

# Effect of Triacylglycerol Composition and Structures on Oxidative Stability of Oils from Selected Soybean Germplasm<sup>1</sup>

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The oxidative stability of soybean oil triacylglycerols was studied with respect to composition and structure. Crude soybean oils of various fatty acid and triacylglycerol composition, hexane-extracted from ground beans, were chromatographed to remove non-triacylglycerol components. Purified triacylglycerols were oxidized at 60°C, in air, in the dark. The oxidative stability or resistance of the substrate to reaction with oxygen was measured by determination of peroxide value and headspace analysis of volatiles of the oxidized triacylglycerols (at less than 1% oxidation). The correlation coefficients ( $r$ ) for rates of peroxide formation ( $r = 0.85$ ) and total headspace volatiles ( $r = 0.87$ ) were related positively to oxidizability. Rate of peroxide formation showed a positive correlation with average number of double bonds ( $r = 0.81$ ), linoleic acid ( $r = 0.63$ ), linolenic acid ( $r = 0.85$ ). Rate of peroxide formation also showed a positive correlation with linoleic acid ( $r = 0.72$ ) at the 2-position of the glycerol moiety. A negative correlation was observed between rate of peroxide formation and oleic acid ( $r = -0.82$ ). Resistance of soybean triacylglycerols to reaction with oxygen was decreased by linolenic ( $r = 0.87$ ) and increased by oleic acid ( $r = -0.76$ )-containing triacylglycerols. Volatile formation was increased by increased concentration of linolenic acid at exterior glycerol carbons 1,3 and by linoleic acid at the interior carbon 2. Headspace analysis of volatiles and high-performance liquid chromatography of hydroperoxides indicated that as oxidation proceeded there was a slight decrease in the linolenic acid-derived hydroperoxides and an increase in the linoleic acid-derived hydroperoxides. The oxidative stability of soybean oil triacylglycerols with respect to composition and structure is of interest to the development of soybean varieties with oils of improved odor and flavor stability.

**KEY WORDS:** Headspace analysis of volatiles, linoleic acid, linolenic acid, oleic acid, oxidative stability, soybean oil reversion, soybean oil volatiles, stereospecific analysis, triacylglycerols.

Much research has been published on the oxidative stability of soybean oil (SBO) (1-15). SBO can undergo oxidation under mild conditions to develop off-flavors and even potentially harmful compounds (4,5,7,15-19). Efforts to improve SBO stability have involved partial hydrogenation, addition of synthetic antioxidants and metal inactivators (8,10,11,20), and natural selection and induced mutation breeding to reduce the linolenic acid (Ln) content (7,14,21-24). However,

oxidative stability problems have not been completely solved (4,5,14,15,25). Moreover, there is presently concern about the nutritional safety of partially hydrogenated oils (23,25,26) and synthetic antioxidants (25). So much SBO is only refined, bleached and deodorized and packaged under nitrogen without any additives to control oxidation (25). It is, therefore, becoming more important to improve oil stability through changes in triacylglycerol (TAG) composition and structure through plant breeding (24,25) or by enzyme-directed interesterification with more stable fatty acids (27).

Literature on the effect on TAG stability of the position of the fatty acids on the glycerol moiety is controversial. Some workers reported that TAGs having unsaturated fatty acids in the 2-position are more oxidatively stable than those with these acids in the 1- or 3-positions (28-30). These authors concluded that when specific fatty acids occupy the 1- and 3-positions of a TAG, oxidative stability is greater than when these same fatty acids occupy the 1- and 2-positions. However, Park *et al* (31-34), in a series of papers, reported that the position of fatty acids in TAG has no effect on oxidative stability. They studied oxidation of LLL [linoleic acid (L)] and reported that reaction with oxygen is not selective (31). They also reported that, when tocopherols were removed from SBO TAGs, randomization had no effect on oxidative stability (32), and they found no difference in stability between PLnP [palmitic acid (P)] and PPLn and between PLP and PPL (34). With synthetic pure and mixed TAGs prepared from L and Ln, we have shown (35-37) that, at oxidation levels pertinent to SBO flavor deterioration, dilinolenoyl-linoleoyl glycerols (Di-LnL) were less stable to oxidation when Ln was in the 1,2 compared to the 1,3 glycerol positions. Also, dilinoleoyl-linolenoyl glycerols (Di-LLn) were less stable when L was in the 1,3- compared to the 1,2-position. In LnLnLn a small preference was observed for the formation of 16-hydroperoxides when Ln was on the 1- and 3-positions compared to the 2-position. However, there has been no study on the oxidative stability of SBO TAG mixtures under conditions and oxidation levels pertinent to flavor deterioration.

Little attention has been given to the effect of the individual TAG interaction in soybean oil on oxidative stability. Park *et al* (33) studied the autooxidative stability of 13 types of TAG molecular species, and they reported that autooxidative stability of each TAG species decreased with increasing degree of unsaturation and increasing chainlength of the saturated acyl chain. Individual TAG of highest oxidative stability were SOP [oleic acid (O); stearic acid (S)], POP, SOO and POO. However, these studies were conducted at oxidation levels excessively high and beyond significance with SBO flavor deterioration.

The current study reports the oxidative stabilities of soybean oils having various fatty acid compositions and triglyceride structures. The oils were extracted from selected soybean varieties and experimental lines produced by induced mutation breeding. Mild oxidation conditions were employed to produce low oxidation levels [peroxide value (PV) <50 and oxidation products less than 1% by weight of oil] associated with soybean oil flavor deterioration (15).

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## EXPERIMENTAL PROCEDURES

**Materials.** Soybeans [*Glycine max.* (L.) Merr.] were commercial cultivars, including plant introductions selected on the basis of fatty acid composition, obtained from Randall L. Nelson, Curator, U.S. Department of Agriculture Germplasm Collection (University of Illinois, Urbana, IL), and experimental lines provided by Walter R. Fehr, (Iowa State University, Ames, IA). Solid-phase extraction columns (SE) (6.5 mL, loaded 2.0 g silica), used for removal of non-TAG components from crude SBO oils, were purchased from Baxter Health Care (Muskegon, MI). The SE columns ("Bond-Elut", 3 mL, loaded 0.2 g silica), used for resolution of lipolysis mixtures, were purchased from Analytichem International (Harbor City, CA). Pancreatic lipase (EC 3.1.1.3, type 2, crude from porcine pancreas, activity 220 units per 1 mg protein with olive oil at pH 7.7), bile salts and sodium cholate were purchased from Sigma Chemical Co. (St. Louis, MO). Thin-layer chromatography (TLC) plates (2.5 × 7.5 cm, 250 μm layer of silica gel A, ultraviolet 254 nm indicator) were obtained from Whatman Manufacturing (Fairfield, NJ). Standard TAG and gas chromatography (GC) reference standard mixture 15A were purchased from Nu Chek Prep (Elysian, MN). All solvents were high performance liquid chromatography (HPLC) grade. Diethyl ether was passed through the 2-g SE column to remove antioxidant.

**Methods.** To facilitate use of germplasm samples of limited size, small-scale oil extraction, chromatographic and analytical methods were developed. Except where otherwise noted, all procedures were performed in duplicate for each soybean variety studied.

**Extraction.** Crude SBO (1.6–2.7 g) was obtained by extraction of 15 g of soybeans in duplicate. Fifteen grams of beans were ground in a Varco coffee bean grinder (Type 228, Mouli Manufacturing Co., Belleville, NJ) and soaked in 30 mL hexane at room temperature for 10 min. Oil was extracted by sonication for 5 min with an ultrasonic Homogenizer Model 4710 sonicator (Cole Parmer Instrument Co., Chicago, IL) with output setting at 7. After sonication, the hexane-bean mixture was cooled in ice to room temperature. This mixture was filtered over 50 mg Celite filter aid, and the filter cake was rinsed 5 times with 80 mL hexane. The filtrate was dried with 4 g sodium sulfate and then filtered through folded filter paper (2V). Solvent was removed in 45 min on a roto-evaporator with bath temperature at 27°C. Recovered crude oil was stored neat, under helium, at –20°C.

**SE chromatography.** The crude SBO was stripped of non-TAG components by SE chromatography. Before chromatography, each oil (1.2 g) was mixed with 485 mg of activated carbon and soaked in 1 mL hexane for 15 min. The hexane-oil-carbon mixture was transferred to the top of the 2-g SE cartridge, previously activated with 6 mL hexane, and introduced into the absorbant under helium pressure. The oil was eluted with 1.5 mL hexane to give fraction 1 (nonpolar material); 15 mL chromatographed diethyl ether: hexane (10:90, v/v) to give fraction 2 (TAG); and 15 mL methanol to give fraction 3 (polar material). Solvent was removed with a roto-evaporator [45 min; water bath temperature 25–27°C; at 0.1 mm (Hg), mechanical vacuum pump]. While this SE procedure gave about 90% recovery of TAG, the fatty acid composition was unaffected as discussed below. Time required for

chromatography and preparation of duplicate oil samples for oxidation was 2 to 3 hr.

The nonpolar, TAG and polar fractions from SE were quickly evaluated by TLC with diethyl ether/hexane (20:80, v/v) developing solvent. Resolved components were visualized by iodine and sulfuric/chromic acid charring. Purity of the TAG was confirmed by HPLC (Spectrophysics, Inc., San Jose, CA) on a Zorbax silica column (0.49 × 25 cm, Dupont, Inc., Wilmington, DE), with eluting solvent of 3% 2-propanol in hexane (v/v), and by using a variable wavelength detector (ultraviolet-visible light absorbance, Schoeffel Instruments Co., Westwood, NJ) against non-TAG components found in crude SBO as reference compounds. The non-TAG reference components and the ultraviolet (UV)-visible light detector wavelength used for each were: phospholipids (215 nm), tocopherols (298 nm), chlorophyll (670 nm), carotene (436 nm), xanthophyll (436 nm), squalane (Refractive Index Detector), squalene (254 nm), sterols (215 nm), diglycerides (215 nm), monoglycerides (215 nm), free fatty acids (215 nm) and TAG hydroperoxides (232 nm).

**Analysis of purified TAG.** Purified TAG was evaluated by PV analysis (38) of triplicate samples (15 mg each). Fatty acid composition was determined by gas chromatography (GC) of the methyl esters (35). A 15-mg sample was transmethylated by reaction with 5 mL of 0.5 N KOH in methanol at 50°C. The reaction mixture was neutralized to pH 7 with dilute hydrochloric acid and extracted once with 5 mL petroleum ether:diethyl ether (1:1, v/v) and dried with 5 mL acetone azeotrope. Fatty acid methyl ester (FAME) samples were analyzed on a direct-injection glass column (183 cm × 0.32 cm i.d.) packed with 10% SP2330, (Supelco, Inc., Bellefonte, PA), in a Hewlett-Packard Gas Chromatograph, Model 5770 (Avondale, PA) equipped with a flame-ionization detector. The column was operated isothermally at 160°C with helium flow rate at 20 mL/min. The injector and detector temperatures were set at 200°C. Sample size was 1 μL of 0.5% solute in diethyl ether. Peak integration was by computer (39). The procedure was quantitated by analysis of standard FAME mixture 15A (NuChek Prep, Inc.). A coefficient of variation (CV) of 0.3 to 5.0% was obtained between weight and area percent per fatty acid.

To determine if any change had occurred due to chromatography, the fatty acid composition of the chromatographed oil was compared to that of the parent crude oil. The deviation in fatty acid composition was 1% or less for unsaturated fatty acids.

TAG molecular species (TAGMS) analysis was performed, in duplicate by using 10-mg samples for reversed-phase HPLC (RP-HPLC) with flame-ionization detection (FID) (40) and two C-18 (5 μ, 0.49 × 50 cm, Zorbax, Dupont Inc.) columns placed in series. The TAGs, 0.5 mg in 5–10 μL methylene chloride, were resolved with a 120-min gradient of 70:30 to 40:60 acetonitrile:methylene chloride (v/v) pumped at 0.8 mL/min. The columns were cleaned between analyses with 100% methylene chloride. The flame-ionization detector was a Tracor Model 945 HPLC detector (Tracor Co., Austin, TX) with block temperature 130°C; detector gas, 140 mL/min hydrogen; cleaning flame: 600 mL/min hydrogen, 300 mL/min oxygen; and compressed air set at 0.4 ft<sup>3</sup>/min. TAGMS were identified by peak retention times referenced to those of synthetic TAGMS (37,40–42).

**Stereospecific analysis.** Stereospecific analysis by lipolysis (43) was performed with a 30-mg sample of purified TAG.

**Oxidation of purified TAG.** Purified TAGs from test samples were oxidized at  $60 \pm 2^\circ\text{C}$  in the dark in a forced-air oven (Precision Scientific Co., Chicago, IL). A control sample of TAG, obtained by chromatography of a standard SBO, was used in each oxidation experiment to confirm reproducibility of oxidation conditions. Samples (225 mg each) were weighed into open 20-mL vials, which were placed in a 150-mL beaker with a cover slip. Two TAG samples per variety and standard SBO were prepared for each oxidation time (24, 48, and 72 hr). Three 15-mg samples were removed from each TAG per time period for peroxide value (PV) determination by the colorimetric ferric thiocyanate method (38) in triplicate; two 50-mg samples for volatile headspace analysis (HS) in duplicate (12); and one 50-mg sample for hydroperoxide analysis by RP-HPLC as described below. The formation rate of peroxides ( $\Delta\text{PV}$ ) was determined from linear regression of the plot of PV versus time. Comparisons between oxidation experiments were valid because the  $\Delta\text{PV}$  of the TAG from the standard oil had a coefficient of variation (CV) of 5% or less.

**TAG oxidation product analysis.** RP-HPLC was performed in duplicate on each oxidized TAG sample at 24, 48 and 72 hr. HPLC conditions were: 3  $\mu$  Zorbax ODS column, 8  $\times$  0.6 cm (Dupont, Inc.); mobile phase was methylene chloride/acetonitrile, (20:80, v/v) at a flow rate of 0.5 mL/min; ultraviolet (UV) detector was set at 235 nm and sensitivity at 2 absorbance units. Sample size was 6.1 mg per 10  $\mu$ L methylene chloride. The formation of oxidation products from LLnLn, LLn, LLL, LnLP + LnOP, LLO, LLP, LOO, LLS and LOP was monitored. These oxidation products were identified by matching HPLC retention times with UV detection at 235 nm with standard autoxidized triacylglycerols, which included LnLLn, LLnLn, LLnL, LLLn, LLL, LOL, LnOO, LLP, OLO and LOP. The autoxidation products of LnLLn, LLnLn, LLnL and LLLn, prepared from pure synthesized individual TAG (41), were characterized previously (37). The autoxidation products of LLL have been characterized (35). The autoxidation products of the remaining autoxidized TAGs, prepared according to synthetic procedures (41), were confirmed according to previous methods (35,37) by capillary GC analysis of the borohydride-reduced and hydrogenated, transmethylated, silylated derivatives of the respective preparative HPLC fractions. After elution of oxidized TAGs, non-oxidized TAGs were eluted with 100% methylene chloride.

**Analysis of volatiles.** Volatile analyses were performed with a Perkin-Elmer Sigma 2000 Capillary GC equipped with a Perkin-Elmer HS-100 Headspace Sampler and flame-ionization detector (FID) (Norwalk, CT). Detector temperature was  $180^\circ\text{C}$ . A 50-mg sample of TAG was sealed in a 20-mL clean vial, heated to  $140^\circ\text{C}$  and held at temperature for 20 min in the magazine of the headspace sampler. The headspace sampler was placed into the injection position and pressurized for 1 min. Volatiles generated were automatically transferred onto a Durabond DB 1701 capillary column (30 m  $\times$  0.32 mm i.d. with 1-micron film thickness) (J&W Scientific, Folsom, CA) after the helium flow was interrupted. Helium velocity for the column was 23.9 cm/sec. The GC oven was held

at  $50^\circ\text{C}$  for 1 min and then temperature programmed to  $100^\circ\text{C}$  at  $30^\circ\text{C}/\text{min}$ . To clean the GC column for the next run, the oven was heated to  $250^\circ\text{C}$  with a 4-min hold. With this procedure, one sample was analyzed every 30 min. Formation of 6 volatiles was monitored as a measure of TAG oxidation. The volatiles monitored and their precursor fatty acid hydroperoxides were: pentane, (L); propanal, (Ln); pentanal, (L); hexanal, (L), *cis* and *trans* 2-heptenal, (L); and 2,4-heptadienal, (Ln). The FID response was monitored by a real-time computer programmed to calculate peak areas (39). The sum of the peak areas of these selected volatiles (TV) and the peak areas for propanal (PR) and hexanal (HX) were used as measures of experimental oxidizability.

**Statistical methods.** Correlation coefficients (44) were calculated (45) for oxidizability {calculated from the fatty acid composition as:  $[0.02 \times (\text{O}) + 1 \times (\text{L}) + 2 \times (\text{Ln})]$ } (46), with respect to rate of formation of peroxides ( $\Delta\text{PV}$ ) and headspace volatiles ( $\Delta\text{TV}$ ,  $\Delta\text{PR}$ ,  $\Delta\text{HX}$ ). Correlations were determined between  $\Delta\text{PV}$ , rate of formation of total selected volatiles ( $\Delta\text{TV}$ ), rate of formation of propanal ( $\Delta\text{PR}$ ), and rate of formation of hexanal ( $\Delta\text{HX}$ ) and average number of double bonds, fatty acid composition, TAG fatty acid position, and TAG molecular species composition. To show apparent TAGMS interaction or grouping that may effect oxidative stability a three-principal-component (PC) analysis was performed (45). A PC is a new variable made up of combinations of old variables. The first PC assigned to each TAGMS is a number that represents its most obvious positive and negative correlations with those of the remaining TAGMS. The first, second, and third PCs account for 57%, 19% and 16%, respectively, of the remaining correlations among the TAGMS.

## RESULTS AND DISCUSSION

Soybean oil typically contains TAG (97.9 wt%), diacylglycerol (1.0 wt%) and non-TAG (1.1 wt%) (47). TAG can vary widely in fatty acid and TAG molecular species composition depending on the germplasm source (47). TAG mixtures of widely different composition were obtained from many soybean varieties for the study of oxidative stability.

**Methodology for oxidative stability studies.** This study required small-scale methodology suitable for limited quantities of soybeans and oil. The major procedures developed involved rapid extraction of oil from ground beans, removal of non-TAG components from the oil, analyses of fatty acid and TAG molecular species composition, and TAG structure of the oil before oxidation. Further, procedures were needed for mild, low-level oxidation of oils. Finally, sensitive and valid methods were required to analyze oils oxidized to levels of less than 1%.

The data for yield, oxidation level and fatty acid composition for the crude oils extracted by sonication compared to that for the same analyses for crude oils extracted by standard Soxhlet procedure (48) showed deviations in oil yield of 0.5% and in fatty acid composition of 0.5 to 2.8%. Crude oils extracted by sonication had an average PV of 1.9 compared to oils obtained by Soxhlet extraction which had an average PV of 5.2. For a typical extraction of duplicate soybean samples, the sonicator extraction required 2.5 hr, compared to 7 hr for the Soxhlet extraction procedure. The non-TAG of crude SBO contains

tocopherols and carotenoids, that, at proper concentration, are free-radical and singlet-oxygen scavengers and can inhibit oxidation (4,25). Also, sensitizers, such as chlorophyll, can promote oxidation (4). Therefore, to study TAG effects on oxidative stability, the non-TAG components were removed from the oil by solid-phase extraction. Recovery of pure TAG, confirmed by polar-phase TLC and RP-HPLC, was about 90%. Differences between fatty acid composition of crude and chromatographed SBO ranged from 2 to 5% for saturated and 0 to 1% for unsaturated acids. Differences between TAGMS composition of crude and chromatographed soybean oil showed a deviation of 5% or less for individual TAG. The difference for fatty acid composition of the two chromatographed oils used in each oxidation experiment was 0 to 1.2%, which permitted valid comparison between duplicate samples.

Since the oxidation of the TAG did not exceed 1% total oxidation, sensitive instrumental and chemical methods were required to evaluate stability. The PV determined by the colorimetric ferric thiocyanate method showed good linear relationship with total HPLC peak areas for oxidation products (37). This method was suitable for measurement of PV from 15-mg samples of lipid that had been oxidized to low levels (0.1 to 5.0%) (37). Volatile headspace analysis of duplicate samples showed a coefficient of variation for  $\Delta$ PV and  $\Delta$ TV of 5% or less. The oxidation reaction was also monitored by RP-HPLC-UV analyses of TAG oxidation products. The RP-HPLC procedure is suitable for the analysis of linoleic acid (L) and linolenic acid (Ln) TAG oxidation products on 1 to 10-mg lipid samples at oxidation levels as low as 0.1% (35–37).

Depending on the oil composition, PV ranged, at 24 hr, from 3.1 to 15.7; at 48 hr, from 5.9 to 26.3; and at 72 hr, from 9.5 to 38.4. These PV ranges are representative of oils that develop off-flavors (3,15) and are thus relevant to oil flavor deterioration.

Typically, duplicates of oil from one soybean variety were oxidized at the same time. To make valid comparisons between oxidation experiments, a common SBO (internal standard oil) was oxidized in all the experiments. For the internal standard oil, the CV ranged from 1.3 to 2.5% for the slope of the linear regression in a plot of PV versus time, indicating that the data could be correlated among oxidation experiments with confidence.

The unsaturated fatty acid compositions of the TAG from twenty soybean varieties are presented in Table 1. Also shown is the oxidizability of each oil, calculated based on the unsaturated fatty acid composition (46) or as experimentally determined from  $\Delta$ PV. Samples are listed in increasing order of calculated oxidizability. Samples 4–6, 8, 16 and 20 have  $\Delta$ PV values that indicate greater stability than predicted by the calculated oxidizability, while  $\Delta$ PV values of samples 2, 3, 10 and 13 indicate less stability than predicted. These observations may be the result of variation in TAG structure.

Results of lipolysis analyses of the TAG structures are presented in Table 2. These data indicate that the greater stability of samples 4 and 5 may be attributable to a higher L/Ln ratio at the internal (carbon 2) position relative to samples 2, 3, and 10, which showed lower stability.

*Correlation of PV and  $\Delta$ PV with TAG composition and structure.* Peroxide values determined at each oxidation period and  $\Delta$ PV obtained by linear regression analysis

TABLE 1

Fatty Acid Composition, Oxidizability and Stability of Soybean Oil Triacylglycerols

Sample number	Fatty acid composition				
	0	L	Ln	OX <sup>a</sup>	$\Delta$ PV <sup>b</sup>
1	28.7	47.5	2.8	0.536	0.135
2	36.2	45.3	4.7	0.553	0.152
3	33.9	46.1	5.6	0.579	0.156
4	27.0	54.8	3.2	0.617	0.137
5	27.0	55.2	3.0	0.617	0.147
6	31.5	47.5	6.8	0.613	0.130
7	32.5	46.3	7.4	0.618	0.229
8	30.7	47.8	6.8	0.620	0.221
9	30.3	49.4	6.4	0.628	0.181
10	29.6	50.0	6.5	0.636	0.256
11	27.9	50.2	6.4	0.637	0.213
12	26.3	52.9	6.3	0.660	0.207
13	19.6	58.8	8.2	0.755	0.490
14	19.4	59.7	8.0	0.760	0.281
15	18.7	53.5	11.6	0.769	0.477
16	19.6	59.3	8.7	0.771	0.213
17	17.6	58.9	9.4	0.780	0.444
18	15.7	57.4	11.4	0.805	0.480
19	15.3	57.3	11.6	0.807	0.495
20	15.3	57.2	11.8	0.810	0.370

<sup>a</sup>Oxidizability = (0.02 [0%] + [L%] + 2 [Ln%])/100 (46).

<sup>b</sup>Slope obtained by linear regression plot of PV versus time.

were correlated with TAG composition and structure for 20 different soybean varieties and the results are presented in Table 3. The  $\Delta$ PV and PV determined at 24, 48 and 72 hr were highly correlated with oxidizability (46), which is based on the unsaturated fatty acid content of the oil and number of double bonds.

Correlations developed for PV and for  $\Delta$ PV with the results of stereospecific analysis are also presented in Table 3. The fatty acid composition of the 20 different TAGs at the internal position (carbon 2) indicated that the ranges for unsaturated fatty acids were: O, 13.3 to 38.6%; L, 57.5 to 76.5%; and Ln, 2.4 to 10.7%. At the external positions (carbon 1,3), ranges were: O, 15.7 to 33.9%; L, 38.4 to 52.4%; and Ln, 3.0 to 13.0%. Both PV and  $\Delta$ PV showed strong positive correlations with L and Ln located on the internal carbon. A low correlation value for  $\Delta$ PV was noted for L located at the external glycerol carbons.

Oleic acid located on the internal carbon had only a slightly larger negative correlation for  $\Delta$ PV and PV compared to its location on the external carbons. Thus, the glycerol position of oleic acid has little influence on oxidative stability.

RP-HPLC showed 22 TAG molecular species in soybean oil (Fig. 1). These results are comparable to those reported by other workers (34,43). The content of specific TAG molecular species varied by a factor of 2 to 4 depending on germplasm source. The ranges of the major soybean oil TAGMS (average of 20 soybean varieties) are presented in Table 4, and were determined to be: LnLL, LLL, LLO, LLP, LOO and LOP. These data confirm the work reported by Phillips *et al.* (40) and Perrin and Prevot (42). Correlation coefficients in Table 4 indicate that  $\Delta$ PV had a significant, positive correlation with those TAG molecular species containing Ln. As indicated by the correlation of  $\Delta$ PV with L content shown in Table 3, there was little positive correlation with LLL. But, in agreement with results of Park *et al.* (33), oleic acid in the TAG improved

## TRIACYLGLYCEROL OXIDATIVE STABILITY

TABLE 2

Fatty Acid Ratios at Glyceride Carbons<sup>a</sup>, Oxidizability and Stability of Soybean Oil Triacylglycerols

Sample number	Glyceride carbon number						OX <sup>b</sup>	ΔPV <sup>c</sup>
	2		1,3		1,2;1,3			
	L/O	L/Ln	L/O	L/Ln	L/O	L/Ln		
1	2.23	27.75	1.38	12.80	1.80	19.40	0.536	0.135
2	1.49	16.42	1.20	7.46	1.32	11.00	0.553	0.152
3	1.78	11.84	1.15	6.71	1.47	9.03	0.579	0.156
4	2.53	23.86	1.77	14.00	2.16	18.84	0.617	0.137
5	3.01	23.39	1.62	15.47	2.26	19.83	0.617	0.147
6	2.07	10.38	1.26	5.49	1.63	7.76	0.613	0.130
7	2.08	8.65	1.14	5.12	1.58	6.80	0.618	0.229
8	2.10	10.38	1.31	5.64	1.69	7.83	0.620	0.221
9	2.00	13.17	1.95	6.22	1.73	8.82	0.628	0.181
10	2.09	12.27	1.48	6.00	1.80	8.62	0.636	0.256
11	2.38	13.16	1.52	6.01	1.94	8.92	0.637	0.213
12	2.87	13.77	1.63	6.41	2.21	9.53	0.660	0.207
13	4.56	11.03	2.38	5.71	3.34	8.00	0.755	0.490
14	4.41	11.50	2.53	5.93	3.38	8.29	0.760	0.281
15	4.56	6.82	2.19	3.64	3.24	5.12	0.769	0.477
16	3.83	10.01	2.65	5.58	3.22	7.48	0.771	0.213
17	4.96	9.54	2.65	5.06	3.70	6.96	0.780	0.444
18	5.75	7.73	2.83	3.92	4.12	5.66	0.805	0.480
19	5.47	7.92	3.00	3.80	4.13	5.59	0.807	0.495
20	5.26	8.05	3.06	3.69	4.09	5.52	0.810	0.370

<sup>a</sup>Composition at glyceride carbon atoms determined by lipolysis procedures (43).<sup>b</sup>Oxidizability = (0.02 [O%] + [L%] + 2 [Ln%])/100 (46).<sup>c</sup>Slope obtained by linear regression plot of PV versus time.

TABLE 3

Correlation Coefficients<sup>a</sup> of ΔPV<sup>b</sup> and PV at Each Time Period with Oxidizability, Degree of Unsaturation, Soybean Oil Triacylglycerol Unsaturated Fatty Acid Content and Position at Glyceride Carbons

Factor	ΔPV	PV		
		24 hr	48 hr	72 hr
Oxidizability <sup>c</sup>	0.85	0.67	0.79	0.82
Degree of unsaturation	0.81	0.64	0.75	0.78
Oleic acid	-0.82	-0.62	-0.74	-0.78
Linoleic acid	0.63	NS <sup>d</sup>	0.55	0.60
Linolenic acid	0.85	0.68	0.81	0.83
Oleic acid/carbon 2	-0.83	-0.67	-0.78	-0.81
Oleic acid/carbon 1,3	-0.80	-0.57	-0.71	-0.75
Linoleic acid/carbon 2	0.72	0.57	0.66	0.70
Linoleic acid/carbon 1,3	0.52	NS	NS	NS
Linolenic acid/carbon 2	0.85	0.73	0.83	0.84
Linolenic acid/carbon 1,3	0.84	0.65	0.79	0.81

<sup>a</sup>Procedure (44).<sup>b</sup>Slope obtained by linear regression plot of PV versus time.<sup>c</sup>Oxidizability = (0.02 [O%] + [L%] + 2 [Ln%])/100 (46).<sup>d</sup>NS = not significant, (P>0.05; >95% confidence level).

its oxidative stability. The addition of oleic to LLL to give LLO increased the oxidative stability. Conversely, the addition of P to LLL slightly decreased oxidative stability. Replacement of P in LOP with S slightly improved oxidative stability, as shown by a higher negative correlation coefficient for LOS ( $r = -0.75$ ). These oxidative stability

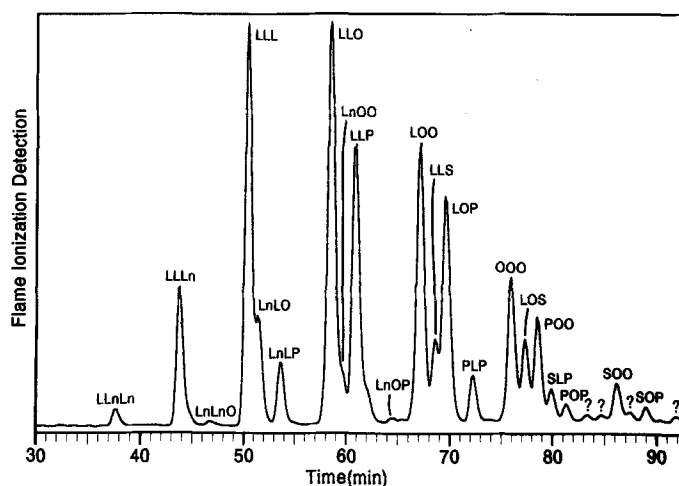


FIG. 1. RP-HPLC analysis of purified soybean oil triacylglycerols. Sample (#8, Table 1): 0.5–1.0 mg; 5  $\mu$  C-18 column (0.49  $\times$  50 cm); 120 min solvent gradient acetonitrile:methylene chloride (70:30 to 40:60 v/v); flow rate, 0.8 mL/min; FID detector. Column cleaned with methylene chloride after analysis.

results for saturated fatty acids at low oxidation levels are the opposite of those found by Park *et al.* (33) for TAG at higher levels of oxidation.

The effect of interaction of the TAGMS on oxidative stability of the TAG was examined by three-principal-component analysis (PC) (Fig. 2). The PC analysis, in



## TRIACYLGLYCEROL OXIDATIVE STABILITY

TABLE 6

Correlation of  $\Delta TV$ ,  $\Delta HX$  and  $\Delta PR$  with Oxidizability, Degree of Unsaturation, and Soybean Oil Triacylglycerol Unsaturated Fatty Acid Content and Position at Glyceride Carbons

Factor	Correlation coefficients <sup>a</sup>		
	$\Delta TV^b$	$\Delta HX^c$	$\Delta PR^d$
Oxidizability <sup>e</sup>	0.87	0.86	0.85
Degree of unsaturation	0.86	0.83	0.82
Oleic acid	-0.84	-0.85	-0.81
Linoleic acid	0.82	0.79	0.70
Linolenic acid	0.73	0.75	0.84
Oleic acid/carbon 2	-0.85	-0.84	-0.80
Oleic acid/carbon 1,3	-0.82	-0.80	-0.81
Linoleic acid/carbon 2	0.82	0.80	0.71
Linoleic acid/carbon 1,3	0.78	0.73	0.64
Linolenic acid/carbon 2	0.69	0.70	0.78
Linolenic acid/carbon 1,3	0.74	0.76	0.85

<sup>a</sup>Procedure (44).

<sup>b</sup>Slope of linear regression plot of sum of FID peak areas for pentane, propanal, pentanal, hexanal, *cis* and *trans* 2-heptenals, and 2,4-heptadienal vs time.

<sup>c</sup>Linear regression of FID peak areas for hexanal vs time.

<sup>d</sup>Linear regression of FID peak areas for propanal vs time.

<sup>e</sup>Oxidizability =  $(0.02 [0\%] + [L\%] + 2 [Ln\%])/100$  (46).

Correlations for  $\Delta HX$ , from L, and  $\Delta PR$ , from Ln, are also presented in Table 6. Both  $\Delta HX$  and  $\Delta PR$  show high positive correlation with oxidizability and degree of unsaturation. A plot of propanal vs hexanal GC peak areas with oxidation time showed that while the amount of both propanal and hexanal increased with oxidation time, the slopes of these curves were slightly different. The slopes of the curves for propanal and hexanal were 0.320 and 0.329 area counts/hr, respectively. Thus, the amount of propanal increased with oxidation time at a rate slightly lower than the rate for hexanal. Moreover, propanal area percent decreased slightly (32.0 to 30.2%) in the mixture of total selected volatiles compared to hexanal (26.7 to 27.4%), which increased slightly with oxidation time. These results with TAG volatiles agree with reported values for methyl ester hydroperoxides from mixtures of O, L, and Ln (4,49). The mixed methyl esters were reported to produce more Ln hydroperoxides at low oxidation levels. At higher oxidation levels, more L hydroperoxides were reported to be produced. Volatile formation thus indicates Ln is a slightly more important oxidation product than L as a precursor at early stages of oxidation. As oxidation proceeds, L becomes slightly more important than Ln as a precursor of oxidation products at later oxidation stages (49).

Further correlations presented in Table 6 indicate that  $\Delta HX$  and  $\Delta PR$  are reduced with oleic acid content and increased with L and Ln content. L at carbon 2 compared to carbons 1,3 increased  $\Delta HX$  and  $\Delta PR$ , while Ln on carbons 1,3 slightly increased  $\Delta HX$  and  $\Delta PR$  compared to Ln on carbon 2. Thus, volatile formation was affected by types of unsaturated fatty acids, their interactions and TAG structure.

*Oxidative stability studies by analysis of TAG oxidation products.* A typical RP-HPLC chromatogram of TAG hydroperoxides formed at low levels of oxidation (PV = 16) is shown in Figure 3. Hydroperoxides (OOH) and cyclic

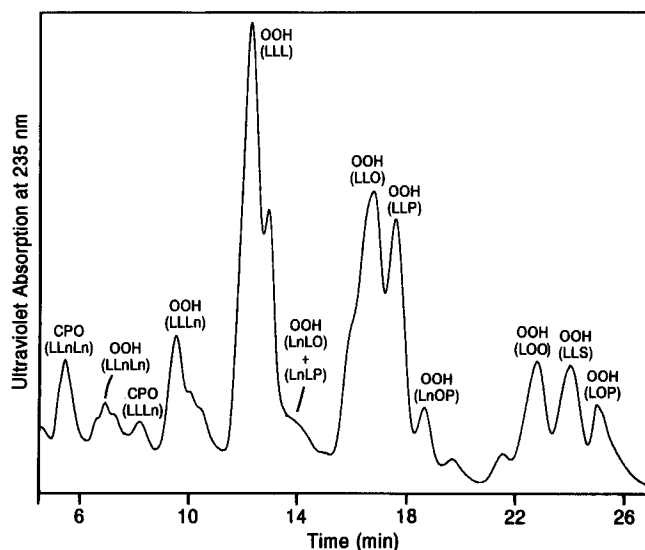


FIG. 3. RP-HPLC resolution of oxidized soybean oil triacylglycerols (PV = 16). Sample: 5–10 mg; 3  $\mu$  C-18 column (0.62  $\times$  8.0 cm); solvent: acetonitrile/methylene chloride (80:20, v/v), isocratic elution at 1.0 mL/min; UV detector at 235 nm. Column cleaned with methylene chloride to remove unoxidized triacylglycerols after analysis. Triacylglycerol oxidation products: —OOH = Monohydroperoxide; —CPO = hydroperoxy epidioxide.

peroxides (CPO) formed from major TAGs were identified by procedures given in the experimental section.

As presented in Table 4, the  $\Delta OOH$  showed a slight reduction as oxidation proceeds for Ln-containing TAG compared to non-Ln-containing TAG. The rate of decrease for the unique Ln oxidation product, LLnLn cyclic peroxide ( $-0.021$  area percent/min), compared to the rate of increase in the unique L oxidation product, LLL-OOH (0.015 area percent/min), was more significant. These results and our findings for L and Ln volatiles support previous work reported by others, which showed that Ln is a more important oxidation precursor at early oxidation time (4,5,49). While, at later oxidation times, L is a more important oxidation product precursor.

The experimental results presented here may be useful for the development of soybean varieties with TAG compositions that exhibit natural resistance to flavor deterioration.

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