Antioxidant Activity of Minor Amounts of γ-Tocopherol in Natural Triacylglycerols

Anna-Maija Lampi*, Anu Hopia, and Vieno Piironen

Department of Applied Chemistry and Microbiology, FIN-00014, University of Helsinki, Helsinki, Finland

ABSTRACT: The effects of minor amounts of γ-tocopherol on the oxidation of natural triacylglycerols (TAG) of rapeseed (RO) and butter oils (BO) were studied. Four different TAG materials were blended from chromatographically purified TAG that contained 100–25% of RO TAG. The RO TAG contained from <1 to 43 μg γ-tocopherol per gram of TAG, which corresponded to ≤6% of the total tocopherols in the original RO. The TAG were held at 40°C in the dark for 4 wk and followed at regular intervals by measurements of hydroperoxide formation by peroxide values and of secondary product formation by *p*-anisidine values at regular intervals. In all TAG, minor amounts of γ-tocopherol retarded oxidation. In RO TAG, concentrations as low as 11 μg/g of γ-tocopherol (1.5% of the total tocopherols in the original RO) were enough to decrease hydroperoxide and secondary product formation to 46 and 39%, respectively. The effect was even more important in TAG mixtures that contained BO TAG. There were no significant differences between oxidation of the RO TAG at 24 μg/g, the 75% RO TAG mixture at 11 μg/g, and the 50% RO TAG mixture at 3 μg/g of γ-tocopherol. Even at these minor levels, γ-tocopherol was a significant antioxidant, which is important in oxidation studies of purified model systems.

JAOCS 74, 549–555 (1997).

KEY WORDS: Antioxidant, natural triacylglycerols, γ-tocopherol.

Tocopherols (TocH) are known to act as antioxidants by donating a hydrogen atom to chain-propagating peroxyl radicals (LOO·):

$$LOO\cdot + TocH \rightarrow LOOH + Toc\cdot \quad [1]$$

$$LOO\cdot + Toc\cdot \rightarrow \text{nonradical products} \quad [2]$$

and are consumed by the reaction of chromanoxy (Toc•) radicals with other peroxyl radicals or with each other (1). It is generally agreed that the order of hydrogen-donating power of different tocopherols decreases $\alpha > \beta > \gamma > \delta$, as was shown by Burton and Ingold (1). Studies of the antioxidative activity of tocopherols *in vivo* and *in vitro*, however, have given contradictory results, which have recently been reviewed by Kamal-Eldin and Appelqvist (2). The effectiveness of tocopherols as antioxidants depends highly on the characteristics of the oxidizing material, such as the phase of the lipid (3–4) and its oxidative status (5–6), and on experimental conditions, such as the concentrations of tocopherols (3,7) and the temperature (4,8).

When the role of tocopherols in relation to edible oils and fats is studied, it is important to have complex natural triacylglycerols (TAG) as the oxidizing material. Methyl esters and monoacyl TAG are inappropriate substrates, because the TAG structure influences the oxidation (9–11). Natural TAG with various acyl groups must be purified from oils to get material without the pro- and antioxidant activities of minor compounds that are normally present in the oils.

Several column-chromatographic methods (6,11–14), as well as activated carbon treatments (15), have been used to purify natural TAG from oils for antioxidant studies. Commercially available corn oil, stripped of tocopherols, has also been used (3,16). Data on the concentrations of important pro- and antioxidants of the TAG, however, are not always given, and the term "not present" is frequently used without detailed information about the determination limits of the analytical methods used.

In our laboratory, purified natural TAG are used for lipid oxidation as well as for pro- and antioxidant studies. When developing the purification method of TAG, we found it difficult to remove all γ-tocopherol from vegetable oils that contained >300 μg/g of γ-tocopherol. On the other hand, α-tocopherol was effectively adsorbed in the column (14). The traces of remaining γ-tocopherol seemed to influence the oxidation of the TAG, and some of the results were controversial. Therefore, the aim of this work was to study the effects of the remaining minor amounts (<50 μg/g) of γ-tocopherol on the oxidation of natural TAG with various fatty acid compositions. The oxidizing materials studied consisted of TAG that were purified from RO and BO. The γ-tocopherol concentrations were ≤6% of the total tocopherols present in original RO prior to tocopherol removal.

EXPERIMENTAL PROCEDURES

Materials. Turnip RO and BO from cultured cream butter were used for the production of natural TAG. The oils were of com-

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

mercial quality and were obtained from Raisio Group Ltd. (Raisio, Finland) and Valio Ltd. (Seinäjoki, Finland), respectively.

RO and BO TAG were purified by a multilayer chromatographic method (14). There was from <1 μg/g to 43 μg/g of γ-tocopherol left in the RO TAG, contributing at most 6% of all tocopherols or 9% of γ-tocopherol in the original RO. Because it was difficult to remove γ-tocopherol from RO, its concentration in the RO TAG was controlled by the amount of RO purified in each batch, ranging from 65 to 75 g. There were no detectable amounts of other tocopherols in the RO TAG and no tocopherols in the BO TAG. Tocopherol concentrations were determined by normal-phase high-performance liquid chromatography (HPLC) with fluorescence detection. The determination limit was 1 μg/g (14). The peroxide value (PV) of the TAG was always <0.5 mEq of peroxides/kg, and the *p*-anisidine value was <0.5 at the beginning of the experiments. Fatty acid compositions of the TAG were determined by capillary gas chromatography (GC) with flame-ionization detection (FID) of the methyl esters of TAG prepared by alkaline transesterification (17).

RO TAG were used for oxidation studies, individually and in mixtures with BO TAG that contained 75, 50, and 25% of RO TAG (Table 1). The TAG mixtures were named according to their RO contents.

Oxidation. From 14 to 20 TAG samples, 5.0 g each, were oxidized in vials (i.d. 1.7 cm) at 40°C in closed 130-mL flasks in the dark for 4 wk as described earlier (18). Two to three TAG samples were taken away and pooled for the analysis of oxidative status at regular intervals.

Analysis of oxidation status. Primary oxidation products were measured by a iodometric PV method (19), and secondary products were measured by *p*-anisidine values (AnV) (20). All measurements were made in duplicate from pooled TAG samples. The precision of duplicate determinations was calculated as

$$P_{0.95} = t_{v(0.05)} \times S_r \quad [3]$$

where $P_{0.95}$ was the maximum relative random error at the 95% level, $t_{v(0.05)}$ was the 5% value of the *t*-distribution with ν degrees of freedom, and S_r was the relative standard deviation of the determinations (21). The $P_{0.95}$ values of PV and AnV determinations were 7.0 and 5.5%, respectively.

RESULTS

There was a marked difference between the oxidation of RO TAG with <1 μg/g γ-tocopherol and those with 11 μg/g γ-tocopherol, which contributed only 1.5% of all tocopherol isomers in the original RO (Fig. 1). In all samples, hydroperoxides were formed immediately, but the oxidation rate was markedly greater in the sample with <1 μg/g of γ-tocopherol than in the other samples. Formation of secondary products as measured by the AnV began after lag phases of up to 10 d. The AnV of the sample with <1 μg/g of γ-tocopherol reached 46, and those containing greater levels of γ-tocopherol reached 10–18. The antioxidant effect of γ-tocopherol from 11 to 43 μg/g was concentration-dependent: the higher the concentration, the less oxidation.

When RO TAG were mixed with less-unsaturated BO TAG, even lower concentrations of γ-tocopherol had significant antioxidative effects (Fig. 2). As little as 3 and 5 μg/g of γ-tocopherol retarded the oxidation of the 50% RO TAG mixture significantly. The PV increased up to 105, 68 and 29 mEq of peroxides/kg in 4 wk with 50% RO TAG mixtures that contained <1, 3, and 5 μg/g of γ-tocopherol, respectively. A concentration-dependent inhibition effect was seen also in the formation of secondary oxidation products. For the AnV curves, the lag phases were longer and the slopes became more shallow as the γ-tocopherol concentration increased.

As the γ-tocopherol concentrations of the various TAG were adjusted to approximately 10 μg/g (5–11 μg/g), the rate of oxidation of the TAG decreased as expected, based on the amount of unsaturation in the acyl groups. Rates of oxidation decreased in the following order RO TAG > 75% RO TAG > 50% RO TAG > 25% RO TAG when measured by PV and AnV (Fig. 3).

The order of susceptibility to oxidation was altered by a change in γ-tocopherol concentration. For example, when the RO TAG contained 24 μg/g, the 75% RO TAG mixture 11 μg/g, and the 50% RO TAG mixture 3 μg/g of γ-tocopherol, the 50% RO TAG mixture oxidized slightly more and the 75% RO TAG mixture slightly less than the RO TAG (Fig. 4).

TABLE 1
Contents of Major Fatty Acyls in the Natural Triacylglycerol (TAG) Materials from Rapeseed Oil (RO) and Butter Oil (BO)

	Fatty acyls (%)		
TAG materials	Oleic acid and other monoenes	Linoleic acid	Linolenic acid
RO	61	22	10
75% RO mixture	52	17	8
50% RO mixture	44	12	5
25% RO mixture	36	7	3

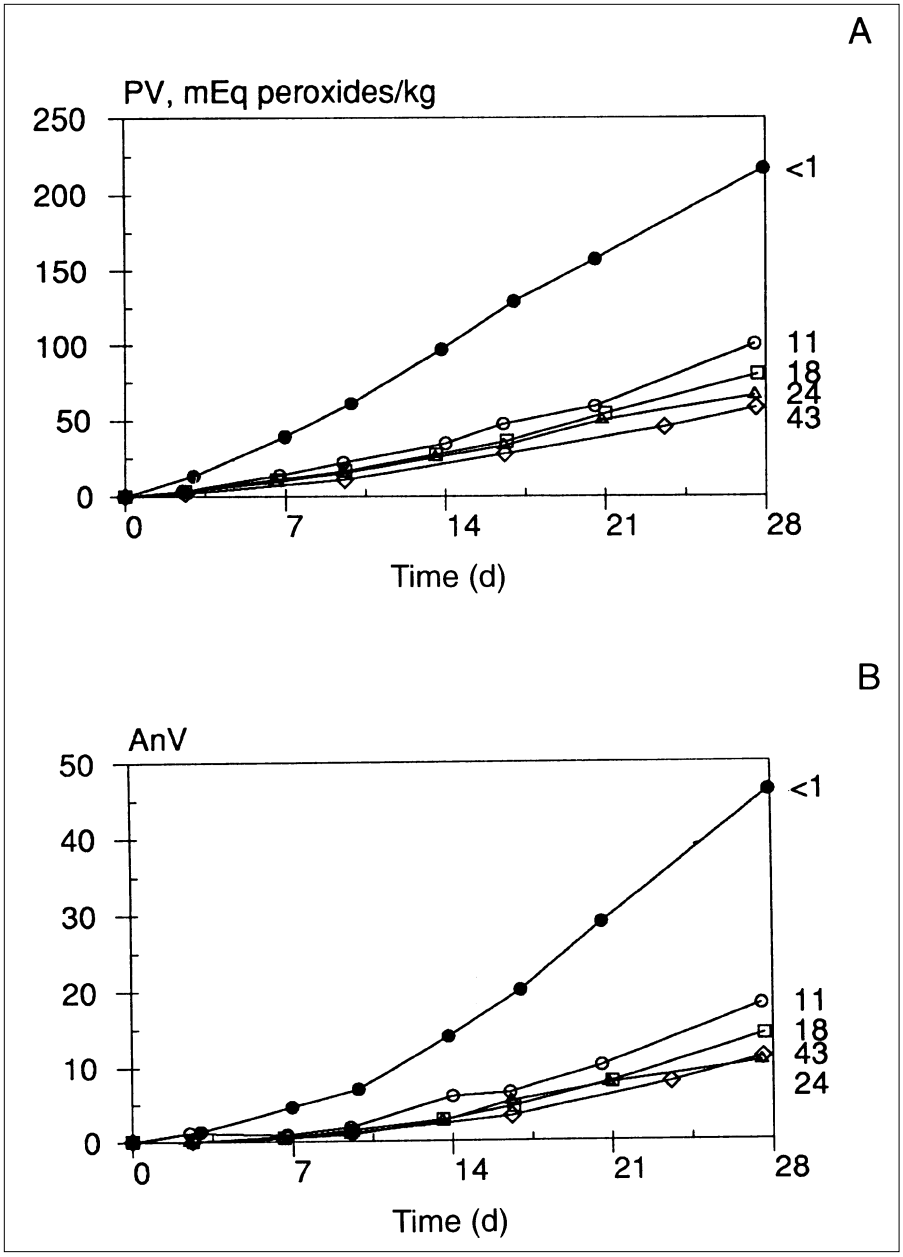

FIG. 1. Oxidation of rapeseed oil triacylglycerols with various γ-tocopherol concentrations, measured by A) peroxide values (PV) and B) *p*-anisidine values (AnV) from pooled triacylglycerol samples in duplicate. ● <1 μg/g, ○ 11 μg/g, □ 18 μg/g, △ 24 μg/g, and ◇ 43 μg/g of γ-tocopherol in triacylglycerols.

DISCUSSION

The effects of minor amounts of γ-tocopherol on the oxidation of the TAG were significant. As little as 11 μg/g γ-tocopherol, representing 1.5% of total tocopherols in the original RO, decreased hydroperoxide formation after 4 wk at 40°C to 46%, compared to the RO TAG with <1 μg/g γ-tocopherol. Similarly, secondary product formation in the RO TAG was decreased to 39%. In the RO TAG that contained 43 μg/g γ-tocopherol, representing 6% of total tocopherols in the original RO, the PV and AnV decreased to 27 and 24%, respectively (Fig. 1). The effect was even more important in less-unsaturated TAG mixtures (Fig. 2), where γ-tocopherol concentrations that were close to the determination limit (3 and 5 μg/g) lowered the final PV to 62 and 28% and the AnV to 45 and 26%, respectively.

The γ-tocopherol concentrations used were markedly lower than those commonly used for tocopherols. Generally, the lowest added γ-tocopherol content is at least 100 μg/g lipid (3,13,22–23), and the reported optimal level for γ-to-

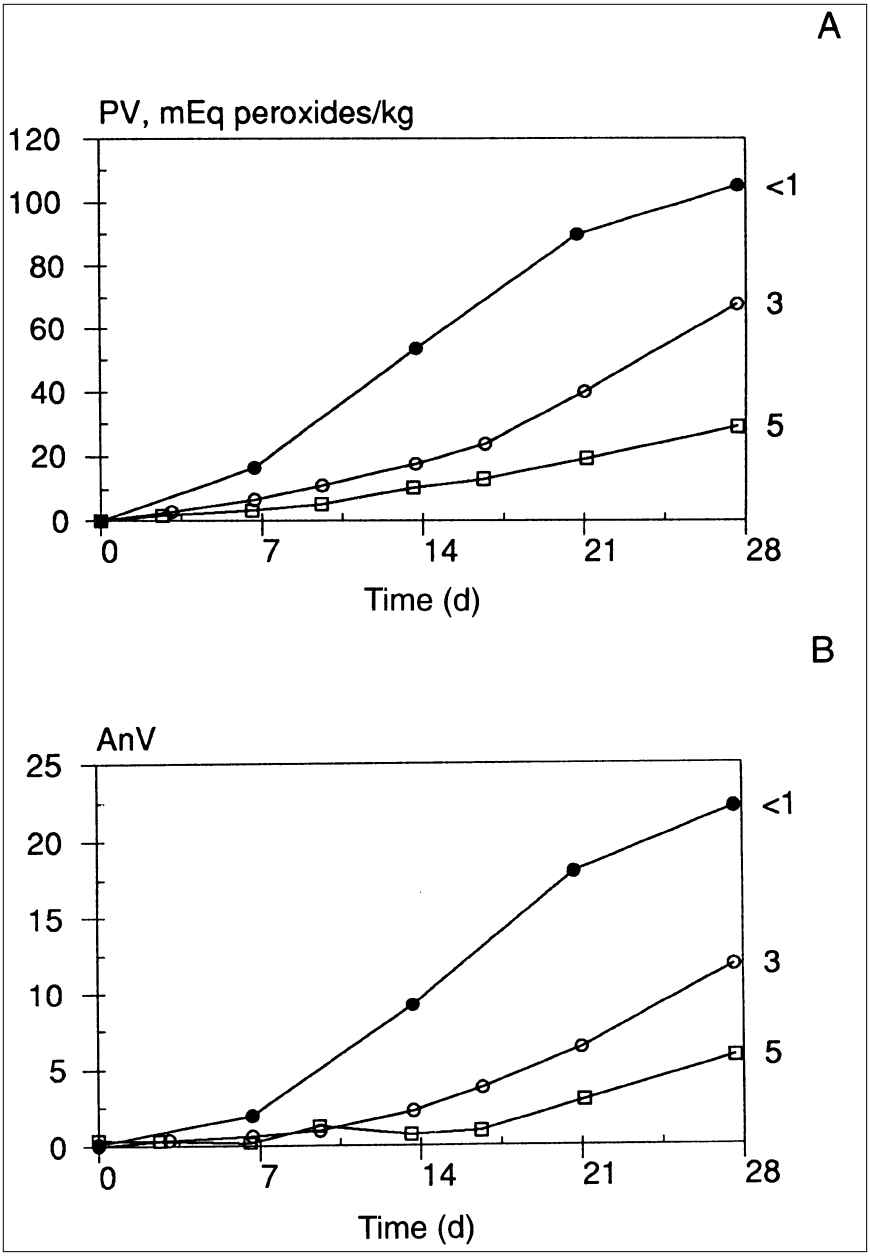

FIG. 2. Oxidation of 50% rapeseed oil triacylglycerol mixtures with various γ-tocopherol concentrations, measured by A) PV and B) AnV from pooled triacylglycerol samples in duplicate. ● <1 μg/g, ○ 3 μg/g and □ 5 μg/g of γ-tocopherol in triacylglycerols. See Figure 1 for abbreviations.

copherol varies from 250 to 500 μg/g in stripped corn oil (3).

The importance of the background effect of trace amounts of γ-tocopherol in the TAG is shown in Figure 4, where the oxidation of three highly different materials with 50–100% RO TAG were unexpectedly similar. In the RO TAG only 3.5% of all tocopherol isomers in the original RO was enough to retard oxidation to the same level as 1.5% in the 75% RO TAG and 0.4% in the 50% RO TAG.

Because purified TAG are more susceptible to oxidation upon removal of nearly all tocopherols, the effects of pro- and antioxidants as well as experimental conditions, are emphasized. It is not only in pro- and antioxidant studies that the effects of residual amounts of tocopherols could lead to misinterpretation of the data. For example, the effect of position of unsaturated acyls in TAG has been studied by interesterifying the TAG. In some studies, interesterification has increased stability (24) and in some studies it was decreased (24–26). Park and his co-workers (25) suggested that the interesterification process might destroy toco-

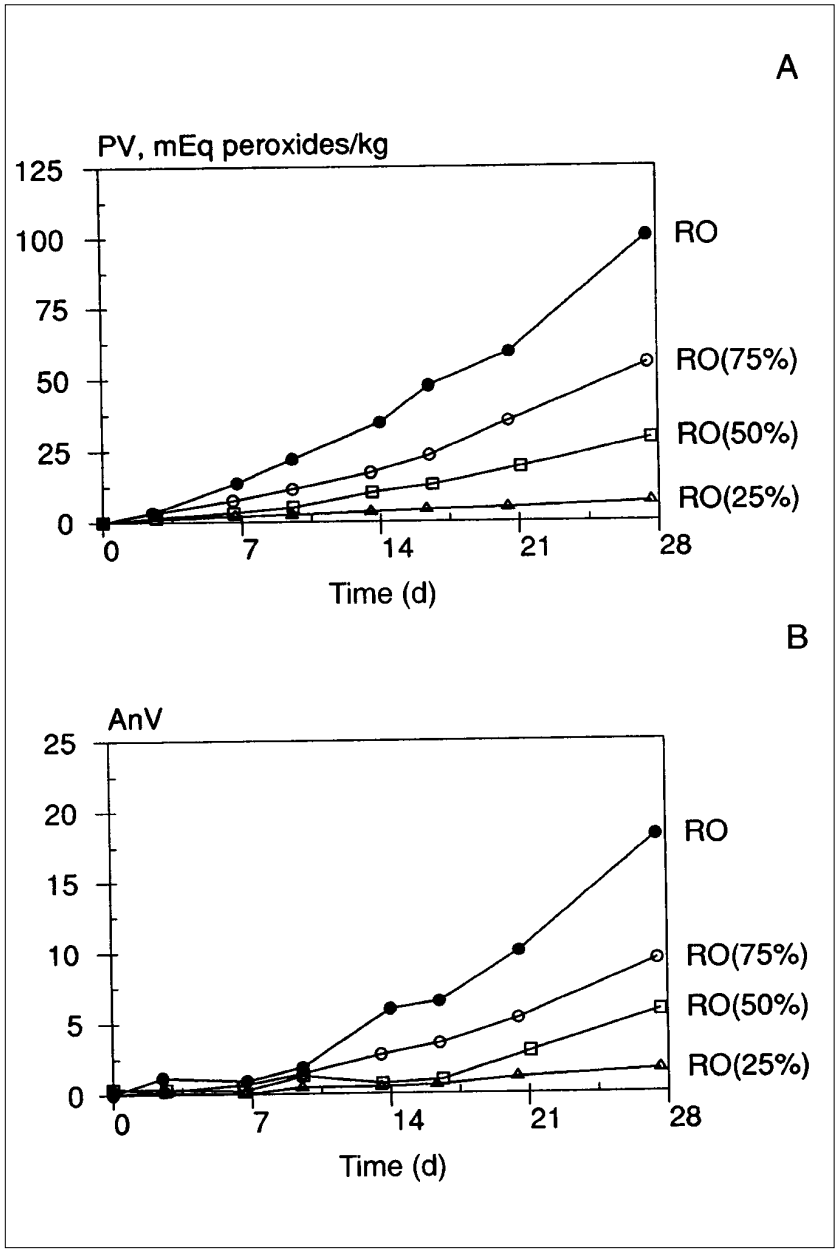

FIG. 3. Oxidation of natural triacylglycerols with various concentrations of rapeseed oil (RO) triacylglycerol measured by A) PV and B) AnV from pooled triacylglycerol samples in duplicate. ● 100% RO triacylglycerol with 11 μg/g of γ-tocopherol, ○ 75% RO triacylglycerol with 11 μg/g of γ-tocopherol, □ 50% RO triacylglycerol with 5 μg/g of γ-tocopherol, and △ 25% RO triacylglycerol with 10 μg/g of γ-tocopherol in triacylglycerols. See Figure 1 for other abbreviations.

pherols, which could have a bigger effect on oxidation than the interesterification *per se*.

Proper characterization of the TAG used for oxidation studies is of great importance. Otherwise, trace amounts of pro-oxidative or antioxidative factors may lead to misinterpretation of the results. They may also cause disagreements between different studies concerning the influence of the same pro- or antioxidative factor.

REFERENCES

1. Burton, G.W., and K.U. Ingold, Autoxidation of Biological Molecules. 1. The Antioxidant Activity of Vitamin E and Related Chain-Breaking Phenolic Antioxidants *in vitro*, *J. Am. Chem. Soc. 103*:6472–6577 (1981).
2. Kamal-Eldin, A., and L.-Å. Appelqvist, The Chemistry and Antioxidant Properties of Tocopherols and Tocotrienols, *Lipids 31*:671–701 (1996).
3. Huang, S.-W., E.N. Frankel, and J.B. German, Antioxidant Ac-

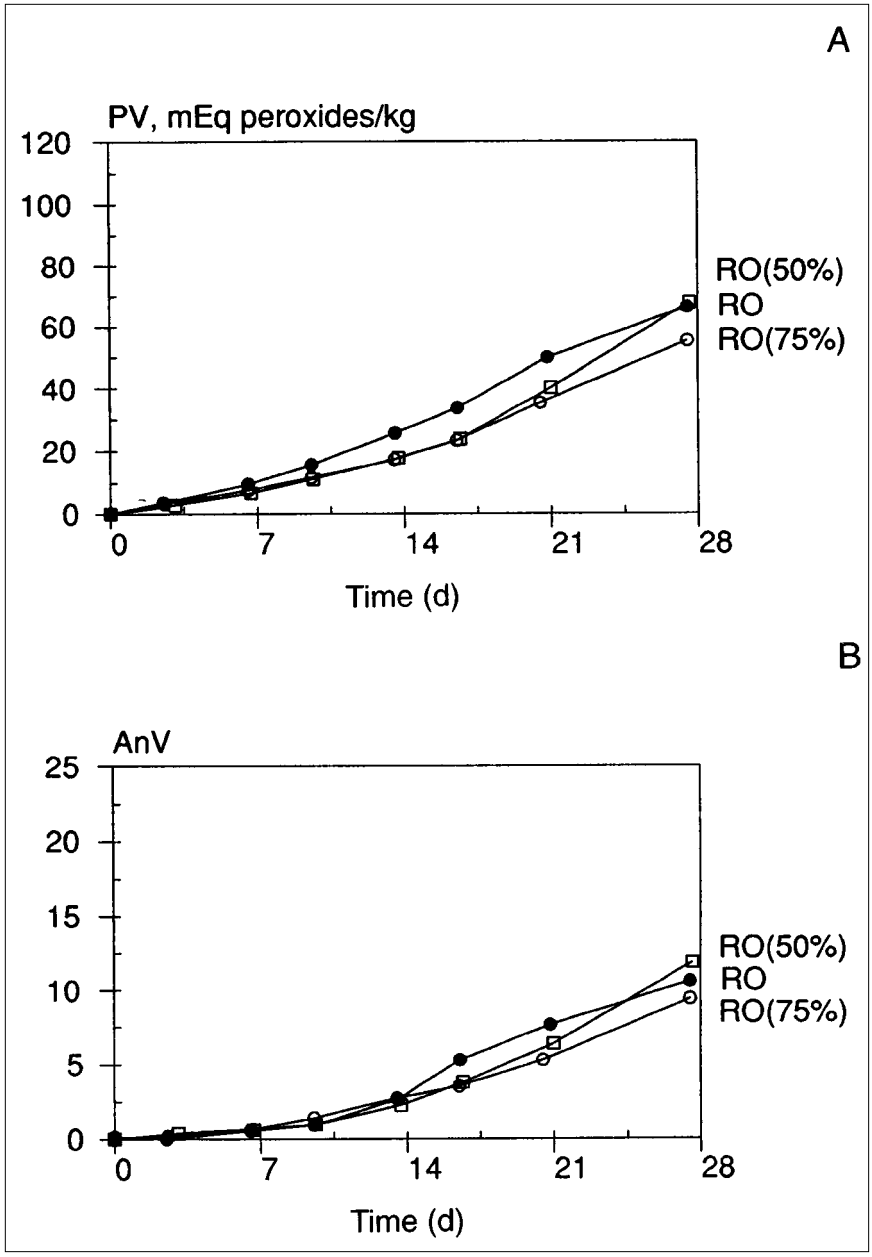

FIG. 4. Oxidation of three triacylglycerols with various concentrations of RO triacylglycerol and γ-tocopherol measured by A) PV and B) AnV from pooled triacylglycerol samples in duplicate. ● 100% RO triacylglycerol with 24 μg/g of γ-tocopherol, ○ 75% RO triacylglycerol with 11 μg/g of γ-tocopherol, and □ 50% RO triacylglycerol with 3 μg/g of γ-tocopherol. See Figures 1 and 3 for abbreviations.

tivity of α- and γ-Tocopherols in Bulk Oils and in Oil-in-Water Emulsions, *J. Agric. Food Chem. 42*:2108–2114 (1994).
4. Huang, S.-W., A. Hopia, K. Schwarz, E.N. Frankel, and J.B. German, Antioxidant Activity of γ-Tocopherol and Trolox in Different Lipid Substrates: Bulk Oils vs. in Oil-in-Water Emulsions, *Ibid. 44*:444–452 (1996).
5. Blekas, G., M. Tsimidou, and D. Boskou, Contribution of γ-Tocopherol to Olive Oil Stability, *Food Chem. 52*:289–294 (1995).
6. Satue, M.T., S.-W. Huang, and E.N. Frankel, Effect of Natural Antioxidants in Virgin Olive Oil on Oxidative Stability of Refined, Bleached, and Deodorized Olive Oil, *J. Am. Oil Chem. Soc. 72*:1131–1137 (1995).
7. Koskas, J.P., J. Cillard, and P. Cillard, Autoxidation of Linoleic Acid and Behavior of Its Hydroperoxides with and without Tocopherols, *Ibid. 61*:1466–1469 (1984).
8. Marinova, E.M., and N.V. Yanishlieva, Effect of Temperature on the Antioxidative Action of Inhibitors in Lipid Autoxidation, *J. Sci. Food Agric. 60*:313–318 (1992).
9. Cosgrove, J.P., D.F. Church, and W.A. Pryor, The Kinetics of the Autoxidation of Polyunsaturated Fatty Acids, *Lipids 22*:299–304 (1987).

10. Frankel, E.N., E. Selke, W.E. Neff, and K. Miyashita, Autoxidation of Polyunsaturated Triacylglycerols. IV. Volatile Decomposition Products from Triacylglycerols Containing Linoleate and Linolenate, *Ibid. 27*:442–446 (1992).
11. Neff, W.E., T.L. Mounts, W.M. Rinsch, H. Konishi, and M.A. El-Agaimy, Oxidative Stability of Purified Canola Oil Triacylglycerols with Altered Fatty Acid Compositions as Affected by Triacylglycerol Composition and Structure, *J. Am. Oil Chem. Soc. 71*:1101–1109 (1994).
12. Fakourelis, N., E.C. Lee, and D.B. Min, Effects of Chlorophyll and β-Carotene on the Oxidation Stability of Olive Oil, *J. Food Sci. 52*:234–235 (1987).
13. Min, D.B., and E.C. Lee, Factors Affecting Singlet Oxygen Oxidation of Soybean Oil, edited by G. Charalambous, *Frontiers of Flavor, Proceedings of the 5th international Flavor Conference*, Porto Karras, Greece, July 1–3, 1987. Elsevier Science Publishers, Amsterdam, 1988, pp. 473–498.
14. Lampi, A.-M., A. Hopia, P. Ekholm, and V. Piironen, Method for the Preparation of Triacylglycerol Fractions from Rapeseed and Other Oils for Autoxidation Studies, *Food Sci. Technol. 25*:386–388 (1992).
15. Boki, K., T. Wada, and S. Ohno, Effects of Filtration Through Activated Carbons on Peroxide, Thiobarbituric Acid, and Carbonyl Values of Autoxidized Soybean Oil, *J. Am. Oil Chem. Soc. 68*:561–565 (1991).
16. Huang, S.-H., E.N. Frankel, and J.B. German, Effects of Individual Tocopherols and Tocopherol Mixtures on the Oxidative Stability of Corn Oil Triglycerides, *J. Agric. Food Chem. 43*:2345–2350 (1995).
17. Hyvönen, L., A.-M. Lampi, P. Varo, and P. Koivistoinen, Fatty Acid Analysis, TAG Equivalents as Net Fat Value, and Nutritional Attributes of Commercial Fats and Oils, *J. Food. Comp. Anal. 6*:24-40 (1993).
18. Hopia, A., A.-M. Lampi, V.I. Piironen, L.E.T. Hyvönen, and P.E. Koivistoinen, Application of High-Performance Size-Exclusion Chromatography to Study the Autoxidation of Unsaturated Triacylglycerols, *J. Am. Oil Chem. Soc. 70*:779–784 (1993).
19. Determination of Peroxide Value, British Standard Institution, London, 1976, Bs 684:Section 2.14.
20. AOCS Official Method Cd 18-90 *p*-Anisidine Value, *Official Methods and Recommended Practices of the AOCS*, 4th edn., American Oil Chemists' Society, Champaign, 1990.
21. Minkkinen, P., Monitoring the Precision of Routine Analyses by Using Duplicate Determinations, *Anal. Chim. Acta. 191*:369–376 (1986).
22. Gottstein, T., and W. Grosch, Model Study of Different Antioxidant Properties of α- and γ-Tocopherol in Fats, *Fat Sci. Technol. 92*:139–144 (1990).
23. Yoshida, H., N. Hirooka, and G. Kajimoto, Microwave Heating Effects of Relative Stabilities of Tocopherols in Oils, *J. Food Sci. 56*:1042–1046 (1991).
24. Kimito, H., Y. Endo, and K. Fujimoto, Influence of Interesterification on the Oxidative Stability of Marine Oil Triacylglycerols, *J. Am. Oil Chem. Soc. 71*:469–473 (1994).
25. Park, D.K., J. Terao, and S. Matsushita, Influence of Interesterification on the Autoxidative Stability of Vegetable Oils, *Agric. Biol. Chem. 47*:121–123 (1983).
26. Neff, W.E., M.A. El-Agaimy, and T.L. Mounts, Oxidative Stability of Blends and Interesterified Blends of Soybean Oil and Palm Olein, *J. Am. Oil Chem. Soc. 71*:1111–1116 (1994).

\

[Received August 26, 1996; accepted February 6, 1997]