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Effects on the Flavor and Oxidative Stability of Stripped Soybean and Sunflower Oils with Added Pure Tocopherols

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Effects of tocopherols on the oxidative stability of stripped vegetable oils were studied by adding pure tocopherols—α, β, γ, and δ—in their naturally occurring proportions in soybean and sunflower oils to the triacylglycerols (TAG) of soybean and sunflower oils. Soybean and sunflower oils were purified by stripping all minor constituents, leaving the triacylglycerols. Pure tocopherols in the proportion typical of sunflower oil—high α, low γ, and low δ—were added to purified sunflower oil and to purified soybean oil. Pure tocopherols in the proportion typical of soybean oil—low α, high γ, and high δ—were added to the purified oils. Oils were subjected to accelerated autoxidation using oven storage at 60 °C in the dark and accelerated photooxidation at 7500 lx light intensity at 30 °C. Oxidation levels of aged oils were measured by the formation of both peroxides and volatile compounds and by flavor analysis. Results from substituting the tocopherol profile from one oil type to another varied on the basis of whether they were oxidized in the dark or in the light. For example, during autoxidation in the dark, soybean oil with the typical soybean tocopherol profile had the lowest levels of peroxides and total volatile compounds, whereas sunflower oil with the sunflower tocopherol profile had the highest levels. In flavor analyses of the same oils, sunflower oil with the soybean tocopherol profile was the most stable. Soybean oil with the profile of sunflower tocopherols was the least stable in dark oxidation. In contrast to the data from autoxidation in the dark, addition of tocopherols typical of sunflower oil significantly improved light stability of both oil types compared to the addition of soybean tocopherols to sunflower oil. The tocopherol profile typical of soybean oil was significantly more effective in inhibiting autoxidation in the dark; however, the tocopherol profile typical of sunflower oil inhibited light oxidation significantly more than the soybean tocopherol profile.

KEYWORDS: α-Tocopherol; δ-tocopherol; γ-tocopherol; flavor; oxidation; oxidative stability; soybean oil; sunflower oil; tocopherol; volatile compounds

INTRODUCTION

Lipid oxidation, a major cause of flavor deterioration in oils and oil-containing foods, can be inhibited by various factors such as types of fatty acids; endogenous minor oil constituents such as sterols, tocopherols, and pigments; and additives including antioxidants and metal chelators. The fatty acid composition of oil is well-known to affect the quality and stability of oils, but it does not account for oxidative stability entirely. Zambiazi suggested that only about half of the stability of oil can be explained by fatty acid composition (*1*). The relative contribution of the minor oil constituents to oxidative stability is not known.

Tocopherols are well recognized as effective antioxidants both endogenously and as additives. Jung reported that optimum concentrations of tocopherol homologues were 100, 250, and 500 ppm for α-, γ-, and δ-tocopherols, respectively (*2*). However, if these compounds are in too high concentrations, they can act as pro-oxidants. For example, Satue et al. (*3*) found that if α-tocopherol was >250 ppm in olive oil, it acted as a pro-oxidant as measured by peroxide values. In addition, Jung found that 500 ppm of added α-tocopherol in soybean oil was a pro-oxidant by peroxide formation (*2*). The difference between these two findings might be due to differences in naturally occurring antioxidants and/or in fatty acid compositions of the oils used. Other researchers have also reported that fatty acid composition may not be the only determinant of oil quality (*4*, *5*).

In previous oil stability tests, we found that soybean oil was more oxidatively stable in dark oxidation than sunflower oil, although the soy oil contained 8−9% of the highly unstable linolenic acid, whereas sunflower oil had no linolenic acid (*6*). In contrast, sunflower oil had greater light stability than soybean oil (*6*). The susceptibility of soybean oil to photo-oxidation is well-known (*7*−*9*). Presumably, the factors influencing susceptibility to photo-oxidation and autoxidation extend beyond just fatty acid composition. Soybean and sunflower oils have widely different tocopherol profiles. Soybean oil is low in α-tocopherol

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Table 1. Compositions of Soybean and Sunflower Oils before Stripping

	soybean oil	sunflower oil
	Percent Fatty Acid Composition	
C16:0	12	7
C18:0	4	5
C18:1	24	14
C18:2	53	74
C18:3	7	0
	Parts per Million Tocopherol Composition	
α	120	610
β	10	10
γ	610	30
δ	260	10

(120 ppm) and high in γ- (610 ppm) and δ-tocopherols (260 ppm); sunflower oil is high in α-tocopherol (610 ppm) and low in γ- (30 ppm) and δ-tocopherols (10 ppm). To determine if these differences in tocopherol profiles may help explain variations between light and dark stabilities of sunflower and soybean oils, the effects of α-, γ-, and δ-tocopherols were studied by adding the tocopherol profile typical of soybean oil to purified sunflower oil and soybean oil and the tocopherol profile typical of sunflower oil to purified soybean oil and sunflower oil.

MATERIALS AND METHODS

Materials. Refined, bleached, and deodorized soybean and sunflower oils were obtained from commercial sources with only citric acid as an additive. α-, β-, γ-, and δ-tocopherols at 99% purity were purchased from Matreya, Inc. (Pleasant Gap, PA).

Oil Purifying/Stripping. To purify the oils and remove all minor oil constituents, oils were stripped of naturally occurring compounds such as pigments, tocopherols, and sterols by first mixing 100 g of oil with a 1 L mixture of 95:5 ethyl ether and MeOH. The solvents/oil mixture was then passed through a 60 cm (height) × 10 cm (diameter) glass column packed with 80% activated alumina, 10% Celite, and 10% carbon black. Solvents were removed from the oil by rotoevaporation after the solvents/oil mixture was eluted through the column. This process was repeated five times with fresh column packing and solvents each time. Oils were deodorized under laboratory conditions at 220 °C for 3 h as previously described (*10*) and held at −20 °C until needed for oxidation tests. No additives were added to the oil. The purity of the oil was tested by thin-layer chromatography.

Fatty Acid Compositions. Fatty acid compositions of the oils were determined before and after stripping by capillary gas chromatographic (GC) analysis with a Hewlett-Packard 5890 GC (Wilmington, DE) equipped with an SP2330 column (30 m, 0.20 mm i.d., 0.20 μm film thickness) (Supelco, Bellefonte, PA). The column temperature was held at 190 °C for 5 min and programmed to 230 °C at 20 °C/min. Other GC conditions were as follows: injector temperature, 250 °C; flame ionization detector temperature, 260 °C.

Addition of Pure Tocopherols. Pure tocopherols (α, β, γ, and δ) were added to the purified soybean oil in the proportions typical of soybean oil and sunflower oil (**Table 1**). The levels of tocopherols added were determined from tocopherol analysis of the original oils. The process of adding pure tocopherols was repeated with the addition of tocopherol levels characteristic of soybean and of sunflower oil to purified sunflower oils.

Analysis of Tocopherols. Stripped oils were analyzed for the presence of residual tocopherols. These oils were also analyzed for levels of tocopherols after the addition of pure tocopherols. Tocopherols were measured by high-performance liquid chromatography (HPLC) (Varian, Palo Alto, CA) using a NH_2 column with 98:2 hexane/2-propanol and a fluorescence detector set at 298 nm excitation and 345 nm emission.

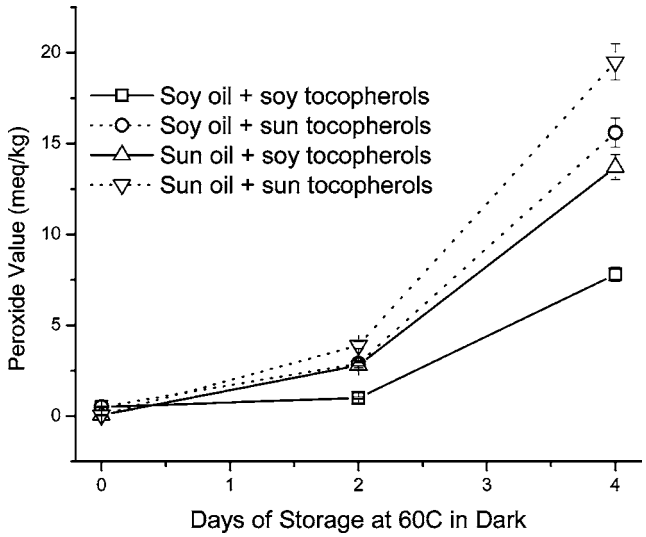

Figure 1. Peroxide values of purified soybean and sunflower oils containing pure tocopherols with profiles typical of soybean oil or sunflower oil and aged for 0, 2, and 4 days at 60 °C in the dark.

Oxidation Tests. Oils were oxidized under accelerated light and temperature conditions in duplicate. Oils were aged according to AOCS oven storage method Cg 5-97 (*11*) at 60 °C for 0, 4, and 8 days in the dark with air in the headspace of the storage container. Oils were aged in the light at 30 °C and 7500 lx with air in the headspace of the storage container using AOCS light stability method Cg 6-01 (*11*). A light intensity of 7500 lx is ∼10× that of ambient room lighting intensity.

Measurement of Oxidation Levels. Oxidation levels of all fresh and aged oils were determined by peroxide value (PV) (AOCS method Cd 8-53) (*11*) and by gas chromatographic volatile compound analysis using static headspace capillary gas chromatography with flame ionization detection (AOCS method Cg 4-94) (*11*). All volatile compounds eluting in a range from 2-propenal to 2,4-decadienal were included to determine total volatiles. A 14-member trained, experienced oil panel evaluated the fresh and aged oils for overall flavor intensity (*12*) (AOCS method Cg 2-83) (*11*). All sensory evaluations were conducted in a panel room with individual booths, temperature control, and red lighting. All data are results of two trials.

Statistical Analysis. Data were evaluated by analysis of variance on duplicate runs of the oxidation tests and duplicate analysis of oxidation levels (*13*). Statistical significance was expressed at the $P < 0.05$ level unless otherwise indicated.

RESULTS AND DISCUSSION

Oil Compositions. The soybean and sunflower oils were analyzed for fatty acid composition before and after stripping of minor constituents. Data in **Table 1** show fatty acid compositions typical of regular soybean and sunflower oils before stripping. Analysis of fatty acids after stripping varied by <1% from the levels in the unpurified oils (data not shown). The tocopherol homologues in the oils before stripping showed 660 ppm of total tocopherols in sunflower oil and 1000 ppm in soybean oil (**Table 1**).

Oxidative Stability of Oils in the Dark. The substitution of the tocopherol profile of soybean oil into sunflower oil and that of sunflower oil into soybean oil significantly changed the stability of these oils as measured by PV (**Figure 1**). Peroxide values of the four oil/tocopherol combinations all had low initial PV of 0.4−0.5. After 2 and 4 days of storage in the dark, soybean oil with added soybean tocopherols had significantly lower PVs than the other three samples, whereas the sunflower oil with added sunflower tocopherols had significantly higher

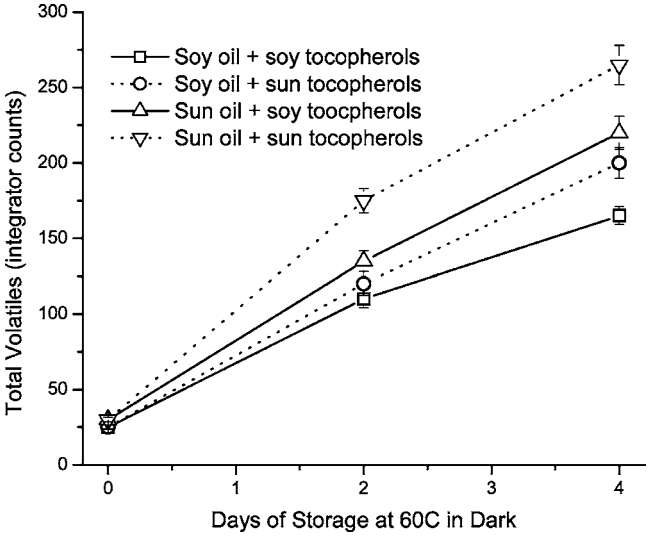

Figure 2. Total volatile compounds in purified soybean and sunflower oils containing pure tocopherols with profiles typical of soybean oil or sunflower oil and aged for 0, 2, and 4 days at 60 °C in the dark.

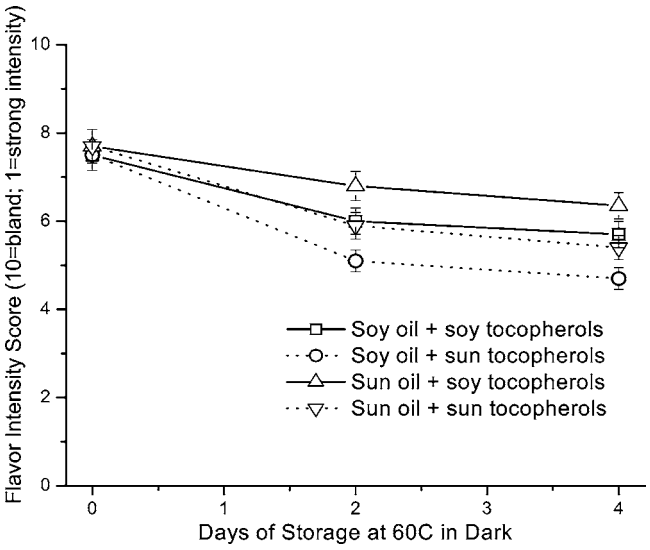

Figure 3. Flavor intensity scores for purified soybean and sunflower oils containing pure tocopherols with profiles typical of soybean oil or sunflower oil and aged for 0, 2, and 4 days at 60 °C in the dark.

PVs than the other three samples. The substitution of sunflower tocopherols into soybean oil significantly increased the PV at both 2 and 4 days compared to that of soybean oil with soybean tocopherols. In contrast, adding the soybean tocopherols into sunflower oil significantly decreased the PV for that oil compared with sunflower oil with sunflower tocopherols. The same positive effect of substituting soybean tocopherols into sunflower oil was also noted for total volatile compounds (**Figure 2**). Soybean oil was significantly less oxidatively stable as measured by volatile compound formation when sunflower tocopherols were added rather than soybean tocopherols.

Overall flavor intensity scores (**Figure 3**) showed similar effects from substituting the tocopherol profiles as in data from the PV and total volatile compounds analyses (**Figures 1** and **2**). Oils were evaluated for flavor intensity using the AOCS scale with 10 = bland and 1 = strong; therefore, the higher the score, the less flavor in the sample. In sunflower oil, at both 2 and 4 days of storage, substituting the natural tocopherol

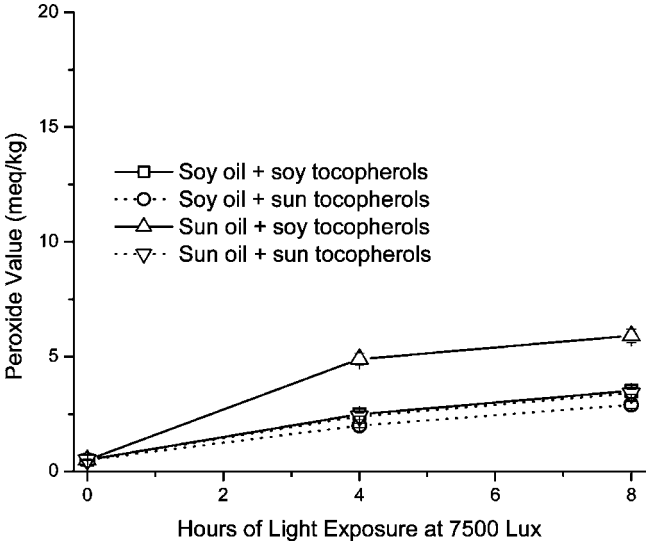

Figure 4. Peroxide values of purified soybean and sunflower oils containing pure tocopherols with profiles typical of soybean oil or sunflower oil and aged for 0, 4, and 8 h at 7500 lx and 30 °C.

proportion of sunflower oil with soybean tocopherols produced an oil with significantly less overall flavor intensity than the other oils. When the sunflower tocopherol profile was added to stripped soybean oil, the flavor intensity significantly increased compared to the soybean oil with soybean tocopherols. No significant differences were noted between soybean oil with soybean tocopherols and sunflower oil with sunflower tocopherols.

In dark oxidation, oils with the soybean tocopherol profile of low α, high γ, and high δ had significantly better stability than oils with the high α, low γ, and low δ proportion common to sunflower oil. The chemical structures of γ- and δ-tocopherols may in part explain their effectiveness in inhibiting dark oxidation. The literature cites many examples of the higher antioxidative capabilities of γ- and δ-tocopherols compared to α-tocopherol. Olcott and Van der Veen (*14*) reported that stability tests at 37 and 50 °C with either menhaden oil or squalene showed γ- and δ-tocopherols to be superior to α-tocopherol as evaluated by weight increases during oxidation. Yanishlieva and Marinova (*15*) reported that adding 0.05% (500 ppm) α-tocopherol to soybean oil did not improve its oxidative stability. Also, Warner et al. (*6*) compared the oxidative and flavor stabilities of soybean and sunflower oils at 60 °C in the dark and found that the soybean oils were significantly more stable than sunflower oils in three different samples of each oil type.

Photo-oxidation of Oils. In contrast to the effect of tocopherols under autoxidation conditions in the dark, the results of photo-oxidation studies showed that oils containing the soybean tocopherols had less light stability than oils containing the sunflower tocopherols. PV analysis showed that sunflower oil with soybean tocopherols had significantly less light stability than any of the other three samples in this test (**Figure 4**). Sunflower and soybean oils with sunflower oil tocopherols had significantly better light stability than the same oil types containing soybean tocopherols as measured by formation of total volatile compounds (**Figure 5**). Results of the sensory analysis of the oils exposed to light agreed with those from the volatile compound analysis (**Figure 6**). When sunflower tocopherols were added to either oil, flavor intensity scores were better than for oils with soybean tocopherols. These results agree

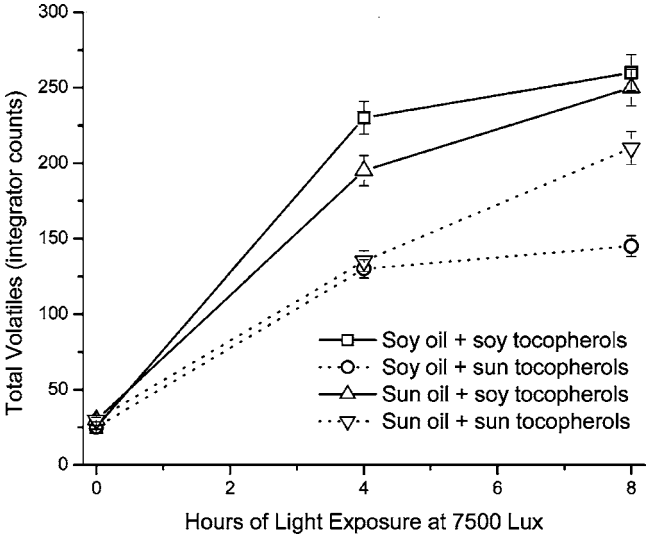

Figure 5. Total volatile compounds in purified soybean and sunflower oils containing pure tocopherols with profiles typical of soybean oil or sunflower oil and aged for 0, 4, and 8 h at 7500 lx and 30 °C.

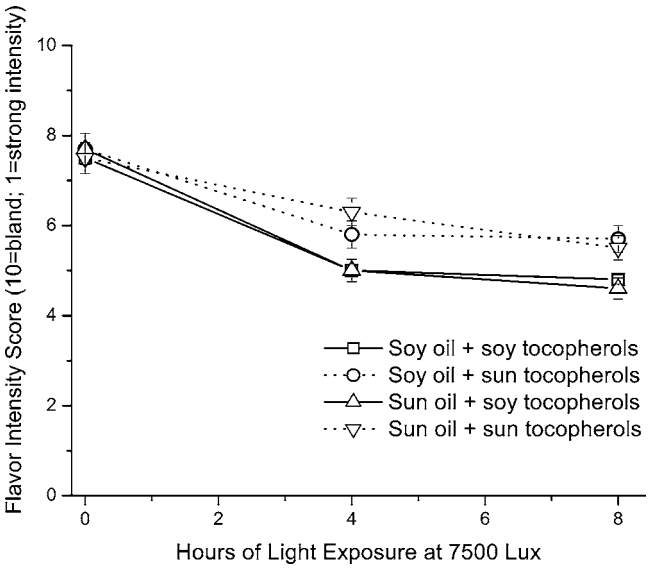

Figure 6. Flavor intensity scores for purified soybean and sunflower oils containing pure tocopherols with profiles typical of soybean oil or sunflower oil and aged for 0, 4, and 8 h at 7500 lx and 30 °C.

with those in previous work in which sunflower oil had significantly better stability to light oxidation than did soybean oil (*6*).

The results of these tests showed that the oxidative and flavor stability of sunflower oil in dark autoxidation could be improved if the tocopherol profile found in soybean oil were added to sunflower oil. In contrast, the stability of soybean oil in photooxidation could be improved if the tocopherol proportions typical of sunflower oil were added. The chemical structures of the tocopherols may in part explain their effectiveness in inhibiting dark oxidations. However, α-tocopherol is a much better singlet oxidation quencher than either γ- or δ-tocopherol (*16*, *17*), which may help to explain why the oils with low α-tocopherol have less light stability than oils with higher levels of α-tocopherol.

Of course, additions of tocopherols may not be economically practical for commodity vegetable oils. However, plant geneticists may consider altering the types and proportions of tocopherols found in sunflower from primarily α-tocopherol to a mixture that also includes γ and δ in order to simulate the enhanced oxidative stability of soybean oil. In addition, soybean oil, which is naturally light sensitive and has low levels of α-tocopherol could benefit from increased levels of α-tocopherol. This new knowledge about the role of tocopherols is especially important now as oil processors and food manufacturers look for oxidatively stable alternatives to hydrogenated oils.

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