Effects of Tocopherols, Ascorbyl Palmitate, and Lecithin on Autoxidation of Fish Oil

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ABSTRACT: The effects of α-, γ/δ, and δ-tocopherol concentrates (0.2–2.0%) alone and in combination with ascorbyl palmitate (0.1%) and lecithin (0.5%) on oxidative stability and flavor of fish oil were studied. Stability was assessed on oil stored in air at 20°C by peroxide value (PV) and off-flavor formation. Polymer content, *para*-anisidine value, and conjugation were used to characterize selected samples. When used alone, the protective effect of the tocopherols, as measured by PV, was δ >> γ/δ >> α, especially at the 2% concentration. Binary systems of ascorbyl palmitate–lecithin and lecithin–γ/δ or –δ-tocopherol were strongly synergistic in delaying peroxidation. The ternary blends provided the greatest protection against autoxidation. Refined fish oil with 2% δ-tocopherol, 0.1% ascorbyl palmitate, and 0.5% lecithin showed no significant peroxidation at 20°C over a period of 6 mon. The original antioxidant effect noted for the ternary systems in delaying peroxidation was not reflected in improved flavor stability. Off-flavors developed within 3 wk, making the oils unsuitable for use at high concentrations in ambient products that are unprotected from air.

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Increasing interest in the nutritional role of n-3 long-chain polyunsaturated fatty acids (LCPUFA) in the diet has prompted significant research into methods of stabilizing unhydrogenated fish oil against oxidation and off-flavor development. As far as we are aware, the problem of preventing the rapid development of fishy flavor and hydroperoxide formation in fish oil exposed to air has not been overcome. Many commercial products use encapsulation to make fish oil palatable. However, even encapsulated products give a false sense of security with regard to autoxidation. Some products have contained high levels of hydroperoxide and autoxidation by-products (1).

Good-quality crude fish oil with a free fatty acid content < 4%, *p*-anisidine value (PAV) < 20, and peroxide value (PV) < 5 will give a refined fish oil that is essentially bland (2,3). In the presence of air, however, the unprotected oil will develop fishy and rancid flavors in a matter of hours or days. The dilution of fish oil by vegetable oil is effective in retarding the development of fishy off-flavors in some products (2).

The effectiveness of antioxidants for protecting fish oils is of relatively recent interest, compared to the wide literature on antioxidants in vegetable oils and fats and in lard and tallow. The majority of these publications describe oxidative stability tests that are accelerated by elevated temperatures (4–8) to demonstrate the effectiveness of the preferred systems. Stability tests nearer ambient temperature have involved chemiluminescence (9) and thin films (10). Single antioxidants have proved ineffective in significantly retarding the autoxidation of fish oil (9).

A number of publications on stabilizing polyunsaturated vegetable oils do not support the notion that using extremely high levels of antioxidant should adequately protect fish oil. For example, research on tocopherols (11–13) demonstrated that the optimal concentrations of α-, γ-, and δ-tocopherols in soybean oil triglycerides were 100, 250, and 500 ppm, respectively (11). Greater levels than this showed pro-oxidant effects. The optimal level for soybean tocopherols lies between 400 and 1000 ppm (12,13). In a more recent study (14), the antioxidant activities of α- and γ-tocopherols in bulk corn oil triglycerides and in emulsions were determined at 60°C. The conclusions drawn depended on whether hydroperoxide or hexanal was used to measure oxidative degradation. When hydroperoxides were used as a measure, α-tocopherol was slightly pro-oxidant at levels >250 ppm in oil and >500 ppm in emulsions. γ-Tocopherol did not display pro-oxidant activity in either system. Hexanal formation was, however, increasingly inhibited with increased concentrations of either tocopherol up to 1000 ppm.

Koskas *et al.* (15) studied the stabilizing effect of α-, γ-, and δ-tocopherols on pure linoleic acid. A pro-oxidant effect was observed with 3.8% α-tocopherol. Mild antioxidant effects were observed at 0.38 and 0.038%; but γ- and δ-tocopherols exhibited antioxidant activity even at 3.8% concentration.

The effect of synthetic antioxidants has been widely studied in conventional oils and fats (13). Their ability to stabilize fish oil has been studied to a limited extent, and of those examined, *tert*-butylhydroquinone proved to be the most effective (4). This antioxidant is not permitted for use in edible oils in most European countries, although it is permitted in the United States of America. A range of natural antioxidants was evaluated in cod

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liver oil (9) with limited success but did provide useful comparisons between methods of evaluating oxidative stability.

The most notable success in fish oil stabilization has been achieved with ternary antioxidant systems that contained α- or γ-tocopherol concentrates, ascorbic acid (or ascorbyl palmitate), and lecithin (5–7,9,16–19). This ternary mixture can be regarded as an extension of the binary system tocopherol/phospholipid in which synergistic effects were observed in lard, soybean, and rapeseed oils (18,20–22) and the nonpolar lipid fraction of sardine oil (23). Synergy between δ-tocopherol, lecithin, and ascorbic acid has also been reported when the ascorbic acid is present in an aqueous microemulsion in fish oil (8,10).

Virtually all publications on the ternary antioxidant system have relied on high-temperature (50–100°C) accelerated tests, e.g., the Rancimat test, to evaluate oxidative stability (5–7). A selected series of results from various authors is given in Table 1 and demonstrates the significant protection that is afforded by ternary systems based on tocopherol, ascorbic acid/ascorbyl palmitate, and lecithin.

In the studies reported here, we have determined the antioxidant properties at 20°C of certain binary blends and ternary blends of tocopherol (α, γ/δ, and δ), ascorbyl palmitate, and lecithin. We have chosen ascorbyl palmitate in preference to ascorbic acid because of its greater solubility in oil. Previous work (24) with refined Chilean fish oil (anchovy) indicated that γ/δ-tocopherol was not pro-oxidative up to 2.6% at ambient temperature. Consideration of these results suggested that examination of the effect of tocopherol in the range 0.2–2.0% would be of interest, especially when combined with ascorbyl palmitate and lecithin in refined fish oil.

The oxidative stability of the systems has been determined at 20°C in air by sensory evaluation and over prolonged periods by PV measurement. The base oils used in these studies were refined menhaden and Chilean fish oils (anchovy). We appreciate that PV alone is not a satisfactory measure of oxidation state because of subsequent potential side reactions and have therefore used a wider range of analytical parameters on selected blends to confirm our conclusions.

EXPERIMENTAL PROCEDURES

Materials. Oils were normal commercially obtained crude fish oils, either menhaden or Chilean (anchovy). Ascorbyl palmitate and α-tocopherol concentrate (Table 2) were purchased from Sigma Chemical Co. (Poole, United Kingdom). γ/δ-Tocopherol and δ-tocopherol concentrates (Table 2) were gifts from Eisai Pharma-chem Europe Ltd. (London, United Kingdom). Soy lecithin (~56% phospholipid in soybean oil) was a gift from Quest International (Maarssen, The Netherlands).

Refining. All fish oils were refined by previously disclosed methods (2), including soda ash/silicate "boiling" after neutralization, bleaching with high (4%) levels of activated earth (R169, Laporte; United Kingdom Inorganics, Widnes, United Kingdom), and deodorizing between 175 and 185°C.

Tocopherol was added either before bleaching (menhaden oil) or after deodorization (Chilean fish oil), depending on the number of antioxidant systems being studied. Ascorbyl palmitate was added to the oil at ~120°C (N_2 sparging) after deodorization, and lecithin was stirred into the oil at ~40°C after deodorization. All oils contained 50 ppm citric acid (aqueous solution), added to the deodorized oil at ~100°C during cooling under high vacuum.

Analyses. PV was measured by AOCS method Cd8-53. PAV was measured by AOCS method Cd 18-90. Fatty acids were measured by AOCS method CE1b-89.

Diene and triene conjugation was measured at 232–238 and 276 nm, respectively, according to Miyakawa and Nomizu (25), in a Hewlett-Packard (Bracknell, United Kingdom) Diodearray Spectrophotometer. Conjugated tetraene was measured at 318 nm according to Bradley and Richardson (26). Polymeric triacylglycerides were analyzed in fish oils in tetrahydrofuran (THP) solution by high-performance size-exclusion chromatography (27) on two columns, 300 mm × 7.8 mm i.d. in series—Phenogel 5 500A followed by Phenogel 5 100A ex Phenomenex (Macclesfield, United Kingdom). Mobile phase was THF, 0.8 mL/min, and detection was by evaporative light-scattering detector [Mk 2 Varex (Rockville, MD)]. Exhaust temperature was set to 50°C, nebulizer gas (N_2) to 30 psi. Quantitation was by reference to volumetric solutions of tripalmitin, run to generate an external standard curve for each sample set.

Lecithin was analyzed in chloroform solution by high-performance liquid chromatography (HPLC) (28) with a quaternary pump linked to a Polymer Laboratories (Church Stretton, United Kingdom) light-scattering detector. Individual

TABLE 1
Published Antioxidant Systems for Protecting Fish Oils During Accelerated Oxidative Stability Tests

Source (ref. number)	Tocopherol (ppm)	Ascorbyl palmitate (AP) (ppm) Ascorbic acid (AA) (ppm)	Lecithin (ppm)	Test conditions	Induction period (control) (h)
Löliger and Saucy (5)	500[a]	1000 (AA)	10,000	Fira-Astell, 80°C	23.2 (0.5)
Koschinski and Macfarlane (6)	1000[b]	500 (AP)	750	Rancimat, 100°C	10.1 (1.5)
Chang and Wu (7)	1000[c]	200 (AA)	2000	Rancimat, 90°C	24.1 (0.9)
Yi, Han, and Shin (8)	2000[d]	400 (AA)	1000	Rancimat, 80°C	40 (4)
Han, Yi, and Shin (10)	4000[d]	200 (AA)	3000	1.8 mm film, 30°C	1114 (50.5)

[a]Mixed tocopherols—not defined.
[b]γ-Tocopherol.
[c]Mixed tocopherols: 12% α-tocopherol (α-T), 56% γ-tocopherol (γ-T), 30% δ-tocopherol (δ-T).
[d]δ-Tocopherol and 0.1% water.

TABLE 2
Tocopherol Concentrates (natural sources)

Type[a]	α-T (%)	γ-T (%)	δ-T (%)	Carrier oil (%)
EM80 (Eisai)	7	33	35	25
Type V (Sigma)	72	1.5		26.5
Eisai δ-T		3	97	
Soy lecithin		0.056	0.03	~45

[a]Eisai; Eisai Pharma-chem Europe Ltd., London, United Kingdom; Sigma: Sigma Chemical Co., Poole, United Kingdom; for abbreviations see Table 1.

lipids were separated on a 10 cm × 4.6 cm 5 µm Lichrosorb Si60 (Merck, Darmstadt, Germany) silica column. Quantitation was by external calibration with a series of standards.

Tocopherols. These were recovered after saponification to remove oil, separated in methanol with a reversed-phase (C_{18}) HPLC column (29) (Jones Chromatography, Hengoed, United Kingdom), and quantitated with a Perkin-Elmer (United Kingdom) LS1 Fluorescence Detector (excitation 295 nm; emission 340 nm).

Copper was analyzed by graphite furnace atomic absorption spectrometry in an uncoated graphite tube at 2700°C by measuring absorption at 324.7 nm (slit 0.7 nm) against known standards.

Iron was similarly analyzed in a niobium-coated graphite tube at 2700°C by measuring absorption at 372.0 nm (slit 0.2 nm) against known standards.

Storage conditions. Oils (100 g) were stored in amber jars with loose-fitting screw-capped lids (free access to air, typical relative humidity 45%) at 20 ± 0.5°C. Oils were thoroughly stirred before sampling for sensory evaluation (1-wk intervals) and PV determinations (2-wk intervals).

Sensory evaluation. Sensory evaluation was carried out on 2 mL of (undiluted) fish oil by an in-house expert in oil off-flavor profiling.

RESULTS

Effect of single antioxidant concentrates. Concentrations of both 0.2 and 2% α-tocopherol (the concentrations given in this paper refer to the amounts of tocopherol concentrates added to the oil; see Table 2, Fig. 1) show a pro-oxidant effect when compared to the control (Fig. 2). This confirms published observations with a wide range of lipid substrates (11,13–15,30,31). However, the protection of fish oil, as defined by PV, increased with increasing γ/δ and δ-tocopherol additions (0.2 to 2%). The most pronounced effect was observed with δ-tocopherol alone, with only a small increase in PV being observed over a period of 20 wk at the 2% level.

Both ascorbyl palmitate and lecithin, when used alone at the 0.1 and 0.5% level, respectively, gave only small improvements in overall oxidative stability (Fig. 2), with ascorbyl palmitate showing a slight initial pro-oxidant effect. Based on these results, further work was mainly focused on using either a mixture of essentially γ- and δ-tocopherols or δ-tocopherol in combination with ascorbyl palmitate and lecithin.

Binary mixtures of ascorbyl palmitate, tocopherol, and lecithin. The concentrations of ascorbyl palmitate and lecithin were fixed at 0.1 and 0.5%, respectively, throughout the study on both binary and ternary systems. At these levels, ascorbyl palmitate and lecithin display strong synergy as a binary mixture. We saw a pronounced and greatly extended induction period, unlike the behavior of the oil that contained the individual components (Fig. 2).

The results for binary mixtures of tocopherol and either ascorbyl palmitate or lecithin are given in Figures 3 (γ/δ-T) and 4 (δ-T) (together with the corresponding ternary systems). Ascorbyl palmitate exhibited a pro-oxidant effect when added to fish oil that contained either 0.2 or 2% γ/δ tocopherol mixture or δ-tocopherol. Lecithin (0.5%) significantly im-

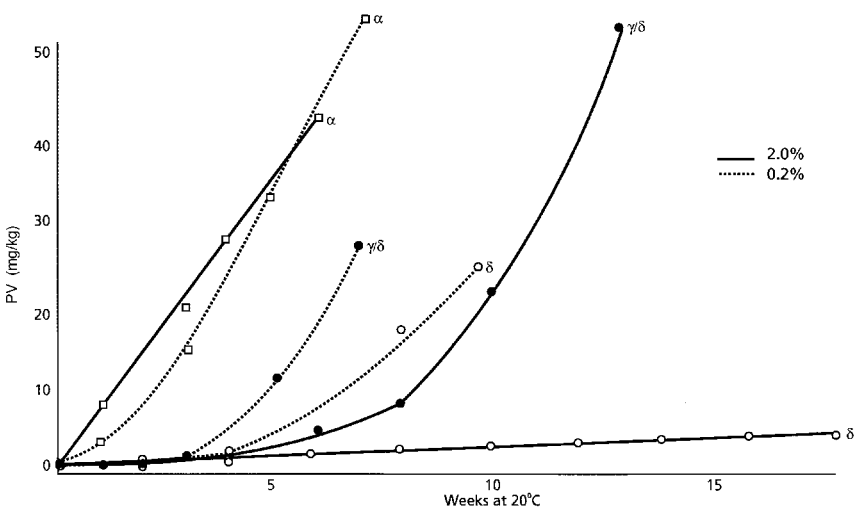

FIG. 1. Effect of tocopherols on the peroxidation in air of Chilean fish oil at 20°C. Percentages refer to proprietary antioxidants as indicated in Table 2. The control is the same as given in Figure 2. PV, peroxide value.

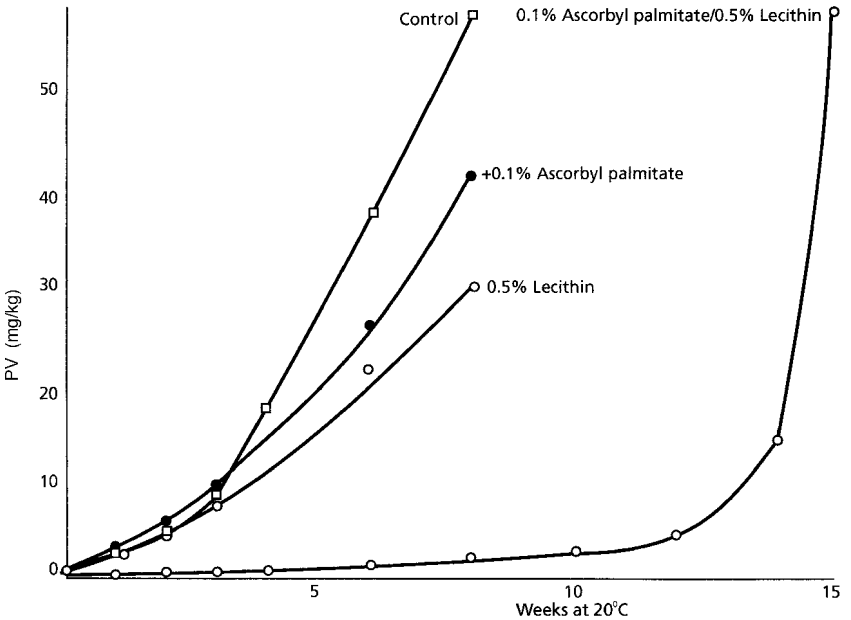

FIG. 2. Effects of ascorbyl palmitate and lecithin on the peroxidation in air of Chilean fish oil at 20°C. For abbreviation see Figure 1.

proved the oxidative stability of the oil at both levels of mixed γ/δ-tocopherols and δ-tocopherol (Fig. 4).

Ternary mixtures of ascorbyl palmitate, tocopherol, and lecithin. For both γ/δ-tocopherol (Fig. 3) and δ-tocopherol (Fig. 4), we saw a major synergism when using the ternary mixture. At both 0.2 and 2% levels of tocopherol addition in the ternary blend, we observed no increase in PV over a period of more than 20 wk at 20°C. Again, the system based on

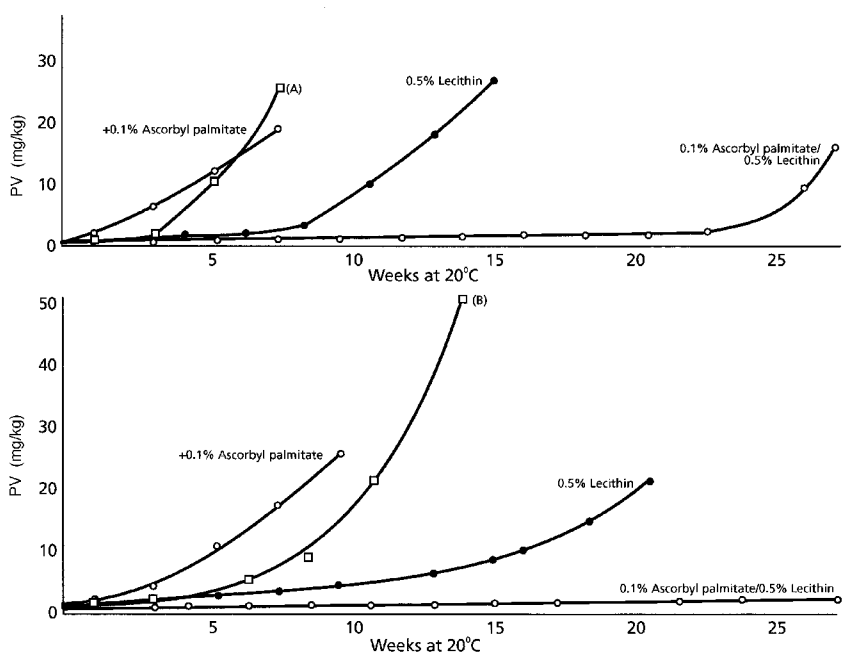

FIG. 3. The effect of binary and ternary mixtures of γ/δ-tocopherol on the peroxidation in air of Chilean fish oil at 20°C. Fish oil (A) (upper panel) contains 0.2% γ/δ-tocopherol concentrate. Fish oil (B) (lower panel) contains 2.0% γ/δ-tocopherol concentrate. Ascorbyl palmitate, lecithin, or a mixture of the two were added to (A) and (B). For abbreviation see Figure 1.

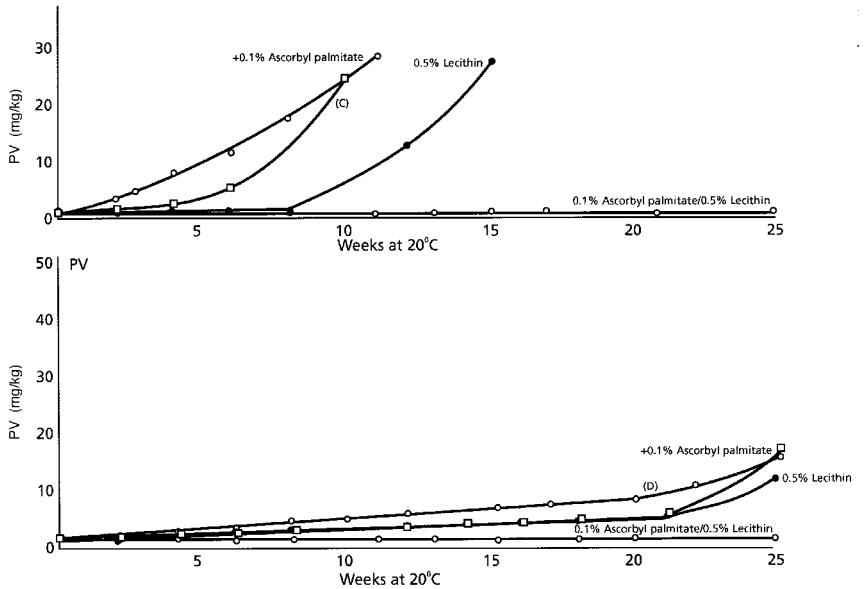

FIG. 4. The effect of binary and ternary mixtures of antioxidants that contained δ-tocopherol on the peroxidation in air of Chilean fish oil at 20°C. Fish oil (C) (upper panel) contains 0.2% δ-tocopherol concentrate. Fish oil (D) (lower panel) contains 2.0% δ-tocopherol concentrate. Ascorbyl palmitate, lecithin, or a mixture of the two were added to (C) and (D). For abbreviation see Figure 1.

δ-tocopherol showed the most protective effect. No increase in PV was observed for the 2% δ-tocopherol ternary system up to 26 wk, when the trial was stopped. The end of the induction period had been reached for the blend with 0.2% γ/δ-tocopherol over the same period.

The results of a separate study on α-tocopherol (Fig. 5) showed the existence of an optimal concentration in providing protection against autoxidation at approximately 0.2%. However, the actual optimum could be slightly lower because concentrations between 0.006 and 0.2% were not studied. The blank oil, which contained 0.006% α-tocopherol, gave a similar induction period to the ternary blend that contained 2% α-tocopherol. Related effects have already been observed for α-tocopherol in vegetable oils (12-14) and for α-rich mixed tocopherols in fish oils (5).

Although the majority of our experiments were on Chilean

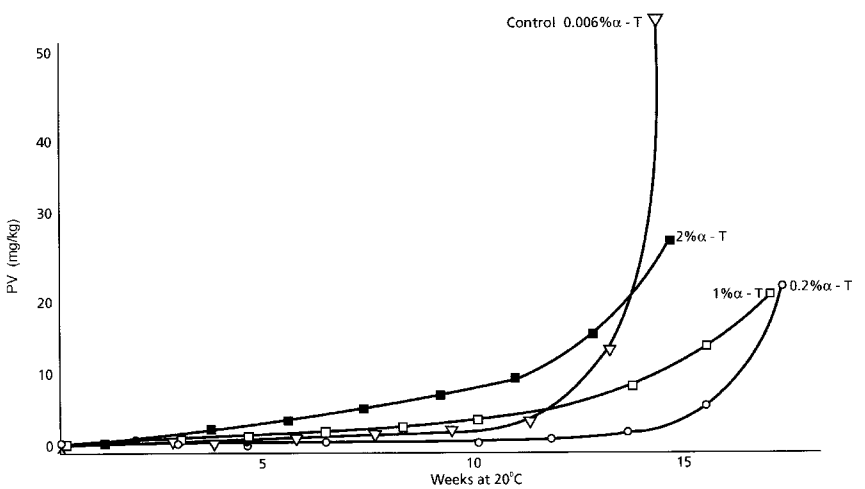

FIG. 5. The effect of α-tocopherol on peroxidation in air of Chilean fish oil that contained lecithin (0.5%) and ascorbyl palmitate (0.1%) at 20°C. Percentages of α-tocopherol refer to Sigma Type V antioxidant (Sigma, Poole, United Kingdom), containing carrier oil as indicated in Table 2.

TABLE 3
Effect of Ascorbyl Palmitate on Peroxide Value (PV) of Chilean Fish Oil (stored 28 wk in air)

Original antioxidant[a]	Extra ascorbyl palmitate (added at wk 28)	PV
2% δ-T	None	36
2% δ-T	0.1% in propylene glycol stirred into oil and left overnight under N_2	28
2% δ-T	0.5% in $CHCl_3$/acetic acid added immediately before PV determination	15.5
2% δ-T/lecithin (0.5%), ascorbyl palmitate (0.1%)	None	1.5
2% δ-T/lecithin (0.5%), ascorbyl palmitate (0.1%)	0.1% in propylene glycol stirred into oil and left overnight under N_2	<0.2
2% δ-T/lecithin (0.5%), ascorbyl palmitate (0.1%)	0.5% in $CHCl_3$/acetic acid added immediately before PV determination.	<0.2

[a]For abbreviations see Table 1.

(anchovy) fish oil, similar results were obtained with menhaden oil for the ternary system of the γ/δ-tocopherol mixture.

We were concerned that the use of PV alone could give a misleading result in terms of the overall oxidation of the oil if significant peroxide breakdown was occurring. Ascorbyl palmitate, for example, can promote hydroperoxide scissions (Table 3). We therefore determined PAV, conjugated diene and triene and polymer levels in the oils during and past the induction period (Tables 4 and 5). These data confirmed that the low PV were, in these tests, indicative of low levels of oxidation. Also, in systems where oxidation is very low, it is possible that PAV could decrease slightly during storage (as shown in Table 5) owing to reactions with phosphatidylethanolamine (PE) in the ternary system.

Changes in tocopherol levels were monitored during the autoxidation of menhaden oil (Table 6). These show, as expected, that the most oxidatively unstable tocopherol is α, although this is only apparent at very high PV values. At low or minimal oxidation, all three tocopherols are lost at a similar low rate.

DISCUSSION

The refining procedure, applied to the fish oils used in this work, is not conventional in that it employs a sodium silicate/soda ash boil after neutralization and a larger than normal amount of bleaching earth (4%). We have not observed any improvement when an extra treatment, involving adsorption on a silica column (6), is used before deodorization in this refining process, in spite of the removal by silica of oxidized polymers. Moreover, the refining process still leaves significant levels of iron and copper in the refined oil (0.18 ppm Fe; ≤0.01 ppm Cu) (32).

The majority of published works on antioxidant evaluation rely on accelerated oxidative stability tests at various elevated temperatures (33). For unhydrogenated fish oil, we would question the relevance of such data until appropriate correlations have been made with ambient-temperature storage results. Certainly the work of Burkow (9), for example, demonstrates that misleading results can be obtained from accelerated tests. Despite these reservations, we observe that, for the

TABLE 4
Changes in Menhaden Oil Stored at 20°C in Air[a]

Antioxidant	Reference frozen under N_2 at –30°C		19 wk	22 wk	29 wk	31 wk
2% γ/δ-T,	PV	0.7	75	98	176	188
0.5% lecithin	Anisidine value	9.8		18.9		40.3
	Conjugated diene (%)	0.51	0.88		1.36	
	Conjugated triene (%)	0.46	0.48		0.05	
	Conjugated tetraene (%)	0.02			0.05	
	Polymer dimer TG (%)	0.26	0.41		1.01	
	Higher polymer (%)	<0.02	<0.02		0.16	
	EPA (%)	12.9		12.8		12.6
	DHA (%)	6.9		6.2		6.1
2% γ/δ-T,	PV	<0.2	3	3.5	40	97
0.5% lecithin,	Anisidine value	9.8		8.6		15.4
0.1% ascorbyl	Conjugated diene (%)	0.51			0.71	
palmitate	Conjugated triene (%)	0.46	0.43		0.44	
	Conjugated tetraene (%)	0.02			0.03	
	Polymer dimer TG (%)	0.26		0.25		0.53
	Higher polymer (%)	<0.02		<0.02		0.08

[a]TG, triacylglycerol; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid. For other abbreviation see Table 3.

TABLE 5
Changes in Chilean FH Stored at 20°C[a]

Antioxidant system		Frozen reference N_2, −30°C	For 22 wk in presence of air 20°C
2% γ/δ-T	PV	1	154
	Anisidine value	9.4	26.6
	Conjugated diene (%)	0.19	1.6
	Conjugated triene (%)	0.08	0.3
	Polymer dimer TG (%)	0.2	0.73
	Higher polymer (%)	<0.02	<0.02
	EPA (%)	11.2	10.7
	DHA (%)	13.2	12.4
0.2% γ/δ-T	PV	1	138
	Anisidine value	9.4	57.4
	Conjugated diene (%)	0.19	1.12
	Conjugated triene (%)	0.08	0.26
	Polymer dimer TG (%)	0.2	1.62
	Higher polymer (%)	<0.02	0.5
	EPA (%)	11.2	10.7
	DHA (%)	13.2	12.7
2% γ/δ-T, 0.5% lecithin, 0.1% ascorbyl palmitate	PV	1	2.5
	Anisidine value	9.4	7.1
	Conjugated diene (%)	0.19	0.5
	Conjugated triene (%)	0.08	0.19
	Polymer dimer TG (%)	0.2	0.21
	Higher polymer (%)	<0.02	<0.02
	EPA (%)	11.2	11.6
	DHA (%)	13.2	13.1
0.2% γ/δ-T, 0.5% lecithin, 0.1% ascorbyl palmitate	PV	1	2.5
	Anisidine value	9.4	8.4
	Conjugated diene (%)	0.19	0.74
	Conjugated triene (%)	0.08	0.22
	Polymer dimer TG (%)	0.2	0.22
	Higher polymer (%)	<0.02	<0.02
	EPA (%)	11.2	11.5
	DHA (%)	13.2	13.2

[a]FH, fish oil. For other abbreviations see Table 4.

different tocopherols in the absence of other antioxidants, the order of effectiveness is $\alpha < \gamma < \delta$, which is in line with previous observations on nonfish oil substrates during tests at elevated (55–140°C) temperatures (11,14,21,34–37). Koskas *et al.* (15) came to similar conclusions for stabilizing linoleic acid in aqueous media.

The addition of lecithin (0.5%) significantly improved oxidative stability at both levels of tocopherol. We did not see any significant advantage, however, in using binary mixtures of the selected antioxidants within the range of concentrations studied.

Pongracz and Szappan (18) were some of the earliest to describe the effectiveness of the ternary system ascorbyl palmitate, tocopherol, and lecithin for lard and vegetable oil. Most of the experimental data on the ternary system in fish oil have been published in patents (5–7), apart from the work of Yi *et al.* (8,10), whose systems included 0.1% water, and the paper by Burkow *et al.* (9). Nearly all this work, including Pongracz and Szappan, is based on accelerated tests. Compared to previous work (Table 1) on fish oil, our work demonstrates that: (i) much higher levels of tocopherol are effective than previously reported; (ii) protection increases with increasing concentration of γ/δ-tocopherols but not with α-tocopherol; (iii) the ternary system offers unusually effective protection of fish oil over a period of 5–6 mon at ambient temperature in air, especially systems based either on γ/δ-tocopherols or, preferably, δ-tocopherol. For δ-tocopherol (2%) in the ternary system, no increase in PV was observed over a period of 26 wk; (iv) for α-tocopherol (Fig. 5), a pro-oxidant effect is observed at high levels of addition, with the optimum being at 0.2% or lower (we did not examine lower levels in this work).

It is impossible to identify a single essential component out of the three, but it is significant that lecithin improves both

TABLE 6
Tocopherol (ppm) in Menhaden Oil Stored at 20°C

Storage (wk at 20°C)	Other antioxidant	α-T	γ-T	δ-T	PV
None	None	2070	6680	5860	0.7
12	None	1950	6410	5680	52
12	0.1% Ascorbyl palmitate	1880	6320	5640	50
29	0.5% Lecithin	1450	5670	5560	176
29	0.1% Ascorbyl palmitate, 0.5% lecithin	1760	6270	5800	40

binary systems in which it is involved. Of the three components, lecithin is also unique in its physical behavior. For this reason, the role of lecithin is discussed below.

Mechanisms for the effectiveness of lecithin in autoxidizing oil include the regeneration by PE of α-tocopherol from its oxidized radical (38,39), but such a function for lecithin in the present system appears unimportant where the system has been overloaded with γ- and δ-tocopherol. The involvement of PE in protecting the oil seems implicated, however, because there is a selective loss during storage (Table 7). This mirrors the known effects of primary amines as antioxidants, particularly spermine, which has been used to protect fish oil (40), and the fact that PE interacts with α-tocopherol (23) and ascorbyl radicals (41) in lipid autoxidation under accelerated conditions. The function of lecithin in merely solubilizing ascorbyl palmitate (16,42) also seems irrelevant, given that lecithin alone was antioxidative while ascorbyl palmitate/tocopherol was either inert (menhaden oil) or initially pro-oxidative (Chilean fish oil). Lecithin and ascorbyl palmitate appear to be acting in tandem in such a way that the pro-oxidative tendency of ascorbyl palmitate is completely reversed, even in the presence of only 0.006% tocopherol. This might be due in part to phosphatidyl sequestering of trace heavy metals (43). However, in parallel experiments, levels of lecithin that were quite adequate for metal complexing purposes have not given good oxidative protection. Moreover, under Rancimat test conditions, other authors have shown that it is the ratio of ascorbyl palmitate to lecithin, rather than the absolute level of lecithin, that is important (16). Ascorbyl palmitate may have antioxidative action in the presence of lecithin due to the ability of the latter to inhibit the activity of the ascorbyl radical in promoting hydroperoxide scission (Table 3). This could be further enhanced by the possible physical interaction of ascorbyl palmitate, lecithin (hydrated), and tocopherol in forming reverse micelles (8,10). These would create a mobile network that is possibly more able to interrupt free-radical propagation. Such interactions may be the basis of the slight brown cast noted in the oils that contain ascorbyl palmitate and lecithin, as also noted by Chang and Wu (7).

There is no evidence from the present data of any synergy between ascorbyl palmitate and high levels of tocopherol, in contrast to work that involved ascorbic acid recycling of α-tocopherol in aqueous media (44–46) and Rancimat testing of lard that contained lower levels of tocopherol (42).

We therefore propose that the effectiveness of the ternary system arises through the ability of PE to interact with free radicals and in particular with ascorbyl radicals. This, combined with the strong antioxidant effect of δ-tocopherol, results in the high degree of protection against autoxidation.

Despite the low PV observed for many of the samples and the fact that the starting point was an essentially bland fish oil, we have observed only a limited extension of flavor stability above the control. Lecithin can be added to oils only after the deodorization stage of the refining process to avoid darkening of the oil. This imparts certain initial levels of off-flavors (sweet, musty, rubbery, toffee, green, bean, paint, nutty, or woody) to the refined fish oil, depending on the quality of the lecithin. Also, although tocopherol can be added before bleaching and deodorization, this was impractical in most of our experiments owing to the number of tocopherol variables being studied, resulting in the further carry-through of some flavor. Flavor evaluation was therefore qualitatively designed to note only major changes in fish off-flavor relative to peroxidation.

It proved possible during the initial 3 wk of storage in air to distinguish two types of fish off-flavor developing along with other off-flavors, such as paint, green, plastic, mushroom, and toffee: (i) an immediate impact of oily/metallic/fishy in samples that did not contain lecithin, similar to that described by

TABLE 7
Phosphatides (mg/100 g) in Stored Chilean Fish Oil

Antioxidant system	Storage	PV	PE[a]	Loss (%)	PI[a]	Loss (%)	PC[a]	Loss (%)
2% δ-T/lecithin (0.5%)	Frozen N_2	1	78.3		50.6		70.3	
2% δ-T/lecithin (0.5%)[b]	20°C/25 wk	11.5	59.9	23.5	46.2	8.7	67.3	4.3
2% δ-T/lecithin (0.5%),[b] ascorbyl palmitate (0.1%)	20°C/25 wk	1	59.2	24.4	47.0	7.1	67.5	4.0

[a]PE, phosphatidylethanolamine; PI, phosphatidylinositol; PC, phosphatidylcholine. For other abbreviations see Tables 1 and 3.
[b]Full PV data, Figure 4.

Swoboda and Peers (47); (ii) a delayed impact of burnt/fishy in samples that contained lecithin, similar to that reported by Karahadian and Lindsay (48) involving 2,4,7-decatrienals.

High levels of tocopherol can favor formation of specific hydroperoxides (15,49) and thus influence off-flavors (50), but because these different profiles also were apparent in fish oils that contained only 60 ppm natural α-tocopherol, it is possible that here lecithin modifies off-flavor profiles in terms of nonradical peroxide breakdown products (51) or participates in oxidation of radicals formed from PE, as discussed by Löliger *et al.* (19). Fish oils that contained lecithin darkened during storage, as observed similarly by Chang and Wu (7) when they used ternary antioxidant systems and by Lovaas (40), who used polyamines.

From a commercial viewpoint, off-flavor formation was such as to render the oils unsuitable for substantial use in ambient products that would remain unprotected from air in 2–3 wk. Flavor evaluation was discontinued after 3 wk, with the no-antioxidant system giving significantly reduced total off-flavor levels. Such a result was disappointing when set against the substantial delay in peroxidation with the ternary antioxidant systems, but this was not surprising, considering the high LCPUFA levels in fish oil and the extremely low (ppb) threshold levels for compounds, such as 1,5*c*-octadiene-3-one (52) and *t,c,c*- and *t,t,c*-2,4,7-decatrienals (48). The control of off-flavor will therefore need to be resolved by the use of sophisticated headspace analysis to characterize what is happening in the oil during the early stages of oxidation (14,48,53–55).

In the absence of substantial flavor improvement at ambient temperature, the ternary antioxidant system nevertheless remains appropriate where long-term peroxidation and maintenance of LCPUFA levels are more relevant issues than flavor. This would apply in the capsule industry and for crude oil production, storage, and transport, and also for frozen foodstuffs in which an unhealthy buildup of stable peroxides could occur during long-term cold storage without off-flavor formation.

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