Protection of α-Tocopherol in Nonpurified and Purified Fish Oil

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ABSTRACT: Menhaden oil was purified by column chromatography to remove minor components. The effect of α-tocopherol (αTOH) (50–500 ppm) on the rate of formation of hydroperoxides in the original menhaden oil and in the purified menhaden triacylglycerol (TAG) fraction was studied at 30°C in the dark. An increase in the initial rate of formation of hydroperoxides was observed at αTOH concentrations above 100 ppm in both substrates. The original menhaden oil oxidized more rapidly than the purified menhaden TAG at all antioxidant levels tested, and the presence of minor components in the menhaden oil was found to contribute only to a limited extent to the peroxidizing effect of αTOH. The αTOH did not display any prooxidant activity at either of the concentrations tested when the control oil was the purified menhaden TAG. Addition of ascorbyl palmitate eliminated the initial peroxidizing effect of αTOH, and this emphasizes the participation of the α-tocopheroxyl radical in the reactions causing an accumulation of hydroperoxides at high concentrations of αTOH.

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KEY WORDS: Ascorbyl palmitate, autoxidation, fish oil, menhaden oil, α-tocopherol.

The polyunsaturated fatty acids (PUFA) in fish oils are highly susceptible to oxidation. Lipid oxidation during processing and storage leads to the formation of undesirable fishy flavors, and adequate antioxidant protection is essential for successful utilization of fish oils. The order of antioxidant activity of the tocopherols in many lipid systems has been found to be δ-tocopherol (δTOH) > γ-tocopherol (γTOH) > α-tocopherol (αTOH) (1–3). However, αTOH is the tocopherol homolog displaying the highest activity as vitamin E (1). As the physiological tocopherol requirement is a function of the type and amount of fatty acids ingested (4), the intake of fish oil and other PUFA supplements requires a high intake of vitamin E to protect the body lipids from *in vivo* peroxidation. It is therefore likely that αTOH will remain important as an antioxidant additive to fish oils for human consumption. Means to increase the *in vitro* antioxidant activity of αTOH is thus highly relevant in the effort to improve the stability of fish oils.

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The antioxidant activity of the tocopherols does not increase linearly with concentration, and at sufficiently high levels of addition an inversion of activity may take place. The mechanism responsible for the inversion of αTOH activity, which has sometimes been found to induce a prooxidant effect, has not been clarified. This accumulation of hydroperoxides at high αTOH concentrations, which has been observed in many different lipid systems, has been suggested to be the result of the participation of αTOH and/or the α-tocopheroxyl radical (αTO·) in reactions other than with fatty acid peroxyl radicals (1,5,6). These so-called side reactions may include the interaction with minor oil constituents such as metal ions and preformed hydroperoxides, the generation of new radicals during tocopherol oxidation to the quinone and epoxy quinones, direct reaction with oxygen, and chain transfer with intact fatty acids or hydroperoxides.

The antioxidant properties of αTOH in vegetable oils and fats have been thoroughly studied. In corn oil, an inversion of antioxidant activity on the basis of hydroperoxide formation was found at 100 ppm αTOH, and αTOH showed a slight prooxidant effect at 250 ppm and higher (7). Similar results were obtained in soybean oil (8). Lampi *et al.* (9) also observed a relative increase in hydroperoxide formation in purified rapeseed oil triacylglycerols (TAG) at αTOH concentrations higher than 100 ppm; however, contrary to the previously mentioned findings, αTOH was found not to display prooxidant activity in the purified TAG when compared to the purified control oil with no antioxidant. This is in accordance with a study by Fuster *et al.* (10) using purified sunflower TAG, where αTOH was an antioxidant at concentrations as high as 2,000 ppm. These authors suggested that αTOH is not a prooxidant *per se* but may act as a prooxidant synergist (or cooxidant) when present at high concentrations together with known prooxidants such as transition metal ions or lipid hydroperoxides.

The objectives of this work were to study the influence of αTOH on the rate of formation of hydroperoxides in fish oil, and to determine the extent to which minor fish oil components, as well as the food-acceptible antioxidants citric acid and ascorbyl palmitate, interact with αTOH to change its effectiveness as an antioxidant. Purification by column chromatography removes the majority of antioxidant and prooxidant molecules normally present in an oil, and the use of the purified TAG fraction as the substrate thus allows better control over factors known to influence the autoxidation process.

EXPERIMENTAL PROCEDURES

Materials. Menhaden oil, refined and bleached, was from Omega Protein (Reedville, VA). Immediately upon arrival, the oil was stored at −30°C, and before use it was held at room temperature overnight. The d-α-tocopherol (purity ≥ 99%) was supplied by Acros (Ghent, Belgium), citric acid (purity ≥ 99.9%) by Fisher (Fair Lawn, NJ), and ascorbyl palmitate (purity ≥ 95%) by Sigma (St. Louis, MO). The αTOH product contained no other tocopherol homologs as verified by high-performance liquid chromatography (HPLC). Silica gel was purchased from Aldrich (Milwaukee, WI), Celite 545 from Supelco (Bellefonte, PA), and activated carbon from Fisher. All solvents were analytical grade.

Oil purification. Menhaden oil was purified using a modified version of a previously reported multilayer chromatographic method (11). A chromatographic column (60 × 4 cm i.d.) was packed sequentially with four adsorbents, all suspended in hexane. The bottom layer was 40 g activated silica gel (60–200 mesh), which had been washed with distilled water and with methanol before activation. The next two layers were 40 g of a 1:2 mixture of Celite 545/activated carbon (50–200 mesh) and 40 g of a 1:2 mixture of Celite 545/granulated sucrose. The top layer was 40 g of activated silica gel. The oil (230 g) was dissolved in hexane (230 mL) and applied to the column. Eluent was collected under N_2 until all the oil solution had been drawn down into the column. The product solution was stripped of hexane by bubbling N_2 through it. This kept the solution cold and retarded onset of autoxidation of the purified TAG. The hexane concentrations in the oil samples after stripping ranged from 0.02 to 0.2 ppm, as determined by static headspace gas chromatography (GC) according to the method for analysis of volatile secondary lipid oxidation products by Huang *et al.* (7) . The chromatographic column and flasks used for collecting product and removal of hexane were covered with aluminum foil to prevent light-induced oxidation during the purification process. The purified menhaden TAG were analyzed with regard to fatty acid composition, remaining αTOH, primary oxidation products (as the peroxide value; POV), secondary oxidation products (as the *p*-anisidine value; *p*AV), %TAG, color, and the concentrations of iron and copper.

Analyses. Fatty acid compositions were determined by capillary GC with flame-ionization detection (FID) of the methyl esters prepared by transesterification using BF_3-methanol (12). The POV was measured according to American Oil Chemists' Society (AOCS) method Cd 8b-90 (13) using a 1:10 dilution of the $Na_2S_2O_3$ solution, and *p*AV was determined according to AOCS method Cd 18-90 (13). Color intensity was measured by reading the oil absorbance against isooctane at 460 nm using a Hewlett-Packard HP 8453 UV-Visible spectrophotometer (Palo Alto, CA). Lipid compositions of menhaden oil and menhaden TAG fractions were determined by Iatroscan thin-layer chromatography-FID according to the method of Sigurgisladottir *et al.* (12). The iron and copper contents were determined by electrothermal atomic absorption spectrophotometry equipment with Zeeman background correction. The oil samples were dissolved in methyl isobutyl ketone and diluted with 2-propanol. Ammonium hydrogen phosphate and magnesium nitrate dissolved in 2-propanol were used as the matrix modifiers in the determination of iron. Acidified palladium nitrate and magnesium nitrate dissolved in 2-propanol were used as the matrix modifiers in the determination of copper.

Conjugated dienes were determined by dissolving weighed oil samples (approximately 30 mg) in isooctane (50 mL) and reading the sample absorbance at 234 nm using a Hewlett-Packard HP 8453 UV-Visible spectrophotometer. The $E^{1\%}_{1\ cm}$ was calculated from the absorbance reading and the sample oil concentration, and the result is reported as the increase from the measurement obtained at time zero of that particular experiment. Normal-phase HPLC with fluorescence detection, according to AOCS method Ce 8-89 (13), was used to measure αTOH, with the exception that a Partisil 5-µm column (11 cm x 4.7 mm; Whatman, Clifton, NJ), equipped with a guard column, was employed.

Oxidation tests. Antioxidant was added immediately following oil purification. The αTOH was dissolved in hexane and the concentration determined spectrophotometrically as described in AOCS method Ce 8-89 (13). Accurate amounts of αTOH were added to the oil by transferring a known volume of αTOH solution to a glass flask and evaporating the solvent under a stream of N_2 before adding the desired amount of oil. The αTOH was then mixed with the oil by bubbling N_2 through the samples for 20 min, and αTOH concentrations were verified by HPLC as already described. Citric acid and ascorbyl palmitate were added to the purified oils as freshly prepared solutions in acetone, the solvent being immediately removed by N_2 stripping. This allowed antioxidant addition without having to heat the oil samples. Portions (1.5 g) of the different oil samples were stored in 20-mL uncapped glass vials (40 × 25 mm i.d.) at 30°C in the dark in a thermosttated oven. Triplicate portions of each oil treatment were removed from the oven at regular intervals for analysis of conjugated dienes and, when evaluating the effect of ascorbyl palmitate, for analysis of residual αTOH as well.

Preliminary oxidation tests, scanning the effectiveness of a wide range of αTOH concentrations (0–2,000 ppm), showed a change in the activity of αTOH in the lower part of the concentration range. Accordingly, the oxidative stabilities of samples of menhaden oil and purified menhaden TAG with 50, 100, 250, and 500 ppm αTOH were compared. Purified menhaden TAG were then used as the substrate in the following experiments: (i) a study of the effect of addition of citric acid (50 ppm) on the stability of the substrate with 0, 50, 100, 250, and 500 ppm αTOH, (ii) a study of the effect of addition of ascorbyl palmitate (250 ppm) on the stability of the substrate with 0, 100, 250, and 500 ppm αTOH.

Statistical analysis. The initial rates of hydroperoxide formation, R_i, were estimated by linear regression of the hydroperoxide levels on storage time (storage time ≤ 3 d) using StatMost™ (DataMost Corporation, Salt Lake City, UT). The slope

estimates were compared using a *t*-test. Rates of hydroperoxide formation were considered different when $P < 0.05$.

RESULTS AND DISCUSSION

Purification of menhaden oil. Menhaden oil and purified menhaden TAG were characterized with regard to content of eicosapentaenoic acid and docosahexaenoic acid, oxidation products, lipid composition, color, αTOH concentration, and iron and copper content (Table 1). Cholesterol esters and free fatty acids were not detected in either of the substrates as analyzed by Iatroscan. The purified menhaden TAG were practically colorless and odorless, and lipid components more polar than TAG, such as αTOH, mono- and diacylglycerols, and preformed oxidation products, had been removed. Purification did not alter the amount of iron in the oil, whereas copper was below the limit of detection in both the nonpurified and purified fractions. In the purified menhaden TAG, formation of hydroperoxides was very rapid with no apparent induction period, but the nonpurified menhaden oil exhibited an induction period of about 4 d. This demonstrates the contribution of αTOH or other antioxidants naturally present to the oxidative stability of commercial fish oils.

Comparison of menhaden oil and purified menhaden TAG. Addition of αTOH to nonpurified menhaden oil to a concentration of 100 ppm did not have an effect on the initial rate of hydroperoxide formation (Fig. 1A). The induction period of 4 d, however, was prolonged by about 1 d. Further increases in the αTOH concentrations resulted in an increased initial rate of hydroperoxide formation, but these oil samples remained in the induction period throughout the experiment. Hence, of the concentrations tested, an inversion of antioxidant activity of αTOH was observed at 100 ppm.

In the purified menhaden TAG (Fig. 1B), the pattern of hydroperoxide formation was similar to that in the menhaden oil, with an inversion of activity taking place at 100 ppm αTOH. The αTOH inhibited hydroperoxide formation at all levels of addition. Figure 2A shows a plot of the initial rate of hydroperoxide formation, R_i, vs. αTOH concentration in both

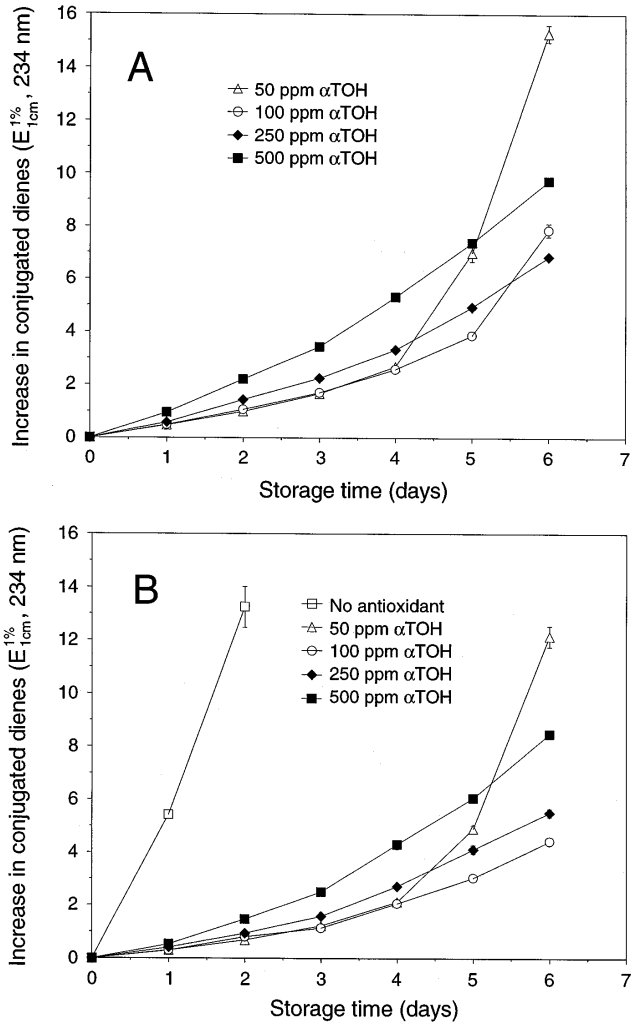

FIG. 1. Formation of hydroperoxides in menhaden oil (A) and purified menhaden triacylglycerols (B) with different levels of α-tocopherol (αTOH) during storage at 30°C in the dark. Data points are means ± standard deviation ($n = 3$).

TABLE 1
Characterization of Menhaden Oil and Purified Menhaden TAG

Analysis[a]	Menhaden oil	Purified menhaden TAG
1. EPA (%)	10.5 ± 0.1	10.3 ± <0.1
2. DHA (%)	13.1 ± 0.1	13.3 ± 0.1
3. Peroxide value (meq/kg)	6.0 ± 0.6	<0.5
4. *p*-Anisidine value	30.0 ± 0.2	0.4 ± <0.1
5. TAG (area%)	98.7	100
6. Color (A at 460 nm)	0.41	0.05
7. αTOH (ppm)[b]	47.9 ± 0.4	ND
8. Iron (ng/mL)	35 ± 6	38 ± 6
9. Copper (ng/mL)[c]	ND	ND

[a]Analyses 1–4: means ± SD, $n = 2$; analysis 5: mean of 10 Iatroscan rods; analyses 7–9: means ± SD, $n = 3$.
[b]Detection limit 1 ppm.
[c]Detection limit: 6 ng/mL. Abbreviations: SD, standard deviation; A, absorbance; ND, not detected; TAG, triacylglycerols; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; α-TOH, α-tocopherol.

the nonpurified and purified oils. The rate of formation of hydroperoxides was higher in the menhaden oil than in the purified menhaden TAG at all αTOH levels tested. The effect of purification appeared only to a limited extent to be influenced by αTOH concentration, purification being slightly more benefical to oil stability at the higher αTOH levels.

The concentration of iron in the original menhaden oil was very low and was not reduced by purification (Table 1). Citric acid was therefore added in an attempt to eliminate a possible influence of this metal on the antioxidant activity of αTOH. Citric acid is considered a very effective metal chelator and is widely used during processing of vegetable oils (14). Citric acid addition (50 ppm) did not have an effect on the rate of oxidation of purified menhaden TAG without any TOH or at any of the four αTOH levels tested, and thus did not alter the concentration for inversion of activity of the tocopherol (results not shown). The citric acid was also added to another commercial fish oil with a higher iron concentration, which resulted in a substantial reduction in the rate of

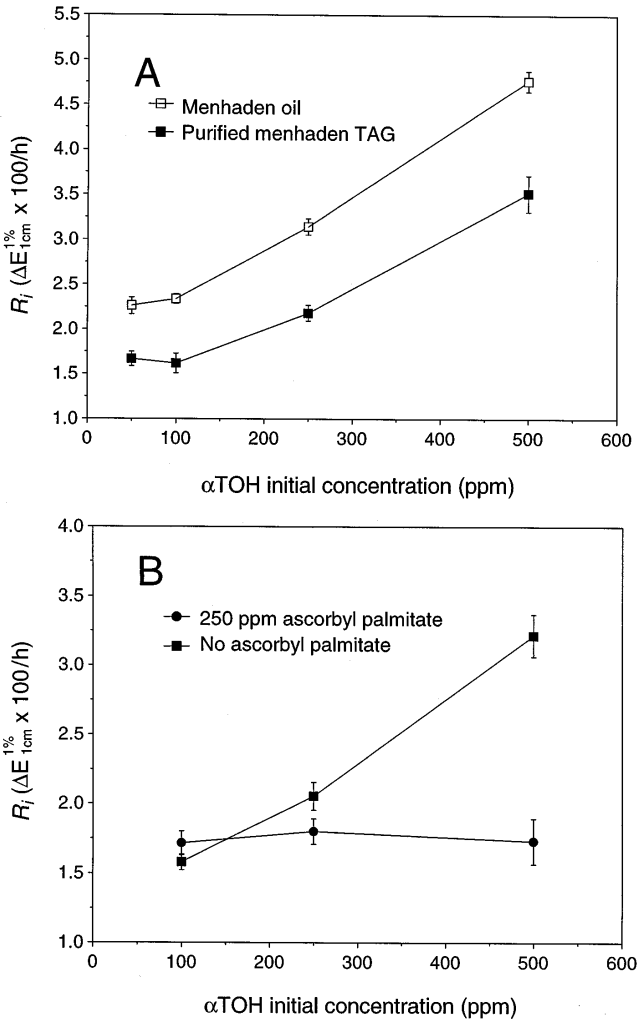

FIG. 2. Relationships between initial rates of hydroperoxide formation, R_i, and initial αTOH concentration: (A) Effect of menhaden oil purification, (B) effect of ascorbyl palmitate addition to purified menhaden triacylglycerols (TAG). Data points are slopes ± standard error. See Figure 1 for other abbreviation.

hydroperoxide formation; this experiment serving as a positive control for the properties of the specific citric acid used.

The presence of minor constituents in menhaden oil did have an effect on the rate of autoxidation of its fatty acids, but appeared only to a limited extent to influence the relative antioxidant activity of αTOH. A peroxidizing effect of αTOH at levels of addition above 100 ppm has also been observed in vegetable oils by several investigators (7–9), whereas a somewhat lower concentration for maximal antioxidant activity was determined in a more saturated substrate, purified butter oil TAG (15). A limited number of TOH concentrations were tested in these studies, as well as in the present study, and the true optimal level may thus vary slightly among the different vegetable oils and between vegetable oils and fish oil, reflecting the different degrees of susceptibility toward oxidation. However, for practical purposes, the influence of substrate composition on the antioxidant behavior of αTOH in bulk oils, with regard to concentration for inversion of activity, appears to be small.

An initial prooxidant effect of αTOH in menhaden oil at 250 ppm and higher, based on rate of hydroperoxide formation, was observed, but αTOH inhibited hydroperoxide formation at all levels of addition in the purified menhaden TAG. The classification of αTOH as a prooxidant or antioxidant clearly depends on the composition of the oil to which the comparison is made, and these results illustrate the importance of properly defining the composition of the control oil. In this study, αTOH concentrations above 100 ppm caused an increased rate of hydroperoxide formation, but the time the samples remained in the induction period was prolonged. When evaluating antioxidants by the duration of the induction period only, valuable information about the oxidation status of the oil while in the induction period may be lost.

The αTOH inhibits lipid autoxidation by scavenging lipid peroxyl radicals (ROO·) according to reactions 1 and 2 (16):

$$ROO\cdot + \alpha TOH \rightarrow ROOH + \alpha TO\cdot \quad [1]$$

$$\alpha TO\cdot + ROO\cdot \rightarrow \text{nonradical products} \quad [2]$$

Proposed mechanisms for the frequently observed decrease in the strength of this antioxidant at high concentrations, involving the participation of αTOH and αTO· in certain side reactions, have been reviewed by several authors (1,5,7) and appear to be related to the high hydrogen-donating power of αTOH compared to other phenolic antioxidants. One of these proposed side reactions involves the decomposition of lipid hydroperoxides. At low temperatures favoring hydrogen-bonding, αTOH can donate hydrogen atoms to lipid hydroperoxides (ROOH), decomposing them to alkoxyl radicals (RO·) and thereby generating radicals that may act as chain-carriers (reviewed in Ref. 1):

$$\alpha TOH + ROOH \rightarrow RO\cdot + H_2O + \alpha TO\cdot \quad [3]$$

Hopia *et al.* (17), on the other hand, found that αTOH markedly inhibited the decomposition of methyl linoleate hydroperoxides in a concentration-dependent manner.

In the present study, the rate of formation of hydroperoxides was greater in the menhaden oil than in the purified menhaden TAG at all αTOH levels tested. Decomposition of hydroperoxides generates radicals which then propagate the autoxidation chain reaction, and a higher concentration of preformed hydroperoxides (POV 6.0 meq/kg) in the original menhaden oil is most likely the main factor responsible for the observed difference in the oxidation rate. A low initial POV has been demonstrated to increase the storage stability of olive oil (18) and methyl linoleate (19). The difference in the initial rate of hydroperoxide formation between the menhaden oil and the purified menhaden TAG appeared somewhat higher at 500 ppm αTOH than at 100 ppm αTOH (Fig. 2A), indicating hydroperoxide-tocopherol interaction (Reaction 3). However, other factors appear to contribute more to the peroxidizing effect of αTOH, particularly at hydroperoxide levels commonly found in refined fish oils.

Transition metal ions (M) can generate radicals and act as prooxidants by the reactions:

$$H_2O_2 + M^{n+} \rightarrow HO\cdot + HO^- + M^{(n+1)+} \quad [4]$$

$$ROOH + M^{n+} \rightarrow RO\cdot + HO^- + M^{(n+1)+} \quad [5]$$

$$ROOH + M^{(n+1)+} \rightarrow ROO\cdot + H^+ + M^{n+} \quad [6]$$

The metal-catalyzed decomposition of preformed hydroperoxides, Reaction 5, is considered the most important reaction in the initiation step of lipid autoxidation (20). It has been demonstrated in a model system that both αTOH and γTOH are easily degraded by Fe^{3+} and Cu^{2+} (21). At high metal concentrations, αTOH and αTO· may act as prooxidants (or prooxidant synergists) by reducing transition metal ions to their lower valence states (Reactions 7 and 8) and thereby recycle Reactions 4 and 5.

$$\alpha TOH + M^{(n+1)+} \rightarrow \alpha TO\cdot + H^+ + M^{n+} \quad [7]$$

$$\alpha TO\cdot + M^{(n+1)+} \rightarrow \alpha TO^+ + M^{n+} \quad [8]$$

Even traces of metal ions, particularly iron and copper, may influence the rate of oxidation of unsaturated fatty acids. The prooxidant effect of trace metal ions has been shown to depend on the type of their associated ligands (22), and it is likely that the efficiency of metal chelators is affected by the form of metal ligands as well. The form in which the metals occur in the menhaden oil is not known, and an influence of metal ions on the properties of αTOH in purified menhaden TAG thus cannot be excluded on the basis of lack of effect of citric acid. However, it is suggested that when the metal concentration is very low, other side reactions of αTOH/αTO· may be of equal or greater importance than Reactions 7 and 8. This is in agreement with several investigators who have observed a peroxidizing effect of αTOH in bulk oils even though the metal concentrations were below the limits of detection (<10 ppb iron, <1 ppb copper in Ref. 10; <10 ppb iron, <10 ppb copper in Ref. 9). Addition of 50 ppm iron, as $FeSO_4$, to purified sunflower TAG did, however, lead to a stronger positive correlation between amount of αTOH destroyed during oxidation and initial tocopherol concentration (10), indicating that tocopherol oxidation by metals may be of greater importance at relatively high metal concentrations.

Effect of ascorbyl palmitate. The αTOH-mediated accumulation of hydroperoxides has also been related to undesirable side reactions of the tocopheroxyl radical. The αTO· may reinitiate the chain reaction by abstraction of a hydrogen atom from a fatty acid (RH, Reaction 9). The rate constant for this reaction is low compared to competing reactions of αTO·, but it has been suggested to be important at high αTOH concentrations (23).

$$\alpha TO\cdot + RH \rightarrow \alpha TOH + R\cdot \quad [9]$$

Further reactions of αTO· during lipid autoxidation yield two main groups of nonradical products (17,24,25). The first consists of 8a-substituted tocopherones, which may rearrange to form α-tocopherolquinone, while epoxyhydroperoxy-α-tocopherones and their hydrolysis products epoxy-α-tocopherolquinones make up the second group of oxidation products. Suggested pathways for the formation of α-tocopherolquinone (26) and epoxyhydroperoxy-α-tocopherones (27) both involve abstraction of hydrogen from intact unsaturated fatty acids. The alkyl radicals generated are expected to react very rapidly with oxygen, and hydroperoxide accumulation may be induced.

It is well known that ascorbic acid, as well as its oil-soluble derivative ascorbyl palmitate, can act as an antioxidant synergist to αTOH (28). It readily reduces αTO·, thereby regenerating αTOH and increasing the effectiveness of the tocopherol. Figure 2B shows a plot of the initial rate of formation of hydroperoxides vs. αTOH start concentration in purified menhaden TAG without and with ascorbyl palmitate (250 ppm). The samples remained in the induction period throughout the experiment. At the αTOH concentration for inversion of activity, 100 ppm, ascorbyl palmitate had no effect on the rate of oxidation. At higher αTOH concentrations, the presence of ascorbyl palmitate reduced the initial rate of hydroperoxide formation to approximately that in purified menhaden TAG with 100 ppm αTOH. The addition of ascorbyl palmitate to purified menhaden TAG with no αTOH reduced the rate of formation of hydroperoxides slightly, but the autoxidation still proceeded with no apparent induction period. Ascorbyl palmitate protected αTOH from being consumed when the initial αTOH concentration was 500 ppm and also had an initial sparing effect on αTOH when the tocopherol start concentration was 100 ppm (Fig. 3). The data presented in Figure 3 also demonstrate that the addition of more ascorbyl palmitate to purified menhaden TAG already containing 500 ppm αTOH and 250 ppm ascorbyl palmitate further reduced the rate of αTOH consumption.

Ascorbyl palmitate was found to suppress an observed prooxidant effect of αTOH in an aqueous dispersion of linoleic acid (29) and in methyl linoleate (19) when tested at one αTOH concentration only. An important finding in the

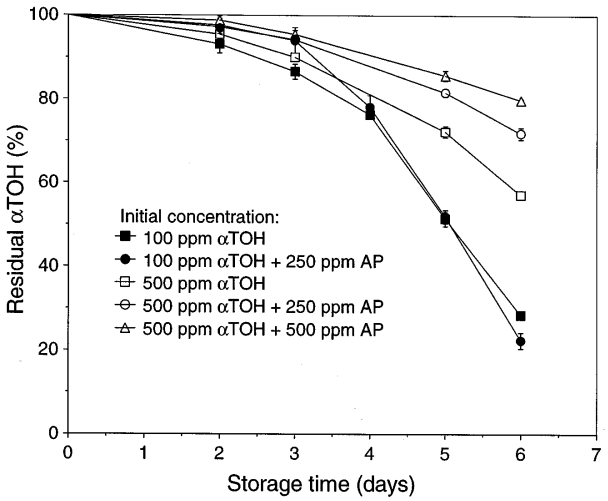

FIG. 3. Effect of ascorbyl palmitate (AP) on consumption of αTOH during storage of purified menhaden TAG at 30°C in the dark. Data points are means ± standard deviation (n = 3). For abbreviations see Figures 1 and 2.

present study was the dependence of the antioxidant activity of ascorbyl palmitate on the concentration of αTOH in the oil. The addition of ascorbyl palmitate to purified menhaden TAG eliminated the initial peroxidizing effect of αTOH when the TOH level was above that for maximal antioxidant activity. It is also noteworthy that ascorbyl palmitate exerted a strong influence on the rate of hydroperoxide formation in the initial phase of the storage period (≤3 d), when very little of the added αTOH had been consumed (Fig. 3). At 100 ppm αTOH, ascorbyl palmitate did not reduce the initial rate of hydroperoxide formation, but did have an initial sparing effect on the αTOH. A subsequent increase in the rate of αTOH consumption to approximately that of αTOH in the samples without ascorbyl palmitate is thought mainly to reflect depletion of the ascorbyl palmitate.

Lambelet and Löliger (3) used electron spin resonance (ESR) spectroscopy to study the behavior of tocopheroxyl radicals generated in oxidized chicken fat. The intensity of the ESR signal of αTO• increased with increasing αTOH starting concentration. However, at a high αTOH concentration, the αT• generated were rapidly destroyed, and the duration of the radical signal was shorter than at a lower αTOH concentration. These results suggest that there is an increase in the rate of generation of αTO• at αTOH concentrations above 100 ppm in menhaden oil and TAG. This was in accordance with the observed higher overall rate of consumption of αTOH when the level of addition was 500 ppm (38.8 ppm/d) than when the level of addition was 100 ppm (11.9 ppm/d). It is suggested that ascorbyl palmitate regenerates αTOH from αTO•, thus reducing the amount of αTO• available for further oxidation and participation in side reactions. The effect of ascorbyl palmitate therefore increases with increasing rate of αTO• generation. These results emphasize the participation of αTO•, as opposed to αTOH itself, in the hydroperoxide-forming side reactions in unsaturated fatty acid substrates with relatively high αTOH concentrations, possibly by the generation of radicals according to Reaction 9, or upon further reactions of αTO• to α-tocopherolquinone or the epoxy quinones. By using ascorbyl palmitate in addition to αTOH as an antioxidant in fish oil, the excellent hydrogen-donating power of this tocopherol is utilized, while at the same time the oil is protected from αTOH-mediated accumulation of hydroperoxides.

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