. Table 15 shows that BHA and a mixture of equal amounts of BHA and BHT were inferior to BHT in their ability to stabilize vitamin A.

Table 14. Effect of DPPD on the stability of vitamin A oil alone and together with lecithin

Oil	DPPD %	Lecithin %	Time for 50 % destruction of vitamin A, hours	Peroxide number in the oil when 50 % of the vitamin A initially present is destroyed	Relative stability
C	0	0	965	17	1
C	0.01	0	1 875	16	1.94
C	0.01	0	2 000	18	2.07
M	0	0	205	48	1
M	0.001	0	222	No test	1.08
M	0.003	0	262	» »	1.28
M	0.01	0	340	38	1.66
M	0.01	0	314	48	1.53
M	0.01	0.1	370	50	1.80
M	0	0.1	254	50	1.24

Table 15. Comparison between BHA and BHT

Oil	Antioxidant mixture		Time for 50 % destruction of vitamin A, hours	Peroxide number in the oil when 50 % of the vitamin A initially present is destroyed	Relative stability
M	Lecithin	0.1 %	329	49	1.34
	BHA	0.02 %			
	Citric acid	0.01 %			
M	Lecithin	0.1 %	375	49	1.55
	BHA	0.01 %			
	BHT	0.01 %			
	Citric acid	0.01 %			
M	Lecithin	0.1 %	428	50	1.77
	BHT	0.02 %			
	Citric acid	0.01 %			

In this experiment the temperature in the constant temperature oven fell to room temperature and remained at that level for 24 h. The figures are valid only when compared with each other.

Some experiments were made with propyl gallate, NDGA and tioidiphenylamine. The results are summarized in Tables 18 and 19. NDGA and propyl gallate had distinctly antioxidative effects but tioidiphenylamine had no effect. The latter substance even seemed to be antagonistic to lecithin.

Table 16. Effect of BHT on the stability of vitamin A oil together with lecithin and/or citric acid

Oil	BHT %	Lecithin %	Citric acid %	Time for 50 % destruction of vitamin A, hours	Peroxide number in the oil when 50 % of the vitamin A initially present is destroyed	Relative stability
M	0	0	0	205	48	1
M	0	0.1	0	254	50	1.24
M	0.02	0.1	0	288	63	1.40
M	0.02	0.1	0.01	296	65	1.44
M	0.02	0.1	0.01	295	55	1.44
M	0.02	0.1	0.01	362	46	1.77
C	0	0	0	965	17	1
C	0	0	0.01	1 064	16	1.10
C	0.02	0.1	0.01	1 255	17	1.30
N	0	0	0	272	58	1
N	0	0	0.01	314	60	1.14
N	0.02	0	0.01	337	64	1.24
N	0	0.1	0.01	355	65	1.30
N	0.02	0.1	0.01	375	66	1.38

Table 17. Effect of BHA on the stability of vitamin A oil together with lecithin

Oil	BHA %	Lecithin %	Time for 50 % destruction of vitamin A, hours	Peroxide number in the oil when 50 % of the vitamin A initially present is destroyed	Relative stability
M	0	0	205	48	1
M	0	0.1	254	50	1.24
M	0.02	0.1	275	55	1.34
M	0.02	0.1	288	54	1.40

Table 18. Effect of propyl gallate and NDGA on the stability of vitamin A oil together with citric acid

Oil	Antioxidant	Citric acid	Time for 50 % destruction of vitamin A, hours	Peroxide number in the oil when 50 % of the vitamin A initially present is destroyed	Relative stability
L	—	0	247	55	1
L	—	0.01	268	55	1.08
L	Propyl gallate 0.01 %	0.01	294	62	1.19
L	NDGA 0.01 %	0.01	300	56	1.21

Table 19. Effect of tioldiphenyl amine on the stability of vitamin A oil alone and together with lecithin

Oil	Tioldiphenyl amine %	Lecithin %	Time for 50 % destruction of vitamin A, hours	Peroxide number in the oil when 50 % of the vitamin A initially present is destroyed	Relative stability
M	0	0	205	48	1
M	0.01	0	202	43	0.99
M	0	0.1	254	50	1.24
M	0.01	0.1	236	46	1.15

Some commercial antioxidant mixtures were also tested. The results from these experiments are summarized in Table 20. A mixture containing BHT, BHA, propyl gallate and lecithin citrate gave the best results. When added at a concentration of 0.05 %, as recommended by the manufacturer, this mixture increased the time for 50 % destruction of vitamin A by 50 %.

Table 20. Effect of some commercial antioxidant mixtures on the stability of vitamin A oil

Oil	Trade name	Conc. = maximum recommended by manu- facturer	Time for 50 % destruction of vitamin A, hours	Peroxide number in the oil when 50 % of the vitamin A initially present is destroyed	Relative stability
J	—	—	750	38	1
J	Tenox II	0.05	803	37	1.07
M	—	—	205	48	1
M	Tenox II	0.05	267	51	1.30
J	—	—	750	38	1
J	"Keep It" Green	0.05	770	37	1.03
J	"Keep It" Blue	0.05	722	35	0.96
M	—	—	205	48	1
M	G4	0.05	270	55	1.32
M	G15	0.05	315	61	1.54
M	G15	0.05	300	46	1.46
M	Sustane F	0.02	263	52	1.28

DISCUSSION

The effect of metal ions on the oxidation of various fats and oils has been studied by Lea ⁷, Morris *et al.*⁸, and Evans *et al.*⁹ and others. Lea studied the accelerating effect of metal ions at room temperature in the presence of an aqueous phase while the other investigators used an A.O.M. stability test. Lea found that 1 p.p.m. of Fe and 0.1 p.p.m. of Cu greatly enhanced the oxidation of herring oil. Morris *et al.* found that the same amounts of these metals decreased the stability of lard by 50 %. Evans *et al.* found that 0.3 p.p.m. of Fe greatly accelerated the oxidation of soya-bean oil.

Under the conditions of this investigation the effect of trace metals is less pronounced. To double the rate of destruction of vitamin A, 10 p.p.m. of copper or iron has to be added to the oil when stored in the dark at 34.5°C. Aluminium or nickel must be present at a concentration of about 100 p.p.m. in order to accelerate the destruction to the same extent. As much as 100 p.p.m. of tin or zinc do not usually increase the rate of vitamin A destruction by 100 %. Metal ions seem to have at least two different effects on the oxidation of vitamin A in fish liver oil. One is a direct enhancement of the oxidation of vitamin A. The other is an acceleration of the breaking down of the peroxides formed. This second reaction seems to decrease the rate of oxidation of vitamin A *via* peroxides.

The effects of various antioxidants on the stability of vitamin A have previously been studied by Buxton ^{10,11}, Caspe and Hadjopoulos ¹², Kring ¹³, Swain and Idler ¹⁴, Kamath and Magar ¹⁵, Spruyt ¹⁶, and Wodsack ¹⁷ and others. Different methods have been tried for testing and determining the vitamin A and peroxide values.

In this investigation, citric acid was found to act as a good stabilizer of vitamin A. It is not considered to be a true antioxidant^{6,9}, but is effective as an inactivator of trace metals and as a synergist for true antioxidants. As the fish liver oils tested contained detectable amounts of trace metals and as fish liver oils have been shown to contain antioxidants¹⁰, it is understandable that citric acid should have some stabilizing effect.

Antioxidative properties have been reported for ascorbyl palmitate¹⁷ and for citraconic acid⁶. In the experiments with fish liver oils reported here, however, no antioxidative effect could be detected.

Lecithin has a comparatively good stabilizing effect on vitamin A in fish liver oil. This effect is, however, only slightly increased by the addition of citric acid. The phosphorous acid group of lecithin is itself able to inactivate trace metals and therefore no additional effect of citric acid can be expected.

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