Effect of α- and γ-Tocopherols on Thermal Polymerization of Purified High-Oleic Sunflower Triacylglycerols

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ABSTRACT: The antipolymerization effects of α- and γ-tocopherols were compared in model systems composed of purified high-oleic sunflower triacylglycerols at 180°C. γ-Tocopherol was much more effective as an antipolymerization inhibitor than α-tocopherol, partly due to lower oxidizability/disappearance. Purified triacylglycerols of sunflower, rapeseed, and high-oleic sunflower oils were less stable than their nonpurified forms containing tocopherols. Results confirmed that tocopherols *per se* can act as antipolymerization agents in high-oleic oils at frying temperatures. No synergism was observed when α- and γ-tocopherols were present together although larger amounts of residuals were left for both tocols. Results suggested that high-oleic/high-γ-tocopherol oils (such as high-oleic canola and high-oleic soybean oils) may provide better frying oils than high-oleic/high-α-tocopherol oils (such as high-oleic sunflower oil).
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KEY WORDS: Antioxidant, antipolymerization, high-oleic triacylglycerols, rapeseed oil, sunflower seed oil, thermooxidation, α-tocopherol, γ-tocopherol.

In response to public perception about saturated and *trans* fatty acids in frying oils and possible risks of coronary heart disease and myocardial infarction (1,2), several traditional oils [including sunflower (SFO), rapeseed (RSO)/canola, peanut] were modified to yield stable, high-oleic oils for frying purposes (3). Results from a European Union AAIR project confirmed the safety of using high-oleic sunflower oil (HOSO) as an alternative to palm and partially hydrogenated oils in industrial frying of potato chips and french fries (4).

Further developments in this area may be obtained through stabilization of high-oleic oils by the addition of antioxidants which makes them economically and nutritionally more desirable than saturated oils (5–7). Tocopherols are the most important natural antioxidants for vegetable oils (8,9). α- and γ-Tocopherols increased the oxidative stability of HOSO containing 70% oleic acid and 20% linoleic acid (5). γ-Tocopherol was much more effective than α-tocopherol, especially when added in a concentration range between 100 and 1000 ppm (5).

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The aim of the present study was to compare the antipolymerization effects of α- and γ-tocopherols in model systems composed of purified high-oleic sunflower triacylglycerols (p-HOSO) at 180°C. Purified triacylglycerols were used to eliminate any possible contributions from endogenous antioxidants. Differences in polymerization of SFO, RSO, and HOSO) and their purified triacylglycerols (p-SFO, p-RSO, and p-HOSO, respectively) were also studied. We further investigated the possibility of synergistic interactions between the two tocopherols and analyzed residual tocopherols to see if differences in their performance are due to differences in their rates of consumption.

MATERIALS AND METHODS

Materials and reagents. A sample of HOSO was obtained as a gift from Dr. Maha Misbah (Unilever Research, Vlaardingen, The Netherlands). Commercial samples of SFO and RSO were used, together with HOSO, to study the effects of oil purification on oil stability. The fatty acid (FA) composition, calculated iodine values, and initial tocopherol levels of the three oils are given in Table 1. Fatty acids were analyzed as methyl

TABLE 1
Fatty Acid Composition, Calculated Iodine Values (IV) and Tocopherol Levels (ppm) of the Oils Used in This Study

	Sunflower oil (SFO)	Rapeseed oil (RSO)	High-oleic sunflower oil (HOSO)
Fatty acids (relative wt%)			
Palmitic (16:0)	6.4	5.2	4.0
Stearic (18:0)	4.1	2.0	2.3
Oleic (18:1)	25.2	60.8	81.5
Linoleic (18:2)	62.7	22.4	10.1
Linolenic (18:3)	0.1	6.9	0.1
Others[a]	1.5	2.7	2.0
Calculated iodine value[b] (IV)	130.5	109.1	87.8
Tocopherol levels (ppm)			
α-Tocopherol	526	224	445
γ-Tocopherol	7	341	20

[a]Other minor fatty acids include: myristic, palmitoleic, arachidic, gadoleic, behenic, erucic, lignoceric, and 24:1.
[b]IV were calculated according to the following AOCS (Ref. 10) formula: IV = % 18:1 × 0.860 + % 18:2 × 1.732 + % 18:3 × 2.616.

ester derivatives by gas chromatography: column, NB-351 (25 m × 0.32 mm i.d., 0.2-mm film, Nordion Ltd., Helsinki, Finland), column temperature: 160°C (2 min), 4°C/min, 240°C (5 min), injector temperature: 240°C and flame-ionization detector temperature: 260°C. Alumina (neutral Al_2O_3, 70–120 mesh) used for column chromatography and the α-tocopherol standard (isomer kit, Art. 15496) were purchased from Merck (Darmstadt, Germany). *RRR* α- and *RRR* γ-tocopherols [95% stereochemically pure, high-performance liquid chromatography (HPLC)] were purchased in an isomer kit from Merck (Art. 15496). All other solvents and chemicals (of reagent grade or better quality) were obtained from local suppliers and were used without further purification.

Purification of vegetable oil triacylglycerols. Samples of HOSO, SFO, and RSO were purified from antioxidants and from trace metals and other prooxidants *via* adsorption chromatography using a glass column (40 × 2.5 cm i.d.) packed with 250 g of activated alumina (100°C for 8 h and then at 200°C for 12 h) suspended in *n*-hexane. The oil (100 mL) was dissolved in an equal volume of hexane and passed through the column which was then washed with 200 mL of *n*-hexane. The chromatographic column was wrapped with aluminum foil to prevent light-induced oxidations during the purification process, and triacylglycerols were collected in an aluminum foil-wrapped flask. Analysis of the purified oils by thin-layer chromatography showed that the purified oil only contained triacylglycerols and sterol esters. The oil was void of tocopherols (HPLC, <0.5 ppm) and peroxides (FeSCN colorimetric, <0.6 meq O_2/kg oil). Previous experiments, employing atomic absorption spectroscopy, showed that triacylglycerols prepared from SFO and RSO by this method were void of iron (<0.01 ppm) and copper (<0.001 ppm).

Thermooxidation experiments. Oil samples (1 g) with or without α- or γ-tocopherol were subjected to thermooxidation in 6-mL borosilicate glass vials (40 × 22 mm; Chromacol Ltd., London, United Kingdom) at 180 ± 5°C in a high-temperature oven. Vials were taken from the oven at specific time points and cooled in a dessicator (under reduced air) before samples were taken for analysis. Experiments were performed twice except for those comparing purified and nonpurified oils with each other. Each sample was incubated and analyzed in duplicate.

Analysis of the polymerized material. Portions of the thermooxidized oils obtained from the previous step were dissolved in tetrahydrofuran to a concentration of *ca.* 20 mg/mL. Total dimers and polymers were analyzed by high-performance size-exclusion chromatography (HPSEC) as described by Hopia *et al.* (10). Specifically, portions of the oxidized oils were dissolved in tetrahydrofuran to a concentration of *ca.* 20 mg/mL and analyzed in an HPSEC system consisting of an HPLC pump (Pharmacia LKB Biotechnology, Uppsala, Sweden), a Rheodyne injector fitted with a 20 μL sample loop (Cotati, CA), and a Waters refractive index detector (Waters Associates, Milford, MA) thermostated to 35°C. The separation was performed at 35°C on two 100- and 50-Å columns (PLGEL, 30 cm × 0.8 cm i.d.; Polymers Laboratories Inc., Amherst, MA) connected in series. The mobile phase was HPLC-grade tetrahydrofuran (Merck) stabilized with 0.025% butylated hydroxytoluene and was used at a flow rate of 0.8 mL/min. Peaks were recorded and integrated using the JCL 6000 chromatography data system (Jones Chromatography, Mid-Glamorgan, United Kingdom), and quantitation of each lipid class was based on peak areas assuming equal detector response. The precision of duplicate determinations of polymerized material from two samples in each experiment was calculated as the maximal relative random error at the 95% level (11). It was 16.2% when the total of dimers and polymers (%) was ≥1.0%.

Analysis of residual tocopherols. The amount of residual α- and γ-tocopherols in thermooxidized oils was quantified by HPLC analysis on a Hibar prepacked LiChrosorb NH_2 column (25 cm × 4 mm i.d., particle size 5 mm; Merck). The mobile phase was *n*-heptane/tertiary butylmethyl ether/tetrahydrofuran/methanol (79:20:1:0.1, by vol) at a flow rate of 1.0 mL/min (12). The system consisted of an HPLC pump (Pharmacia LKB Biotechnology, Uppsala, Sweden), a 10-μL injection loop, and a Merck F-1050 Fluorescence spectrophotometer. The peaks were detected at an excitation wavelength of 295 nm and an emission wavelength of 320 nm. Peaks were recorded and integrated using the JCL 6000 chromatography data system, and the amounts of α- and γ-tocopherols were quantified against references used as external standards. The precisions of duplicate determinations of α- and γ-tocopherols were calculated as those of total dimers and polymers and were 24.6 and 28.3% maximum relative random error at ≥10 ppm, respectively (11).

Statistical analysis. The effects of tocopherols on the polymerization and on the stability of tocopherols were statistically analyzed by comparing two groups at a time by *t*-tests performed by Survo software (13).

RESULTS AND DISCUSSION

Only a few literature reports exist about the effects of tocopherols in protecting frying oils and in extending the shelf life of fried foods against oxidative destruction (5,14–17). Previously, we found that the addition of sesame and/or rice bran oil to HOSO increases its performance as a frying oil (6). In this study, we compared the effects of α- and γ-tocopherols (at 500 ppm level) on the stabilization of HOSO during 24 h of continuous heating at 180°C. Figure 1 shows that both α- and γ-tocopherols completely inhibited the polymerization of HOSO until the tocopherols were almost totally consumed. Significantly ($P < 0.05$) more dimers and polymers in samples with tocopherols existed than without tocopherols after 6 and 9 h of heating. These results are in agreement with earlier research showing that tocopherols are consumed rapidly during thermal oxidation and that their destruction is followed by rapid thermoxidation of triacylglycerols (14,17). γ-Tocopherol was more effective as an antipolymerization agent than α-tocopherol. Significantly ($P < 0.05$) more dimers and polymers with α- than with γ-tocopherol existed after 3 and

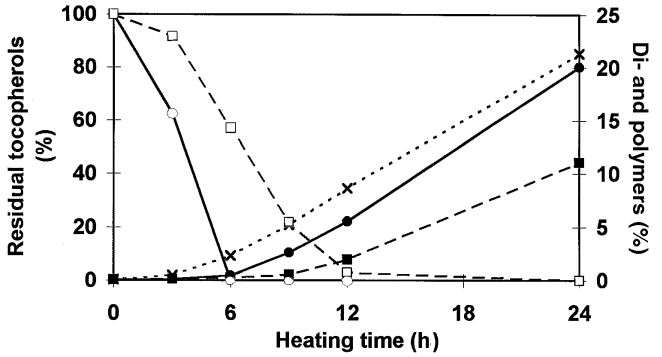

FIG. 1. Effects of the addition of 500 ppm of α- or γ-tocopherol on the thermooxidation of purified high-oleic sunflower triacylglycerols at 180°C. Left-hand y-axis shows the percentages of residual α- (—○—) or γ- (– –□– –) tocopherols and right-hand y-axis shows the percentages of total triacylglycerol dimers and polymers in control samples (····x····) and in samples containing 500 ppm of α- (—●—) or γ- (– –■– –) tocopherols. Results are expressed as means of two repeated thermooxidation experiments.

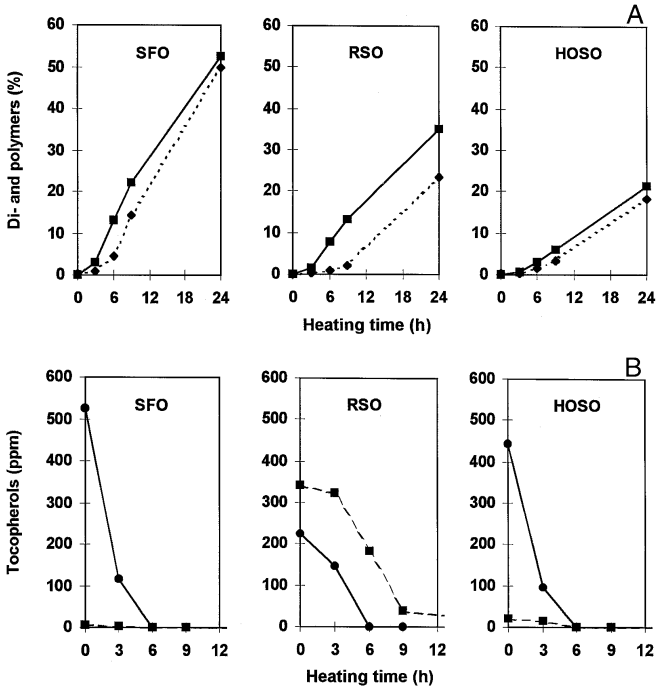

FIG. 2. Thermooxidative (180 ± 5°C) polymerization and tocopherol depletion in commercial sunflower (SFO), rapeseed (RSO) and high-oleic sunflower (HOSO) oils in one experiment. (A) The percentages of total dimers and polymers in natural oils (····◆····) and in their purified triacylglycerols (—■—), and (B) the percentages of residual α- (—●—) and γ- (– –■– –) tocopherols in the commercial oils.

6 h of heating. This effect may be partly due to the markedly higher stability of γ-tocopherol which disappeared at 12 h of heating compared to α-tocopherol which disappeared at 6 h of heating (Fig. 1). The finding that γ-tocopherol was more stable than α-tocopherol agrees with previous results in which thermodecomposition of tocopherols followed the order α-tocopherol > β-tocopherol ≈ γ-tocopherol > δ-tocopherol (18) and also with our previous results that α-tocopherol was consumed faster than γ-tocopherol in purified sunflower triacylglycerols oxidized at 55°C (19). Thus, γ-tocopherol is a better polymerization inhibitor than α-tocopherol during the thermooxidation of oils.

In the next experiment, polymerization and tocopherol consumption were measured in refined SFO, RSO, and HOSO. The stabilities of these oils were compared to the stabilities of their purified triacylglycerols containing no tocopherols. Results shown in Figure 2A demonstrate that the stability of the oils was in the order HOSO > RSO > SFO and that purified oils were less stable than their nonpurified oils. This difference in stability illustrates the importance of tocopherols and other antioxidants that might be present in the unsaponifiable fractions of these oils. Previously, in the sensory comparison of margarines prepared from RSO and SFO with regard to the off-flavor produced during frying or baking, the rapeseed margarine was judged to have significantly less off-flavor and rancidity than the sunflower margarine, and the difference in off-flavor increased with an increase in temperature (20). Results presented in this paper suggest that for the stability of frying oils, the FA composition is more important than the presence of minor antioxidants, in contrast to oxidation at low temperatures where antioxidant components are more important than FA composition (19). The contribution of tocopherols to the stability of oils can be appreciated by considering the times for tocopherol destruction shown in Figure 2B. The finding that the difference in stability between RSO and p-RSO was much greater than the difference in stability between SFO and p-SFO and that between HOSO and p-HOSO is very interesting and may be attributed to the presence of higher amounts of γ-tocopherol and/or to possible occurrence of other antioxidants/synergists in RSO.

Since α- and γ-tocopherols occur together in vegetable oils, an investigation was warranted to determine if mixtures of the two tocols would show any synergistic interaction in purified HOSO triacylglycerols. p-HOSO samples were fortified with 250 and 500 ppm of either α- or γ-tocopherol separately or in mixtures (Table 2). The effects of tocopherols were similar in both experiments, although in one of them, samples thermooxidized slightly more than in the other one. For example, total dimer and polymer content of p-HOSO reached 19.6 and 21.4%, respectively. Results from these experiments revealed the following: (i) the rate of polymerization was significantly reduced by the addition of tocopherols at all heating times ($P < 0.05$), (ii) the degree of protection tended to increase with increasing tocopherol concentration, but the effect was not correlated to the concentration and was not statistically significant, and (iii) γ-tocopherol was more effective than α-tocopherol at both concentrations tested (250 and 500 ppm) having a significant difference at 6 h of heating ($P < 0.05$). The experiment further showed that addition of α-tocopherol (at 250 or 500 ppm) to p-HOSO samples containing 250 or 500 ppm of γ-tocopherol did not improve their stability toward polymerization although it increased the percentage of residual α- and γ-tocopherols at 6 h. The finding that the degree of polymerization was equal for p-HOSO samples containing 250 ppm γ-tocopherol, 250 ppm γ-tocopherol

TABLE 2
Effect of Individual and Mixed α- (α-T) and γ-Tocopherols (γ-T) on Thermopolymerization of HOSO[a]

Measured parameter[b]	Total dimers + polymers (%)			Residual α-T (%)		Residual γ-T (%)		
Heating time (h)	6	12	24	6	12	6	12	24
p-HOSO	3.1	9.0	20.5	—	—	—	—	—
p-HOSO + 250 ppm α-T	0.4	4.4	16.9	1.5	0	—	—	—
p-HOSO + 500 ppm α-T	0.3	3.5	15.9	12.9	0	—	—	—
p-HOSO + 250 ppm γ-T	0.2	1.9	13.8	—	—	31.1	0	0
p-HOSO + 250 ppm γ-T + 250 ppm α-T	0.4	1.6	12.5	2.8	0	56.6	0	0
p-HOSO + 250 ppm γ-T + 500 ppm α-T	0.3	1.4	13.1	20.2	0	80.3	0	0
p-HOSO + 500 ppm γ-T	0.2	0.8	11.0	—	—	54.5	2.9	0
p-HOSO + 500 ppm γ-T + 250 ppm α-T	0.3	0.9	11.2	6.2	0	71.3	2.5	0
p-HOSO + 500 ppm γ-T + 500 ppm α-T	0.3	1.0	11.0	20.8	0	76.1	6.1	0

[a]Abbreviations: p-HOSO = purified high-oleic sunflower triacylglycerols (see the Materials and Methods section), α-T = α-tocopherol, γ-T = γ-tocopherol.
[b]Measured parameters: total dimers + polymers (%) = percentage of total triacylglycerol dimers and polymers in thermooxidized HOSO; residual α-T (%) = percentage of α-tocopherol remaining after thermooxidation; and residual γ-T (%) = percentage of γ-tocopherol remaining after thermooxidation. Results are expressed as means of two repeated thermooxidation experiments.

+ 250 ppm α-tocopherol and 250 ppm γ-tocopherol + 500 ppm α-tocopherol and for p-HOSO samples containing 500 ppm γ-tocopherol, 500 ppm γ-tocopherol + 250 ppm α-tocopherol, or 500 ppm γ-tocopherol + 500 ppm α-tocopherol, despite the sparing effects of mixing on residual tocopherols, suggests that the difference in protective effects between the two tocopherols is due not only to the slower destruction of γ-tocopherol and that other factors, e.g., the oxidation products of the two tocopherols and their interaction with oxidizing FA, might be involved. Previous work in our laboratory on purified sunflower triacylglycerols suggested that the low effectiveness of α-tocopherol compared to γ-tocopherol is possibly a result of α-tocopherol being consumed not only in antioxidation reactions but also in different side reactions (19). When used to stabilize purified sunflower triacylglycerols at 55°C, very weak synergism was observed for mixtures containing 100 ppm each of α- and γ-tocopherol but not for mixtures containing higher tocopherol concentrations (19). The lack of synergism between the two tocols observed in the current and the previous study (19) is supported by the small difference in redox potentials which gives α-tocopherol a negligible chance to act as a regenerator for γ-tocopherol. An additional reason for the lack of synergism stems from the fact that the two tocopherols act by the same basic mechanism to scavenge free radical species. Other antioxidants, e.g., phospholipids (21) and polyphenols (22), inhibited tocopherol destruction *via* other mechanisms including metal chelation.

With the growing production of different high-oleic oils, the results from this study may have important implications. The finding that γ-tocopherol is a better protector for oils than α-tocopherol at high temperature use has great implications for the economical use of antioxidants as stabilizers for high-oleic oils, which suggest that high-oleic/high-γ-tocopherol oils such as high-oleic canola may be better frying oils than high-oleic/high-α-tocopherol oils such as HOSO. High-oleic soybean varieties may be particularly interesting since they contain considerable amounts of δ-tocopherol which is even more stable than γ-tocopherol (8). Further implications of these results come from the finding that polymerization of oils at frying temperatures increases rapidly upon the consumption of tocopherols. This finding may be of economic importance for continuous industrial frying operations. Particularly interesting was the finding that stabilization was not correlated with tocopherol concentration since the addition of 500 ppm of α- or γ-tocopherol did not provide better stabilization effects than those obtained by adding 250 ppm of these tocopherols (Table 2). The study of the effect of oil replenishment before total tocopherol consumption on the rate and degree of polymerization in actual frying and in model systems would be interesting. Furthermore, studies on the interactions between tocopherols and other frying oil stabilizers including trace-metal chelators (e.g., phospholipids and some phenolic compounds), tocopherol regenerators (e.g., ascorbyl palmitate), or oxidation inhibitors (e.g., silicone oil) in model and real frying experiments are highly warranted.

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