Antioxidant Activities of α- and γ-Tocopherols in the Oxidation of Rapeseed Oil Triacylglycerols

Anna-Maija Lampi[a,*], Leena Kataja[a], Afaf Kamal-Eldin[b], and Piironen Vieno[a]

[a]Department of Applied Chemistry and Microbiology, FIN-00014 University of Helsinki, Finland, and [b]Department of Food Science, Swedish University of Agricultural Sciences (SLU), S-750 07 Uppsala, Sweden

ABSTRACT: Antioxidant properties of 5 to 500 μg/g levels of α- and γ-tocopherols, in the oxidation of rapeseed oil triacylglycerols (RO TAG), were studied at 40°C in the dark. Each tocopherol alone and in a mixture was studied for its stability in oxidizing RO TAG. Also the effects of tocopherols on the formation of primary and secondary oxidation products of RO TAG were investigated. Both tocopherols significantly retarded the oxidation of RO TAG. At low levels (≤50 μg/g), α-tocopherol was more stable and was a more effective antioxidant than γ-tocopherol. At higher α-tocopherol levels (>100 μg/g), there was a relative increase in hydroperoxide formation parallel to consumption of α-tocopherol, which was not found with γ-tocopherol. Therefore, γ-tocopherol was a more effective antioxidant than α-tocopherol at levels above 100 μg/g. As long as there were tocopherols present, the hydroperoxides were quite stable and no volatile aldehydes were formed. In a mixture, α-tocopherol protected γ-tocopherol from being oxidized at the addition levels of 5 + 5 and 10 + 10 μg/g but no synergism between the tocopherols was found. α-Tocopherol was less stable in the 500 + 500 μg/g mixture than when added alone to the RO TAG. No prooxidant activity of either tocopherol or their mixture was found.

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α- and γ-Tocopherols are the most abundant natural antioxidants in plant lipids. Tocopherols (TOH) act as antioxidants by donating a hydrogen atom to a peroxyl radical (LOO•) of an unsaturated lipid molecule, forming a hydroperoxide (LOOH) and a tocopheroxyl radical (TO•) (Eq. 1). The tocopheroxyl radical

$$LOO\bullet + TOH \rightarrow LOOH + TO\bullet \quad [1]$$

has a lower capacity to propagate lipid peroxidation compared to the peroxyl radical. Instead, tocopheroxyl radicals react with other peroxyl (Eq. 2) or tocopheroxyl radicals (Eq. 3) forming more stable adducts.

$$TO\bullet + LOO\bullet \rightarrow \text{more stable products} \quad [2]$$

$$TO\bullet + TO\bullet \rightarrow \text{more stable products} \quad [3]$$

The hydrogen atom donating capacity of α-tocopherol is higher than that of γ-tocopherol (1), which means that α-tocopherol should be a more potent antioxidant than γ-tocopherol. However, tocopherols and tocopheroxyl radicals may also participate in reactions other than those with peroxyl radicals when present at high concentrations (2), e.g.,

$$TOH + O_2 \rightarrow \text{unknown products} \quad [4]$$

$$TO\bullet + LH \rightarrow TOH + L\bullet \quad [5]$$

$$TO\bullet + LOOH \rightarrow TOH + LOO\bullet \quad [6]$$

There is disagreement about the relevance of these "side reactions" which claim to lead to the prooxidant activity of tocopherols (3–4). Several recent reviews have discussed the importance of these reactions as determinants of the absolute and relative antioxidant activities of different tocopherols (2, 5–7). Those reviews emphasized the importance of oxidation conditions, such as the temperature and the availability of oxygen, the chemical nature and physical state of the lipid as well as the concentration of tocopherol in the evaluation of the overall role of tocopherols as inhibitors of lipid oxidation.

Several approaches have been used to study the effects of anti- or prooxidants. Yanishlieva and Marinova (8) introduced two concepts of the effect of an antioxidant: efficiency and strength. Efficiency describes how long an antioxidant can prolong the induction period, and strength describes the rate of the inhibited oxidation during the induction period. Using two concepts, the overall antioxidant property can be better characterized than by using either of them alone. These authors found it necessary to follow the stability of an antioxidant. Furthermore, when secondary products of oxidizing lipids are analyzed, much more information can be obtained from an experiment (5,7).

Recently it has been found that antioxidants may have different effects on the formation and the decomposition of hydroperoxides (9,10). In the presence of tocopherols, the rate of hydroperoxide breakdown and induction of further oxidation is markedly inhibited. Hopia *et al.* (11) found that α-tocopherol

*To whom correspondence should be addressed at Department of Applied Chemistry and Microbiology, Viikki-D, P.O. Box 27, FIN-00014 University of Helsinki, Finland.

E-mail: anna-maija.lampi@helsinki.fi

reduced the decomposition of the hydroperoxides of methyl linoleate by donating hydrogen atoms to alkoxyl radicals. They concluded that in the presence of a strong hydrogen atom donor, the decomposition of hydroperoxides *via* alkoxyl radicals prefers hydrogen atom abstraction to β-scission. Thus, the formation of hydroxy compounds, which are a product of a hydrogen abstraction reaction, was increased and that of hexanal, which is a β-scission product of an alkoxyl radical, was reduced.

A concentration as low as 11 μg/g of γ-tocopherol was enough to markedly inhibit hydroperoxide and secondary product formation in rapeseed oil triacylglycerols (RO TAG) (12). α-Tocopherol was a better antioxidant than γ-tocopherol in purified sunflower triacylglycerols at concentrations ≤40 μg/g but a poorer antioxidant at concentrations >200 μg/g (13). The degree of tocopherol consumption was not related to the peroxide value (PV) but was correlated to the initial tocopherol concentrations indicating that the loss of tocopherols was due to reactions other than those with peroxyl radicals. No synergism was observed when α- and γ-tocopherols were added in equal amounts of 100, 300, and 500 μg/g. This study (13) was limited to determinations of PV and residual tocopherols at one time point (7 d) in the presence and absence of ferrous sulfate.

The aim of the current work was to study the stability and the effects of α- and γ-tocopherols alone and mixed with each other on the oxidation of RO TAG at a moderate temperature (40°C) without added catalyst. Emphasis was put on the antioxidant effects of tocopherols added at low levels (5 and 10 μg/g) and at the 500 μg/g level.

MATERIALS AND METHODS

Materials. RO was chosen as the oxidizing material, because it contains both linoleoyl and linolenoyl groups which are sources for different volatile aldehydes: hexanal and heptadienal, respectively. RO (Van der Bergh Foods Ltd., Helsinki, Finland) was purified by a multilayer chromatographic method (14) to produce RO TAG. RO TAG contained ≤0.6 meq/kg of hydroperoxides measured by PV, <0.1 of secondary oxidation products measured by anisidine value (AnV), and <10 nmol/g of volatile aldehydes. There were no residual α- and γ-tocopherols in RO TAG (<1 μg/g). The major fatty acyl groups in the RO TAG were oleoyls (55%), linoleoyls (22%), and linolenoyls (10%), measured by gas liquid chromatography (15). Tocopherols, α and γ, were purchased from Merck (Darmstadt, Germany; purity ≥98%) and Sigma Chemical Co. (St. Louis, MO; purity >96%), respectively, and used as received. α-Tocopherol contained no other tocopherols and γ-tocopherol contained <1% of α- and β-tocopherols as checked by high-performance liquid chromatography (HPLC) *vide infra*. All solvents and reagents were either HPLC or pro analysis grade.

Oxidation experiments. RO TAG samples (5.0 g) were oxidized in vials (i.d. 1.7 cm) enclosed in 130-mL flasks at 40°C in the dark for 16 d. Two random samples were taken every 4 d, pooled, and analyzed for the oxidative status. Duplicate experiments with added α-tocopherol (5, 10, 50, 100, and 500 μg/g), γ-tocopherol (5, 10, 50, 100, and 500 μg/g) and mixtures of tocopherols (5 + 5, 10 + 10, and 500 + 500 μg/g) were conducted. RO TAG without additions served as the control in each of the six experiments. For comparison, RO without purification was also oxidized under the same conditions for 28 d.

Analysis of oxidation status and tocopherol contents. The primary oxidation status of RO TAG was characterized by measuring hydroperoxides using the ferric thiocyanate PV method (16). *p*-Anisidine reactive products (AnV) (17) and volatile aldehydes with from 5 to 10 carbon atoms (18) measured secondary oxidation products. Volatile aldehydes were measured as their 2,4-dinitrophenyl hydrazone derivatives by reversed-phase HPLC with diode array detection (λ = 360 nm) (18). Tocopherol contents were measured by normal-phase HPLC with fluorescence detection (λ_{ex} = 292 nm, λ_{em} = 324 nm) (14). Each chemical analysis was made in duplicate from a pooled sample.

The determination limit of volatile aldehydes was 2 nmol/g. Four RO samples with a series of added alkanals (from pentanal to decanal) were analyzed at the beginning of each oxidation experiment to confirm the stability of the analytical method. The mean coefficient of variation (CV%) of each alkanal was *ca.* 10%. The determination limit of tocopherols was 2 μg/g. The tocopherol contents of all samples from each oxidation experiment were analyzed in one determination period including one RO sample as a reference. Between the experiments, the level of tocopherol analysis was stable, because the CV% of α- and γ-tocopherol contents of RO were 4.6 and 3.8%, respectively (n = 6).

The precisions of duplicate determination of PV, AnV, total aldehyde, and α- and γ-tocopherol contents were 8.7% (PV ≥ 1 meq/kg), 12% (AnV ≥ 1), 15% (total aldehydes ≥5 nmol/g), 3.9% (≥2 μg/g α-tocopherol) and 8.1% (≥2 μg/g γ-tocopherol), respectively. Precision was calculated as the maximum relative random error at the 95% level (19).

Data collected from the six separate experiments were combined, because the RO TAG controls, run as one material in each experiment, oxidized similarly. The CV% of the rate of hydroperoxide formation in these controls was 10% (n = 6). Each material containing tocopherols was studied in two experiments. The results from these experiments were similar, because the PV from the two experiments differed from the mean by 9.2%. Unless otherwise stated, the oxidation status of each material was expressed as a mean of two repeated experiments except for controls (n = 6). Statistical comparisons of the materials at different time points were made by t-tests with a null hypothesis $\mu_1 > \mu_2$. Statements in the text referred to as significant apply to a 95% significance level.

RESULTS AND DISCUSSION

Stability of α- and γ-tocopherols during oxidation of RO TAG. A remarkable result from this study was that α-tocopherol was significantly more stable than γ-tocopherol when present at ≤10 μg/g and less stable when present at ≥100 μg/g (Fig. 1). At the 5 μg/g

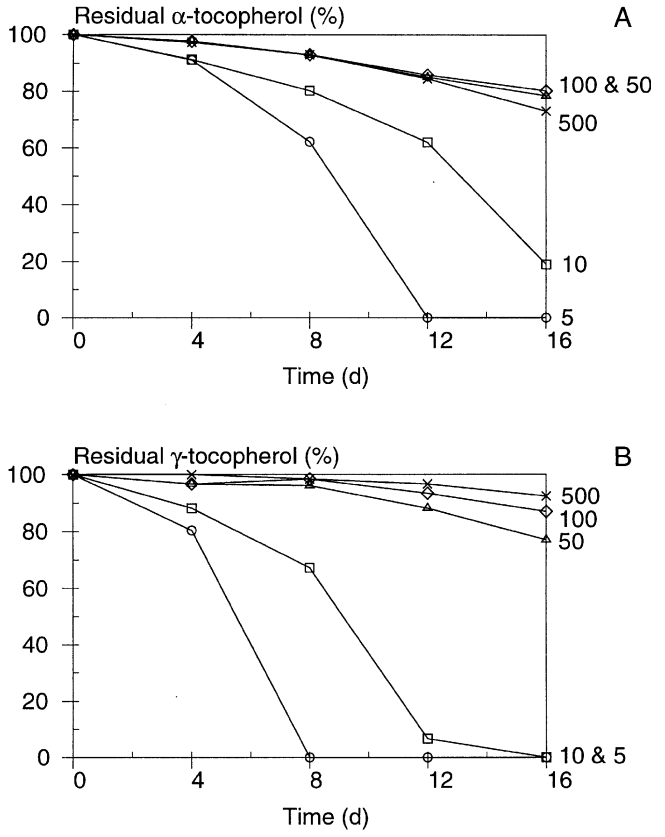

FIG. 1. Residual (A) α- and (B) γ-tocopherol contents of added 5, ..., 500 μg/g levels during oxidation of rapeseed oil triacylglycerols at 40°C in the dark for 16 d; 5 (○), 10 (□), 50 (△), 100 (◇), and 500 μg/g (x) of tocopherol. Means from two experiments.

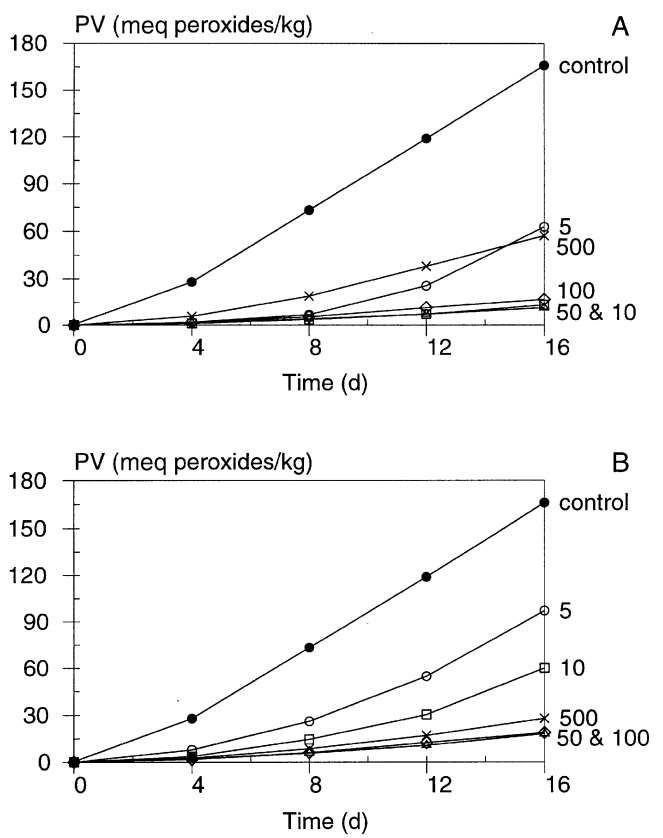

FIG. 2. Effect of added (A) α- and (B) γ-tocopherol at 5, ..., 500 μg/g level on the hydroperoxide formation in the oxidation of rapeseed oil triacylglycerols at 40°C in the dark for 16 d as measured by peroxide value (PV); control (●), 5 (○), 10 (□), 50 (△), 100 (◇), and 500 μg/g (x) of tocopherol. Means from two experiments.

level, α-tocopherol was totally consumed after 12 d of oxidation, while a 19% residual α-tocopherol was recovered after 16 d of oxidation for the 10 μg/g level. γ-Tocopherol was completely used up after 8 and 12 d of oxidation at the 5 and 10 μg/g levels, respectively. From α-tocopherol 73–80% and from γ-tocopherol 77–92% were left after 16 d of oxidation when addition levels of 50 μg/g or higher were used. There was a difference between the absolute amounts of α- and γ-tocopherols used up during oxidation. At addition levels 50, 100, and 500 μg/g, the respective amounts of α-tocopherol consumed were 11, 20, and 135 μg/g while those of γ-tocopherol were 11, 13, and 38 μg/g.

Effect of α- and γ-tocopherols on the formation of primary products. The controls oxidized without a profound induction period. Both α- and γ-tocopherols significantly retarded the formation of hydroperoxides in RO TAG at all levels studied and the effect depended on the concentration (Fig. 2). In the presence of 5 μg/g of either tocopherol, induction periods between 8 and 12 d occurred, and with 10 μg/g of γ-tocopherol, the induction period ended between 12 and 16 d. After the induction period, the oxidation rate of RO TAG increased remarkably in accordance with the disappearance of tocopherols (Fig. 1). At addition levels ≥10 μg/g of α-tocopherol and ≥50 μg/g of γ-tocopherol, the oxidation of RO TAG remained in the induction period during the 16-d experiment, which means that the tocopherols were efficient antioxidants at these levels.

During the induction period, significantly more hydroperoxides were formed in RO TAG with 500 μg/g than with ≤100 μg/g of α-tocopherol, which means that α-tocopherol loses its activity to inhibit hydroperoxide formation at high concentrations. PV were lowest at 10 and 50 μg/g and highest at 500 μg/g of α-tocopherol except for the control, which was oxidized much more (Fig. 2). In fact, the PV of RO TAG with 500 μg/g of α-tocopherol was constantly fourfold that of RO TAG with 50 μg/g of α-tocopherol. In contrast, an addition level of 500 μg/g of γ-tocopherol did not significantly increase hydroperoxide formation compared to the lower γ-tocopherol levels. The PV obtained were lowest at 50 and 100 μg/g of γ-tocopherol and those of samples containing 500 μg/g of γ-tocopherol were only about 40% higher (Fig. 2). Therefore, according to the concept of Yanishlieva and Marinova (8), γ-tocopherol is a stronger antioxidant than α-tocopherol.

No prooxidative effect of either tocopherol was found at any addition level. Even at the beginning of oxidation, tocopherols were antioxidative as the PV of RO TAG after 4 d of oxidation was higher than those with added 500 μg/g of α- and γ-tocopherols, being 28, 5.8, and 2.5 meq/kg, respectively. It is noteworthy that RO TAG containing 500 μg/g of α-tocopherol showed higher PV than those containing 5 μg/g

of α-tocopherol at the initial stage of oxidation, which is a feature of a low strength antioxidant.

In our experiment, control materials began to oxidize immediately. After 4 d, RO TAG without tocopherols was clearly more oxidized (PV = 28 meq/kg) than RO TAG with 500 μg/g of α-tocopherol (PV = 5.8 meq/kg), which means that no initial prooxidant effect was found when purified RO TAG was used as control. When comparing RO TAG containing 500 μg/g of α-tocopherol and that containing 5 μg/g of α-tocopherol, we saw that the former contained more hydroperoxides at every time point before 16 d. After 16 d of oxidation, RO TAG with 5 μg/g of α-tocopherol was in the propagation phase while that with 500 μg/g of α-tocopherol was still in the induction period. In RO TAG with high levels of α-tocopherol, hydroperoxide formation began at once and increased at a constant rate throughout the induction period. We think that the judgment of prooxidativity depends on at least two factors, the first being the tocopherol content of control materials and the second being the time of detection. Controls void of tocopherols should be oxidized immediately without an induction period. It may be that the initial pro-oxidativity found in previous studies (4,20,21) was due to the presence of residual tocopherols in their controls, which led to an induction period.

Effect of α- and γ-tocopherols on the formation of secondary products. Tocopherols significantly retarded the formation of secondary products as measured by AnV (Fig. 3) and volatile aldehydes (Fig. 4). After 16 d of oxidation, the AnV and the content of total volatile aldehydes of RO TAG without tocopherols reached 22 and 290 nmol/g, respectively. In general, as long as there was α- or γ-tocopherol present, the AnV remained below 1.5 and volatile aldehydes below 30 nmol/g except for RO TAG containing initially 500 μg/g of α-tocopherol, which reached an AnV of 3.4 after 16 d of oxidation. Here, the formation of secondary products was negligible compared to the formation of hydroperoxides (PV = 57 meq/kg). In particular, hydroperoxide breakdown to volatile aldehydes was greatly inhibited. In RO TAG at low levels (≤10 μg/g) of α- or γ-tocopherol, volatile aldehydes began to form before AnV measurements could detect oxidation. Thus, the AnV and the contents of volatile aldehydes gave information about secondary products, which are somewhat different.

Hexanal and heptadienal were the two major volatile aldehydes produced in RO TAG with and without tocopherols. When more than 110 nmol/g of volatile aldehydes was formed in RO TAG without tocopherols, the relative proportion of hexanal increased more than 40% (Table 1). However, with ≤10 μg/g of α- or γ-tocopherol, the proportion of hexanal was always >40%. Especially α-tocopherol seemed to have the higher percentage of hexanal.

Comparison of the effects of α- and γ-tocopherols with each other and their interactions. Tocopherols were mixed in

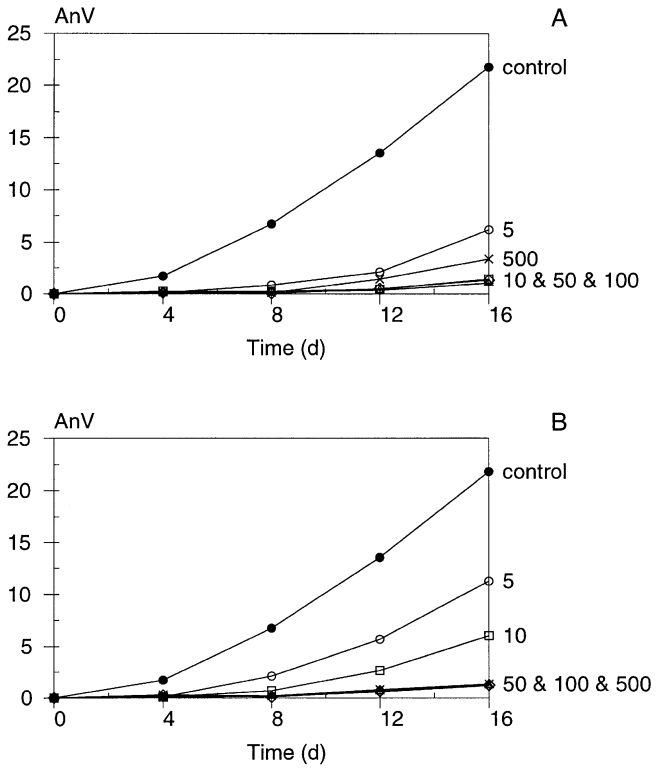

FIG. 3. Effect of added (A) α- and (B) γ-tocopherol at 5, ..., 500 μg/g level on the formation of secondary products in the oxidation of rapeseed oil triacylglycerols at 40°C in the dark for 16 d as measured by anisidine value (AnV); control (●), 5 (○), 10 (□), 50 (△), 100 (◇), and 500 μg/g (x) of tocopherol. Means from two experiments.

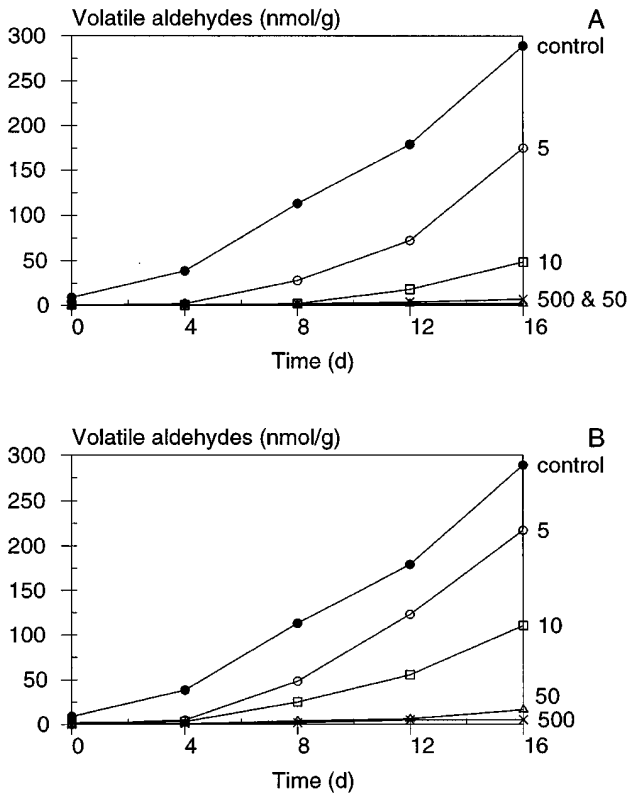

FIG. 4. Effect of added (A) α- and (B) γ-tocopherol at 5, ..., 500 μg/g level on the formation of volatile aldehydes in the oxidation of rapeseed oil triacylglycerols at 40°C in the dark for 16 d; control (●), 5 (○), 10 (□), 50 (△), and 500 μg/g (x) of tocopherol. Means from two experiments.

TABLE 1
Effects of α- and γ-Tocopherols on the Proportions of Hexanal and Heptadienal and Levels of Volatile Aldehydes in Oxidized Rapeseed Oil Triacylglycerols (RO TAG) at Different Oxidation Times[a]

Material and oxidation time in days	Total volatile aldehydes (nmol/g)	Hexanal (%)	Heptadienal (%)
RO TAG, 4 d *	38	14	31
RO TAG, 8 d *	113	32	22
RO TAG, 16 d *	289	49	13
+ 5 μg/g α-T, 8 d	29	76	7
+ 5 μg/g α-T, 16 d	174	56	10
+ 10 μg/g γ-T, 8 d	25	54	24
+ 10 μg/g γ-T, 16 d	110	41	18

[a]Means from two experiments, except for * where it was four. α-T, α-tocopherol; γ-T, γ-tocopherol.

equal amounts at the levels of 5 + 5, 10 + 10, and 500 + 500 μg/g to investigate their interactions with each other. The most striking effect was the change in their stability when added at ≤10 μg/g (Fig. 5). In a mixture, α-tocopherol protected γ-tocopherol from oxidation. When each of them was added separately, γ-tocopherol was oxidized before α-tocopherol, but in a mixture the content of γ-tocopherol was stable until the α-tocopherol had been consumed.

The addition of 5 μg/g γ-tocopherol to RO TAG containing 5 μg/g α-tocopherol increased the antioxidant activity in the system, but no synergism was found. Instead, the effect was additive, because the oxidation of RO TAG with the 5 + 5 μg/g mixture had proceeded to between those with 10 μg/g of either tocopherol. The addition of 10 μg/g of γ-tocopherol to RO TAG with 10 μg/g of α-tocopherol did not improve the oxidative stability of the system, which means that there was no longer an additional beneficial effect from extra tocopherols.

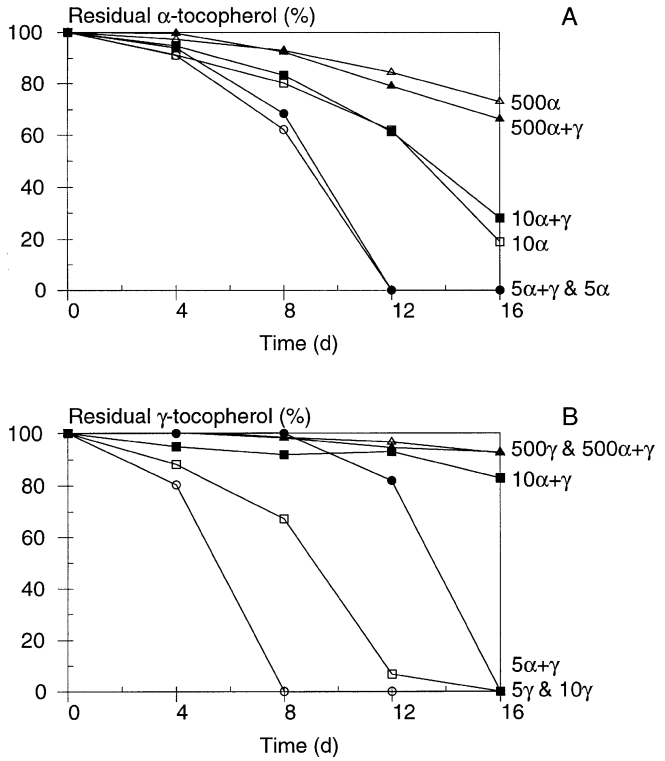

FIG. 5. Interactions of added α- and γ-tocopherols at 5, 10, and 500 μg/g levels on the stability of (A) α- and (B) γ-tocopherol during the oxidation of rapeseed oil triacylglycerols at 40°C in the dark for 16 d; 5 μg/g (○), 10 μg/g (□) and 500 μg/g (△) one tocopherol, and 5 + 5 μg/g (●), 10 + 10 μg/g (■), and 500 + 500 μg/g (◆) of each tocopherol in a mixture. Means from two experiments, except for 10 + 10 μg/g mixture, where it was one.

At the low addition levels of tocopherol (≤10 μg/g), α-tocopherol was more powerful than γ-tocopherol in inhibiting hydroperoxide formation both alone and in a mixture (Table 2). For example, when an additional 5 μg/g of tocopherol was added to RO TAG with 5 μg/g of α-tocopherol, additional α-tocopherol retarded hydroperoxide formation more than did an equal addition of γ-tocopherol. The final PV were 63 meq/kg (5 μg/g α-tocopherol), 13 meq/kg (10 μg/g α-tocopherol), and 32 meq/kg (5 + 5 μg/g). The most stable material was RO TAG with 10 μg/g of α-tocopherol. Measurements of AnV confirm the same trend as that seen with the PV (Table 2).

When added separately and in a 500 + 500 μg/g mixture, 73 and 66%, respectively, of α-tocopherol remained at the end of the study. The respective values for γ-tocopherol were 92 and 93%. The residual α-tocopherol content in a mixture was significantly lower than when added alone at the 500 μg/g level after 16 d of oxidation. This means that at this high total tocopherol content, α-tocopherol consumption was enhanced by the presence of γ-tocopherol. In contrast, the presence of α-tocopherol did not have an effect on the stability of γ-tocopherol in a mixture.

It has been found in an intensively oxidizing system that α-tocopherol can regenerate γ-tocopheroxyl radicals to γ-tocopherol (22). γ-Tocopherol alone inhibited hydroperoxide formation most efficiently. Adding both of the tocopherols caused more oxidation in RO TAG than when adding either of them alone, which is understandable as at these high levels of tocopherols, especially with α-tocopherol, more hydroperoxides are formed than at lower concentrations. Others have found that mixed tocopherols stabilized lard (23) and stripped corn oil (9) similar to γ-tocopherol alone. As in our study, Huang *et al.* (9) found more hydroperoxides with a 500 + 500 μg/g mixture in stripped corn oil than with either of them alone at 500 μg/g.

At the highest addition level (500 μg/g), γ-tocopherol alone inhibited hydroperoxide formation and breakdown

TABLE 2
Comparison of Peroxide Values and Anisidine Values of RO TAG with Added α- or γ-T after 16 d of Oxidation at 40°C in the Dark[a]

	Peroxide value (meq/kg)				Anisidine value		
	RO TAG	5 μg/g α-T	10 μg/g α-T		RO TAG	5 μg/g α-T	10 μg/g α-T
RO TAG	166	63	13	RO TAG	21	6.2	1.4
5 μg/g γ-T	97	32	N.T.	5 μg/g γ-T	11	3.4	N.T.
10 μg/g γ-T	60	N.T.	14*	10 μg/g γ-T	6.0	N.T.	1.3*

[a]Means from two experiments, except for * where it was one. Abbreviation: N.T., not tested. For other abbreviations see Table 1.

most efficiently. The final PV and AnV of RO TAG with 500 μg/g of α-tocopherol, γ-tocopherol, and their mixture were 57, 27, and 70 meq/kg and 3.4, 1.3, and 4.3, respectively.

Effects of natural α- and γ-tocopherol levels on unpurified RO. The importance of tocopherols and other components removed during the purification of RO was confirmed by studying the oxidation of unpurified RO. RO oxidized slightly during 28 d at 40°C. The initial PV of 0.2 meq/kg increased to 0.8 meq/kg during the first 20 d and reached 2.1 meq/kg by the end of the experiment. The formation of secondary oxidation products was negligible. AnV rose only from 1.9 to 2.3; α- and γ-tocopherol contents in RO remained stable being 170 and 390 μg/g, respectively. Both tocopherols are stable under these conditions for 28 d and thus they are able to efficiently retard oxidation.

Change of antioxidant activity of α- and γ-tocopherols. In this experiment, both α- and γ-tocopherols were effective antioxidants at all concentrations studied. At low levels (≤50 μg/g), α-tocopherol was more efficient than γ-tocopherol in terms of inhibiting the formation of both hydroperoxides and secondary products. However, the efficiency of α-tocopherol at high levels (>100 μg/g) was less than that of γ-tocopherol, which was shown by the increased formation of hydroperoxides and increased consumption of the tocopherol. That γ-tocopherol was a better antioxidant than α-tocopherol at high levels is in agreement with our previous findings using purified sunflower oil (13).

At lower levels (≤50 μg/g) of tocopherols, tocopherols are efficiently used up in the reactions with peroxyl radicals and nonsignificant amounts will be available to participate in side reactions. Loss of tocopherols remains unexplained since no kinetic studies have been performed so far on the oxidation products of tocopherols. It is noteworthy that residual α-tocopherol contents at addition levels of 5 and 10 μg/g were higher than those of γ-tocopherol and that lower rates of oxidation were observed in RO TAG containing α-tocopherol than in those containing γ-tocopherol. At these levels, the destruction of tocopherols is due mainly to oxidation reactions with peroxyl radicals while protecting unsaturated fatty acyls. Because of its higher hydrogen-donating ability, α-tocopherol is faster than γ-tocopherol in donating hydrogen to the free radicals and inhibiting oxidation. On the other hand, γ-tocopherol is much slower in its reactions with peroxyl radicals, which will lead to a greater progress of lipid oxidation accompanied by co-oxidation and loss of γ-tocopherol. These findings are in line with previous results showing that tocopherols are more effective and stable in less oxidizable material than in highly oxidizable material (4,24). In general, the stability of γ-tocopherol has been shown to be better than that of α-tocopherol in enhanced conditions such as at high concentration (25), at high temperatures (23,26), in thin layers (27,28), and in microwave heating (29). Similarly, the tocopherols were very stable in the unpurified RO used in this study, where the natural tocopherol mixture together with other antioxidants present retarded oxidation for 28 d.

The change in the order of antioxidant activity of α- and γ-tocopherol at 100 μg/g accompanied by increased relative formation of hydroperoxides at higher α-tocopherol concentrations supports our previous assumption that α-tocopherol is consumed both in the antioxidant reaction and in other "side reactions," which are not fully understood (13). As α-tocopherol concentration increased, relatively fewer molecules were used up in the antioxidant reaction and more were available for side reactions. γ-Tocopherol, being less reactive, participates less in side reactions. There was a slight increase in hydroperoxide formation, compared to the lower addition levels, in the 500 μg/g of γ-tocopherol samples. Studies dedicated to reveal the structures of tocopherol oxidation products by analytical methodologies (mass spectroscopy, nuclear magnetic resonance, etc.) and thereafter to the kinetics of tocopherol consumption in oxidizing polyunsaturated systems are needed to further clarify this issue.

Volatile aldehydes as an indicator of different reactions of hydroperoxides. We found that even at the highest α-tocopherol level, where the hydroperoxide contents were remarkably higher than those at lower tocopherol levels, no volatile aldehydes were formed. In the presence of the initial 500 μg/g of α-tocopherol, hydroperoxides were formed, but they were also stabilized by α-tocopherol and/or alkoxyl radicals which formed abstracted hydrogen atoms from α-tocopherol. Thus β-scission of alkoxyl radicals, which is the most important volatile aldehyde-forming reaction, was inhibited. This is in agreement with several investigations (9–11).

The distribution of volatile aldehydes formed indicated that without tocopherols, the proportion of heptadienal decreased and that of hexanal increased during oxidation. In a previous study (18), we found a similar trend. This could be explained by the fact that at the beginning of oxidation, there were more volatile products from the more oxidizable linolenoyl than from the less oxidizable linoleoyl groups

characterized by heptadienal and hexanal, respectively. As oxidation proceeds, Park *et al.* (30) found that the proportion of hydroperoxides derived from linolenoyl groups in soybean oil TAG decreased with oxidation time. Another reason for the increasing levels of saturated aldehydes during oxidation could be that heptadienal and other unsaturated aldehydes undergo further oxidation and break down, thereby forming smaller saturated aldehydes (31). It was interesting to discover that with tocopherols there was always >40% of hexanal. This could be explained by the controlling effect of tocopherols in hydroperoxide breakdown.

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