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Effects of oxidized α-, γ- and δ-tocopherols on the oxidative stability of purified soybean oil

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Oxidized α-, γ- and δ-tocopherols were prepared in methanol containing methylene blue for 30 h under light. The effects of 0, 100, 250, 500 and 1000 ppm of oxidized α-, γ- or δ-tocopherol on the oxidative stability of purified soybean oil in the dark at 55°C were studied by measuring the peroxide value and headspace oxygen of sample bottles. As the concentrations of oxidized tocopherols increased, the peroxide values increased and headspace oxygen decreased. Tukey's test showed that the oxidized α-, γ- and δ-tocopherols had prooxidant effects ($P < 0.05$) on the peroxide value and headspace oxygen of soybean oil. Although oxidized α-tocopherol had the greatest prooxidant effect, oxidized γ- and δ-tocopherols had similar but lesser effects.

INTRODUCTION

Commercial soybean oil contains about 1000–1500 ppm of tocopherols (Carpenter, 1979; Jung *et al.*, 1989) and the compositions of α-, β-, γ- and δ-tocopherols in soybean oil are 4·0, 1·1, 66·1 and 28·8% respectively (Jung *et al.*, 1989). Tocopherols have been reported as natural antioxidants (Fahrenholtz *et al.*, 1974; Stevens *et al.*, 1974; Sherwin, 1978; Burton & Ingold, 1981; Christopher & Ho, 1985). Optimum concentrations of α-, γ- and δ-tocopherols to increase the oxidative stability of oil are 100, 250 and 500 ppm, respectively, and the tocopherols had significant prooxidant effects at concentrations above these levels (Jung & Min, 1990).

Half of 1250 ppm of α-tocopherol in methyl linoleate is destroyed during 8 days of dark storage at 40°C (Lips, 1957) and 1500 ppm of tocopherols in soybean oil is reduced to 450 ppm during 18 h of dark storage at 100°C by oxidation (Frankel *et al.*, 1959). The oxidation products of α-tocopherol at 55 or 100°C in methyl linoleate are dimer, trimer and dihydroxy dimer, α-tocopherol quinone and quinone oxide (Csallany *et al.*, 1970; Gardner *et al.*, 1972; Grams *et al.*, 1972; Yamauchi & Matsushita, 1977; Yamauchi *et al.*, 1981).

Even though the oxidation of tocopherols in vegetable oils and the identification of oxidized tocopherols have been extensively studied (Lips, 1957; Frankel *et al.*, 1959; Csallany *et al.*, 1970; Gardner *et al.*, 1972; Grams *et al.*, 1972; Yamauchi & Matsushita, 1977; Yamauchi *et al.*, 1981), the effects of oxidized tocopherols on the oxidative stabilities of oils have not been reported. The objective of the present research was to determine the effects of oxidized α-, γ- and δ-tocopherols on the oxidative stability of soybean oil.

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MATERIALS AND METHODS

Materials

Soybean oil was obtained from Capital City Products Co. (Columbus, OH, USA), α- and γ- tocopherols from Hoffman La Roche (Nutley, NJ, USA) and δ-tocopherol from Sigma Chemical Co. (St. Louis, MO, USA).

Purification of soybean oil

Soybean oil was purified by the method of Lee and Min (1988). Fifty grams of soybean oil were passed through a chromatographic column (55 × 4 cm) packed with a series of 100 g of activated silicic acid (100 mesh, Mallinkrodt Co., Paris, Kentucky, USA), 30 g of a 2:1 mixture of activated charcoal (J. T. Baker Chemical Co., Phillipsburg, NJ, USA) and Celite, 120 g of a 2:1 mixture of powdered sugar and

Celite, and 100 g of activated silicic acid. The flow rate of oil through the column was 2 ml/h.

Chemical analyses

Tocopherol contents were analyzed by the high-performance liquid chromatography (HPLC) method of Carpenter (1979). Phospholipids, chlorophylls and free fatty acids were determined by AOCS (1980) methods.

Preparation of oxidized α-, γ- and δ-tocopherols

Oxidized α-, γ- and δ-tocopherols were prepared by the method of Yamauchi *et al.* (1981). One gram of α-, γ- or δ-tocopherol and 125 μmol of methylene blue were dissolved in 200 ml of methanol. Fifty milliliters of the sample were transferred into a 250-ml Erlenmeyer flask and sealed airtight with a Teflon stopper. The samples were stored in a light storage box of 4000 lux for 30 h (Lee & Min, 1988). One hundred milliliters of hexane were added to extract the oxidized tocopherols, and methylene blue was removed by washing several times with water. Hexane was removed with a rotary vacuum evaporator at room temperature to obtain oxidized α-, γ- or δ-tocopherol.

Thin-layer chromatography

The oxidized tocopherols were analyzed using a silica gel thin-layer chromatograph plate (Fisher Scientific, Pittsburgh, PA, USA), and a benzene : methanol (97: 3, v/v) mixture was used as a mobile phase (Yamauchi *et al.*, 1981). The chromatographed compounds were detected by spraying with 50% sulfuric acid and heating at 110°C for 10 min.

Effects of oxidized α-, γ- and δ-tocopherols on the oxidative stability of oil

To study the effects of oxidized α-, γ- and δ-tocopherols on the oxidative stability of soybean oil, soybean oils containing 0, 100, 250, 500 and 1000 ppm of oxidized α-, γ- or δ-tocopherol were prepared. Five grams of sample were transferred into a 30-ml serum bottle and sealed airtight with Teflon septum and aluminum cap. The sample bottles were stored, in duplicate, for 6 days in a Blue M oven (Blueland, IL, USA) at 55°C (Jung & Min, 1990).

Evaluation of the oxidative stability of soybean oil

The oxidative stabilities of purified soybean oils, containing 0, 100, 250, 500 or 1000 ppm of oxidized α-, γ- or δ-tocopherol, were determined by measuring the peroxide value and headspace oxygen of the sample bottles, in duplicate, every day for 6 days.

Peroxide values of the samples were determined using the AOCS (1980) method. The headspace oxygen of the sample was measured by injecting 40 μl of headspace gas of the sample bottle into a 5880 Hewlett-Packard (Avondale, PA, USA) gas chromatograph equipped with a thermal-conductivity detector (Jung *et al.*, 1989). A stainless-steel column (1·8 m × 0·32 cm) packed with 80/100 Molecular Sieve 13X (Alltech Assoc., Inc., Deerfield, IL, USA) was used. The flow rate of helium gas was 20 ml/min. The temperatures of the injection port, oven and detector were 120, 40 and 250°C, respectively. The gas chromatographic oxygen peak area of the 40-μl headspace gas of the sample bottle was determined using a Hewlett-Packard 3390 electronic integrator and then compared to the oxygen peak area of 40 μl of air (Lee & Min, 1988).

Statistical analysis

The peroxide values and headspace oxygen reported in this paper are the mean values of duplicate samples. The analytical data of the effects of oxidized α-, γ- and δ-tocopherols on the peroxide value and headspace oxygen of soybean oil were analyzed by Tukey's range test at a 5% level of significance (SAS, 1982).

RESULTS AND DISCUSSION

Chemical composition of purified soybean oil

The soybean oil that passed through the silicic-acid column was termed purified soybean oil. The purified soybean oil was colorless and did not contain peroxides, chlorophylls, free fatty acids, tocopherols or phospholipids.

Thin-layer chromatograms of oxidized tocopherols

Thin-layer chromatograms of oxidized α-tocopherol formed during light storage in the presence of methylene blue are shown in Fig. 1. Some α-tocopherol was oxidized after storage for 2 h, and it was completely oxidized after 30 h of light storage (Fig. 1). The HPLC analysis of α-tocopherol after 30 h of light storage also showed that α-tocopherol was not present in the sample (data not shown). Thin-layer and HPLC chromatograms showed that γ- and δ-tocopherols were also completely oxidized during the 30 h of light storage (data not shown).

Effects of oxidized α-, γ- and δ-tocopherols on the oxidative stability of oil

The effects of 0, 100, 250, 500 and 1000 ppm of oxidized α-, γ- and δ-tocopherols on the peroxide value of purified soybean oil during storage are shown in the Figs 2, 3 and 4, respectively. As the concentration of oxidized tocopherols in soybean oil increased from 0 to

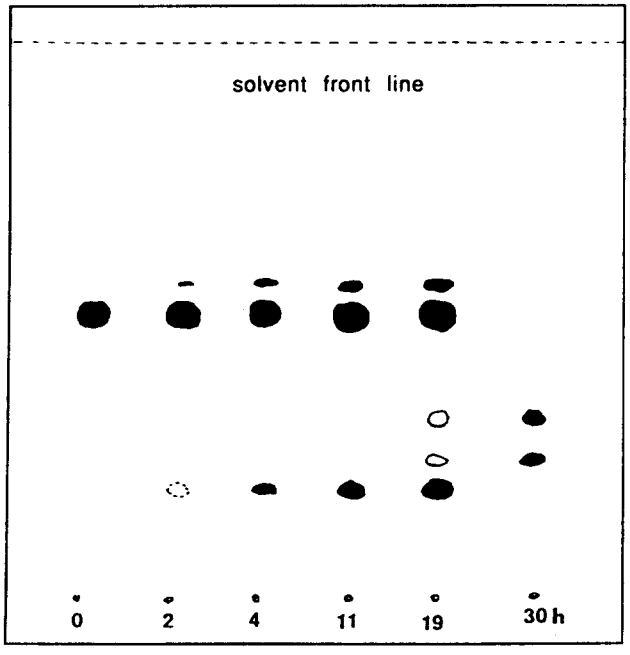

Fig. 1. Thin-layer chromatograms of α-tocopherol in methanol containing methylene blue during light storage at 25°C: stationary phase—silica-gel TLC plate; mobile phase —benzene : methanol (97 : 3, v/v).

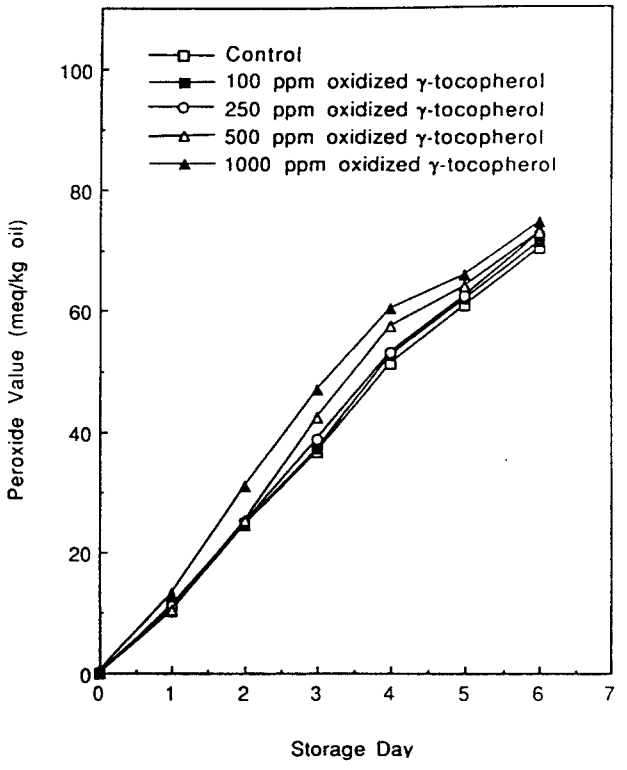

Fig. 3. Effects of 0, 100, 250, 500 and 1000 ppm of oxidized γ-tocopherol on the peroxide value of purified soybean oil during storage at 55°C.

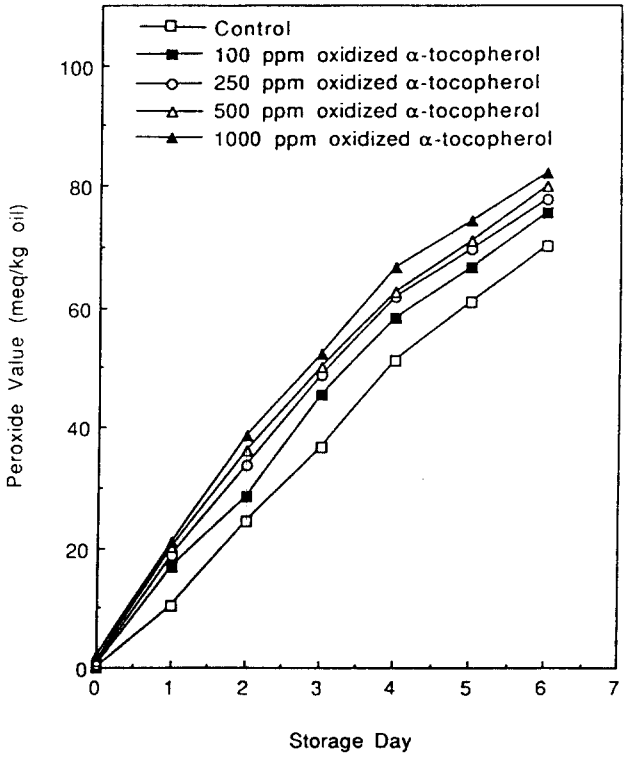

Fig. 2. Effects of 0, 100, 250, 500 and 1000 ppm of oxidized α-tocopherol on the peroxide value of purified soybean oil during storage at 55°C.

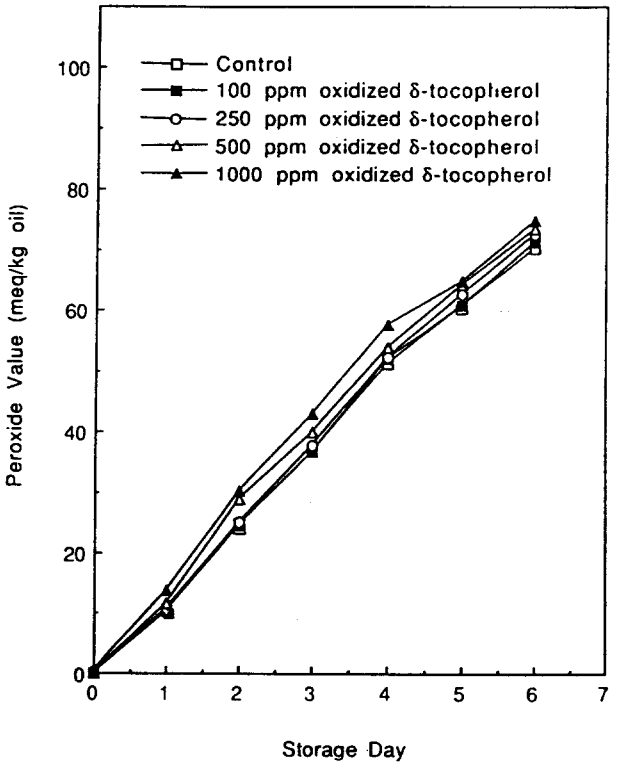

Fig. 4. Effects of 0, 100, 250, 500 and 1000 ppm of oxidized δ-tocopherol on the peroxide value of purified soybean oil during storage at 55°C.

Table 1. Effects of 0, 100, 250, 500 and 1000 ppm of oxidized α-, γ, and δ-tocopherols on the headspace-oxygen content of purified soybean oil during dark storage at 55°C

Oxidized tocopherol (ppm)	Headspace oxygen (%)						
	1 day	2 day	3 day	4 day	5 day	6 day	Means[a]
α-Tocopherol							
0	19·33	17·30	15·65	13·65	12·33	10·72	14·83[b]
100	18·53	15·83	14·20	12·19	11·10	9·58	13·57[c]
250	18·27	15·60	13·90	11·93	10·70	9·42	13·30[c,d]
500	18·15	15·38	13·53	11·78	10·50	9·21	13·09[d]
1000	17·91	14·69	13·11	11·22	10·20	8·93	12·68[e]
γ-Tocopherol							
0	19·33	17·30	15·65	13·65	12·33	10·72	14·83[b]
100	19·25	17·24	15·55	13·65	12·13	10·45	14·71[b]
250	19·07	17·32	15·24	13·56	12·20	10·36	14·63[b,c]
500	19·23	17·03	14·36	12·72	11·50	10·10	14·16[c]
1000	18·65	16·21	13·57	11·83	10·90	9·88	13·50[d]
δ-Tocopherol							
0	19·33	17·30	15·65	13·65	12·33	10·72	14·83[b]
100	19·25	16·94	15·50	13·63	12·29	10·46	14·67[b]
250	19·19	16·85	15·34	13·35	12·06	10·33	14·52[b,c]
500	19·01	16·76	15·22	12·74	11·70	9·93	14·23[c]
1000	18·84	16·13	14·50	11·95	11·20	9·85	13·75[d]

[a] Mean of contents of samples after 1, 2, 3, 4, 5 and 6 days of storage.
[b–e] Means with different letters in a column with the same oxidized tocopherol are different at $P < 0.05$ (i.e. 14·83[b] is different from 13·57[c] at $P < 0.05$). The headspace-oxygen content of 0-day sample was 20·73%.

100, 250, 500 and 1000 ppm, the peroxide values increased. The peroxide values of purified soybean oil containing oxidized α-tocopherol were higher than those of the oil containing oxidized γ- or δ-tocopherol during storage.

The effects of oxidized α-, γ- and δ-tocopherols on the headspace oxygen of purified soybean oils during storage at 55°C are shown in Table 1. The coefficient of variation of headspace analysis by gas chromatography was 3%. The content of headspace oxygen of a fresh sample bottle was 20·73%. As the oxidized tocopherols increased from 0 to 100, 250, 500 and 1000 ppm, the headspace oxygen of soybean oil decreased during storage. This suggests that oxidized tocopherols acted as prooxidants. The headspace oxygen of soybean oil containing oxidized α-tocopherol was lower than that of oil containing oxidized γ- or δ-tocopherol. This indicates that oxidized α-tocopherol has stronger prooxidant activity than the oxidized γ- or δ-tocopherol. Tukey's range test for the effects of oxidized α-, γ- and δ-tocopherols on the headspace oxygen of soybean oil containing 100 ppm of oxidized α-tocopherol was different ($P < 0.05$) from those of the control oil containing no oxidized tocopherol, but the headspace oxygen of soybean oil containing 100 ppm of oxidized γ- or δ-tocopherol was not different ($P > 0.05$) from the control oil. However, the headspace oxygen of soybean oil containing 500 ppm of oxidized γ- or δ-tocopherol was significantly different ($P < 0.05$) from the control sample. The results of Tukey's test for the effects of oxidized α-, γ- and δ-tocopherols on the peroxide values were the same as those of the headspace oxygen. The linear correlation coefficient (r) between the headspace-oxygen contents of Table 1 and the peroxide values of Figs 2–4 was −0·99. That is, as the headspace oxygen decreased, the peroxide value increased as expected.

The combined results of headspace-oxygen contents (Table 1) and peroxide values (Figs 2–4) indicate that oxidized tocopherols act as prooxidants in soybean oil. Oxidized α-tocopherol had the highest prooxidant effect and the oxidized γ- and δ-tocopherols had similar, but lesser, prooxidant effects. The optimum concentration of tocopherols for the oxidative stability of soybean oil is 400–600 ppm (Frankel *et al.*, 1959), and tocopherols at concentrations of 1500 ppm act as prooxidants in oil during storage (Cillard *et al.*, 1980). Tocopherols are oxidized during storage to form oxidized tocopherols (Lips, 1957; Csallany *et al.*, 1970; Gardner *et al.*, 1972; Cillard & Cillard, 1980). Therefore, it may be reasonable to assume that the more tocopherols in soybean oil, the more the oxidized tocopherol compounds (which are prooxidants) are formed during storage. Cillard and Cillard (1980) suggested that the prooxidant effects of tocopherols at high concentration might be due to the oxidation of tocopherols. However, it requires more detailed and systematic studies to understand and explain logically the prooxidant mechanisms of tocopherols at high concentrations in oil during storage.

The prevention of tocopherol oxidation and the

removal of oxidized tocopherols during processing could improve the oxidative stability of soybean oil.

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