Effects of Thermally Oxidized Triglycerides on the Oxidative Stability of Soybean Oil

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Soybean oil purified by silicic acid column chromatography did not contain peroxides, free fatty acids, phospholipids or oxidized polar compounds. The purified soybean oil was thermally oxidized at 180°C for 96 hr in the presence of air. The thermally oxidized compounds (31.3%) were separated from the purified soybean oil by gradient elution silicic acid chromatography. Thermally oxidized compounds contained hydroxyl groups, carbonyl groups and *trans* double bonds according to the infrared spectrum. Thermally oxidized compounds were added to soybean oil and purified soybean oil at 0, 0.5, 1.0, 1.5 and 2.0% to study the effects of these compounds on the oxidative stability of oil. The oxidative stabilities of oils were determined by gas chromatographic analysis of volatile compound formation and molecular oxygen disappearance in the headspace of oil bottles. The thermally oxidized compounds showed prooxidant effects on the oxidative stabilities of both refined, bleached and deodorized soybean oil and purified soybean oil. Duncan's Multiple Range Test showed that thermally oxidized compounds had a significant effect on the volatile compound formation and oxygen disappearance in the headspace of oil at α = 0.05.

The oxidative stability of oil is greatly influenced by minor components such as phospholipids (1, 2), tocopherols (3, 4), iron (5), fatty acids (6) and mono- and diglycerides (15). Billek et al. (7) reported that refined soybean oil contained 1.2% oxidized triglycerides. Oxidized compounds could be formed by oxidation and polymerization of oils during processing, frying and storage (7, 10, 17, 18). The nonvolatile oxidized compounds include cyclic carbon-to-carbon linked dimers, noncyclic hydroxy dimers, carbon-to-carbon linked trimers, dimers and trimers joined through carbon-to-carbon or carbon-to-oxygen linkage, and trimers joined through carbon-to-oxygen linkage (10, 17, 18). Paulose and Chang (10) reported that 26.3% nonvolatile oxidized compounds were isolated from trilinolein after heating for 74 hr at 180°C, and these compounds contained hydroxyl groups, carbonyl groups and *trans* double bonds. Billek et al. (7) also isolated 26.2% thermally oxidized compounds from soybean oil heated for 64 hr at 180°C.

Even though the effects of thermally oxidized compounds on health have been studied extensively (19, 20), the effects of oxidized triglycerides on the oxidative stability of soybean oil have not been studied systematically. Therefore, the objective of this research is to study the effects of thermally oxidized triglycerides on the oxidative stabilities of refined, bleached and deodorized (RBD) soybean oil and purified soybean oil.

MATERIALS AND METHODS

Materials. Refined, bleached and deodorized (RBD) soybean oil was obtained from a local oil refinery. All the reagents used were of analytical grade unless otherwise specified.

Purification of soybean oil. Soybean oil (500 g) was passed through a 4.4 × 55 cm glass column packed with 300 g activated silicic acid. Silicic acid was 100 mesh (Mallinckrodt, Paris, Kentucky) and activated according to the method of Sahasrabudhe and Chapman (11). The oil which passed through the chromatographic column was designated as purified soybean oil (6, 10, 15).

Preparation and isolation of thermally oxidized triglycerides. Purified soybean oil (100 g) was thermally oxidized in a 250-ml beaker at 180 C for 96 hr in an oven. The thermally oxidized compounds in the soybean oil were isolated by passing the thermally oxidized soybean oil through a 2 × 30 cm silicic acid column (7, 10). The compounds retained on the column were then first washed with 200 ml hexane to elute the residual unoxidized purified soybean oil. The thermally oxidized compounds retained on the silicic acid column after hexane elution were then eluted with 500 ml methanol. The methanol-eluted compounds were designated as thermally oxidized compounds, and methanol was removed by rotary vacuum evaporator at 40°C.

Chemical and spectrometric analyses. Acid value, peroxide value and phosphorus contents in RBD soybean oil, purified soybean oil and thermally oxidized purified soybean oil were determined by AOCS methods (12). To characterize the thermally oxidized compounds and oils, the samples were analyzed by the Beckman Acculab 2 I.R. Spectrometer (Beckman Instruments, Inc., Fullerton, California) (15).

Test of thermally oxidized compounds, effects on oxidative stability. To study the effects of thermally oxidized compounds on the oxidative stability of soybean oil, these compounds were added to purified soybean oil and RBD soybean oil at 0, 0.5, 1.0, 1.5 and 2.0% (w/w) level.

Fifteen g of the prepared sample was transferred into a 30-ml serum bottle and sealed airtight with a Teflon-coated rubber septum and an aluminum cap. The samples were stored in a forced-draft air-oven for 10 days at 55°C (14).

Gas chromatographic analysis of oxidative stability. The oxidative stability of the oil was determined by measuring volatile compound formation and molecular oxygen disappearance in the headspace of oil bottles by gas chromatography (14). Volatile compounds in the headspace were determined by injecting one ml of headspace gas into a Hewlett-Packard 5880A Gas Chromatograph (Avondale, Pennsylvania) equipped with a flame ionization detector. A stainless steel column (10′ × 1/8″O.D.) packed with 80/100 mesh Tenax GC coated with 10% polymetaphenoxylene (Alltech Associates, Inc., Deerfield, Illinois) was used. Temperatures of oven, injector and flame ionization detector were 120, 200 and 250°C, respectively. Flow rate of nitrogen carrier gas was 20 ml/min.

The oxygen content in the headspace was determined by injecting one ml of headspace gas into a Hewlett-Packard 5880A Gas Chromatograph (Avondale, Pennsylvania) equipped with a thermal conductivity detector (14). A stainless steel column (6′ × 1/8″ OD) packed with Molecular Seive 13 X (Supelco, Inc., Bellefonte, Pennsylvania) was used. The flow rate of nitrogen carrier gas was 30 ml/mim. Temperatures of oven, injector and thermal conductivity detector were 35, 200 and 250°C, respectively. Calibrations of gas chromatography for volatile compounds and oxygen were done using 1% ethyl hexanoate acid in ethyl ether and air, respectively. GC peaks were determined by electronic integrator (Hewlett Packard, Avondale, Pennsylvania) and expressed in electronic counts.

Statistical analysis. The analytical data of the effects of thermally oxidized compounds on the oxidative stability of oil were analyzed by Duncan's Multiple Range Test (13).

RESULTS AND DISCUSSION

Purification of RBD soybean oil. The soybean oil eluted from the silicic acid column was designated purified soybean oil. The purified soybean oil was colorless, tasteless and odorless. The chemical compositions of RBD soybean oil, purified soybean oil and thermally oxidized purified soybean oil are shown in Table 1. Silicic acid chromatography removed free fatty acids, phospholipids, peroxides and polar compounds from soybean oil.

Preparation and isolation of thermally oxidized compounds. The contents of thermally oxidized compounds in the RBD soybean oil, purified soybean oil and thermally oxidized purified soybean oil were 1.1, 0 and 31.3%, respectively, as shown in Table 1. Paulose and Chang (10) reported that trilinolein heated for 74 hr at 180°C contained 26.3% thermally oxidized compounds. Billek et al. (7) reported that soybean oil heated for 64 hr at 180°C contained 26.2% polar compounds. The value of 31.3% for purified soybean oil was similar to earlier reports (7, 10).

The thermally oxidized compounds isolated by silicic acid chromatography were viscous and dark yellowish-red. The infrared spectra of RBD soybean oil, purified soybean oil and the thermally oxidized compounds are shown in Figure 1. Thermally oxidized compounds have an absorption at 2.9 microns, which is due to intermolecular hydrogen bonding of hydroxyl group (16). The intermolecular hydrogen bonding would increase the viscosity of the oxidized compounds. The absorption band at 10.3 microns of thermally oxidized compounds suggested the presence of *trans* double bonds (16).

The absorption at 5.8 microns, due to the carbonyl group stretching vibration (16) of thermally oxidized compounds, was larger than that of either RBD soybean oil or purified soybean oil. The increased peak size of 5.8 microns absorption suggested that carbonyl groups were formed during thermal oxidation.

Paulose and Chang (10) reported that hydroxyl groups, carbonyl groups and *trans* double bonds increased as the trilinolein was thermally oxidized at 180°C.

Effects of thermally oxidized compounds on the oxidative stability. The quantitative effects of thermally oxidized compounds on volatile compounds formation and oxygen disappearance in the headspace of RBD soybean oil are shown in Figures 2 and 3, respectively. The results show that the higher the amount of thermally oxidized

TABLE 1

Acid Value, Peroxide Value, Phosphorus Content and Polar Component Content in RBD Soybean Oil, Purified Soybean Oil and Thermally Oxidized Purified Soybean Oil

	RBD[a]	PSO[b]	TOPO[c]
Acid value	<0.1	0	a[d]
Peroxide value (meq/kg oil)	0.6	0	0
Phosphorus (ppm)	1.0	0	0
Polar component (%)	1.1	0	31.3

[a]Refined bleached and deodorized soybean oil.
[b]Purified soybean oil.
[c]Thermally oxidized purified soybean oil.
[d]Not determined.

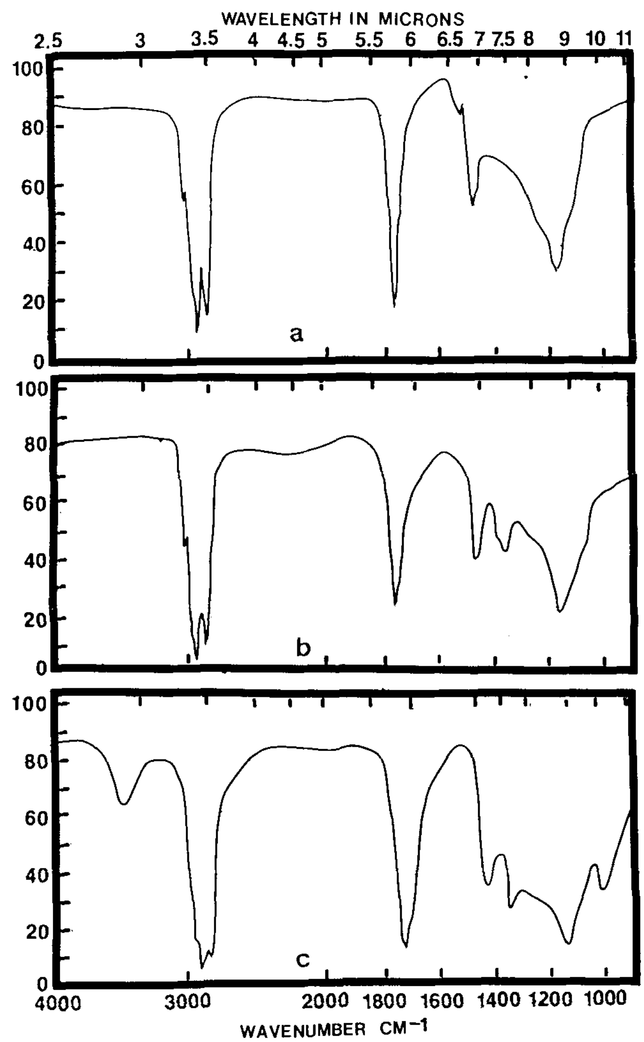

FIG. 1. Infrared spectra of a) RBD soybean oil; b) purified soybean oil, and c) thermally oxidized compounds.

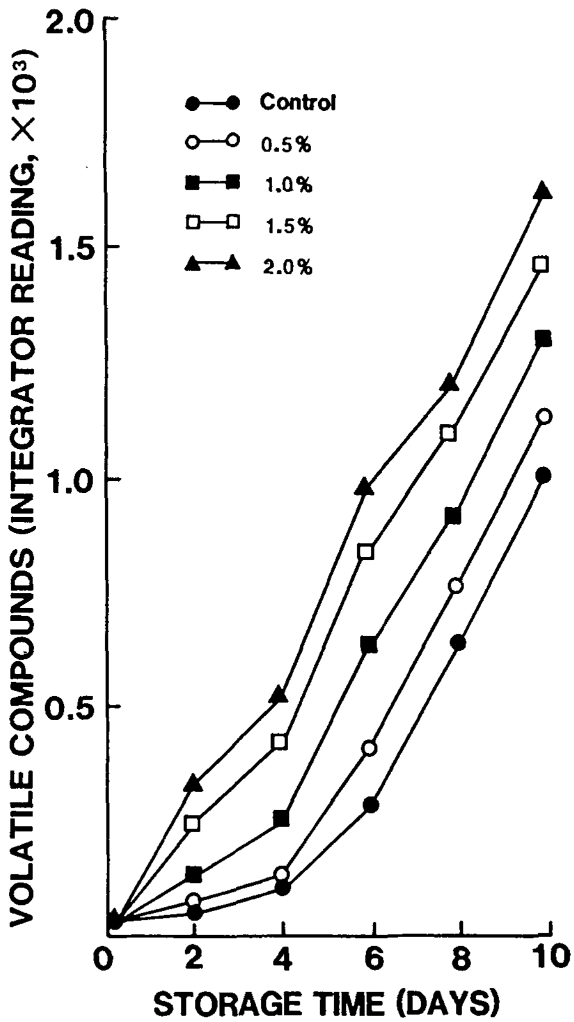

FIG. 2. Effects of different levels of thermally oxidized compounds on the volatile compounds formation in RBD soybean oil during storage at 55°C.

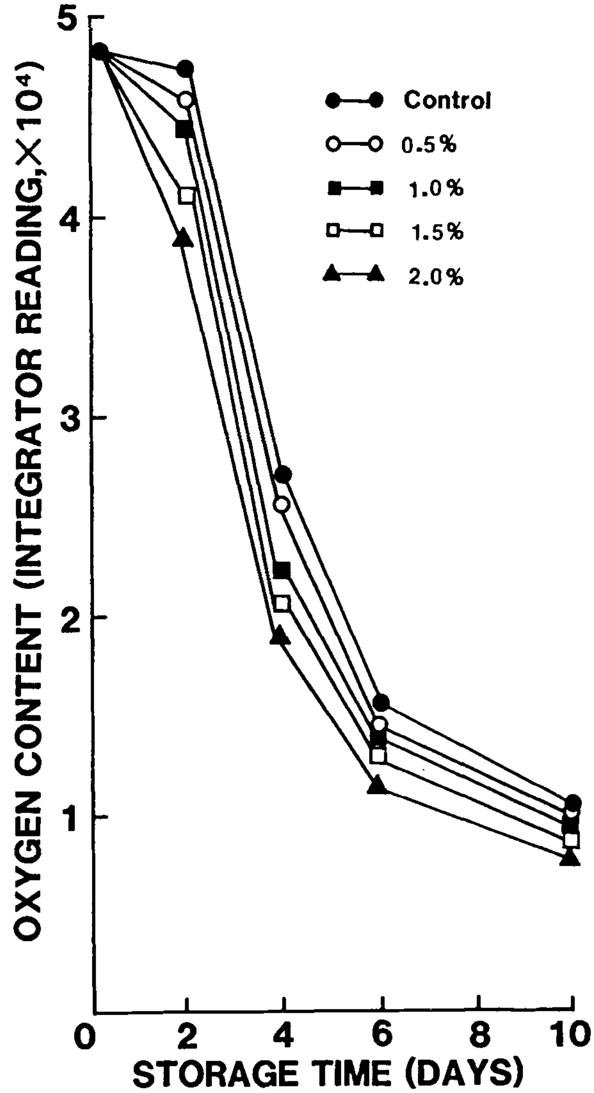

FIG. 3. Effects of different levels of thermally oxidized compounds on oxygen disappearance in the headspace of RBD soybean oil during storage at 55°C.

compounds in the soybean oil, the greater was the formation of volatile compounds and the faster the disappearance of oxygen in the oil. The results suggest that the thermally oxidized compounds acted as prooxidant to decrease the oxidative stability of RBD soybean oil.

The quantitative effects of thermally oxidized compounds on the volatile compound formation and oxygen disappearance in the headspace of the purified soybean oil are shown in Figures 4 and 5, respectively. The results were very similar to those of RBD soybean oil. The higher the amount of thermally oxidized compounds in the purified soybean oil, the greater was the formation of volatile compounds and the faster the disappearance of oxygen in the purified soybean oil. The volatile compounds in purified soybean oil containing 2% thermally oxidized compounds were 2,000 electronic units compared to 980 in the purified soybean oil containing 0% thermally oxidized compounds after 10 days storage at 55°C.

Duncan's multiple range test for the effects of different levels of added thermally oxidized compounds on the volatile compound formation of RBD soybean oil and purified soybean oil are shown in Table 2. The formations of volatile compounds in the oils containing different levels of thermally oxidized compounds were significantly different at $\alpha = 0.05$.

Duncan's multiple range test for the effects of different levels of thermally oxidized compounds on the oxygen disappearances in the headspace of RBD soybean oil and purified soybean oil during storage are shown in Table 3. The oxygen disappearances in the headspaces of oils containing different levels of thermally oxidized compounds both in RBD soybean oil and purified soybean oil were significantly different at $\alpha = 0.05$.

The results of Tables 2 and 3 also show that purified soybean oil was less oxidatively stable than RBD soybean oil. The lower oxidative stability of purified soybean oil as compared to RBD soybean oil might be due to the removal not only of prooxidants such as thermally oxidized compounds but also naturally occurring antioxidants such as tocopherols and phospholipids by silicic acid column chromatography.

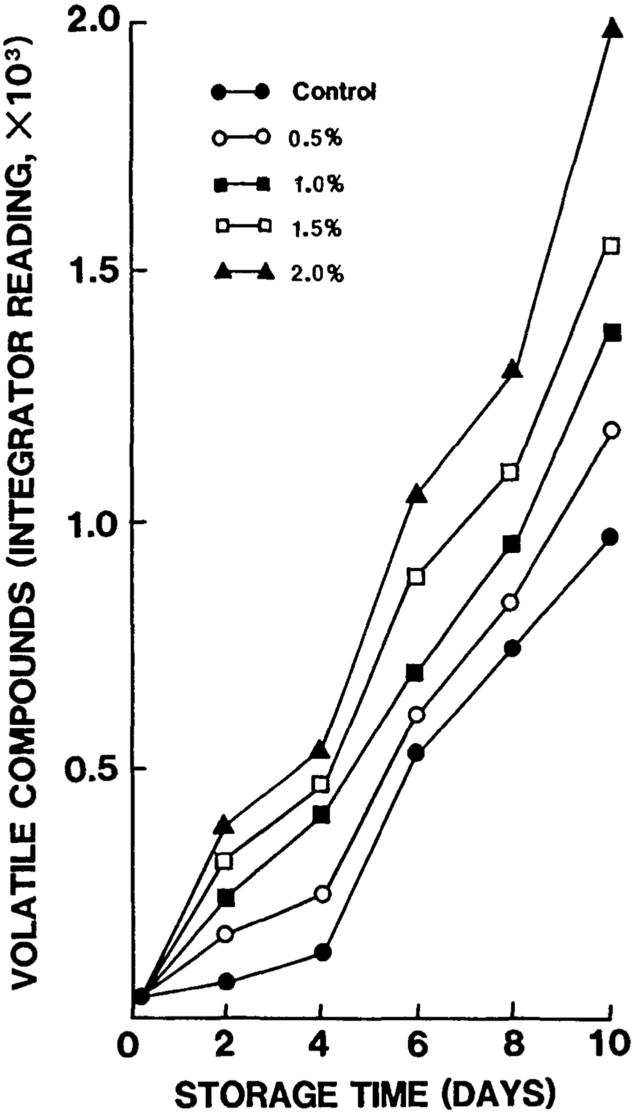

FIG. 4. Effects of different levels of thermally oxidizd compounds on volatile compounds formation in purified soybean oil during storage at 55°C.

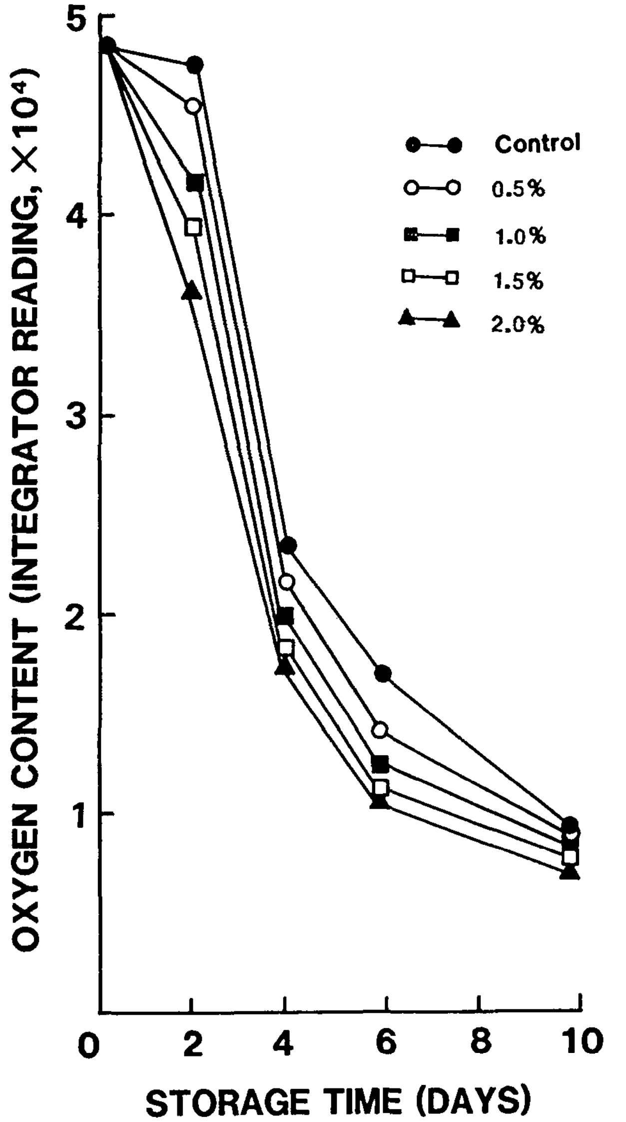

FIG. 5. Effects of different levels of thermally oxidized compounds on oxygen disappearance in headspace of purified soybean oil during storage at 55°C.

TABLE 2

Duncan's Multiple Range Test for the Effects of Different Levels of Thermally Oxidized Compounds on the Volatile Compounds Formations in Soybean Oil and Purified Soybean Oil During 10 Days Storage at 55°C

Thermally oxidized compounds (%)	Volatile compounds in the headspace			
	RBD soybean oil		Purified soybean oil	
	Mean	Group[a]	Mean	Group[a]
0	355	A	501	A
0.5	501	B	596	AB
1.0	645	CD	740	CD
1.5	820	EF	873	E
2.0	926	F	1059	F

[a]Means with the same letter are not significantly different at $\alpha = 0.05$ (Duncan's tests were carried out for RBD soybean oil and purified soybean oil, independently).

TABLE 3

Duncan's Multiple Range Test for the Effects of Different Levels of Thermally Oxidized Compounds on the Headspace Oxygen Disappearances in RBD Soybean Oil and Purified Soybean Oil During 10 Days Storage at 55°C

Thermally oxidized compounds (%)	Oxygen contents in the headspace			
	RBD soybean oil		Purified soybean oil	
	Mean	Group[a]	Mean	Group[a]
0	29858	A	23862	A
0.5	24027	B	23164	B
1.0	22843	BC	20802	CD
1.5	20852	CD	19296	DE
2.0	19341	DE	18205	E

[a]Means with the same letter are not significantly different at $\alpha = 0.05$ (Duncan's tests were carried out for RBD soybean oil and purified soybean oil, independently).

The results show that the oxidized compounds are prooxidant. Therefore, we should minimize the formation of oxidized compounds during processing and storage to improve the oxidative stability of soybean oil.

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