Effect of Triacylglycerol Composition and Structures on Oxidative Stability of Oils from Selected Soybean Germplasm[1]

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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 autoxidative stability of 13 types of TAG molecular species, and they reported that autoxidative 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), chorophyll (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, Zorbex, 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^3/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 ± 2°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 (ΔPV) was determined from linear regression of the plot of PV *versus* time. Comparisons between oxidation experiments were valid because the ΔPV 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 μ Zorbax ODS column, 8 × 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 μL methylene chloride. The formation of oxidation products from LLnLn, LLLn, 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°C. A 50-mg sample of TAG was sealed in a 20-mL clean vial, heated to 140°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 × 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°C for 1 min and then temperature programmed to 100°C at 30°C/min. To clean the GC column for the next run, the oven was heated to 250°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: $[0.02 \times (O) + 1 \times (L) + 2 \times (Ln)]$} (46), with respect to rate of formation of peroxides (ΔPV) and headspace volatiles (ΔTV, ΔPR, ΔHX). Correlations were determined between ΔPV, rate of formation of total selected volatiles (ΔTV), rate of formation of propanol (ΔPR), and rate of formation of hexanal (ΔHX) 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 ΔPV and ΔTV 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 ΔPV. Samples are listed in increasing order of calculated oxidizability. Samples 4–6, 8, 16 and 20 have ΔPV values that indicate greater stability than predicted by the calculated oxidizability, while ΔPV 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 ΔPV with TAG composition and structure. Peroxide values determined at each oxidation period and ΔPV obtained by linear regression analysis were correlated with TAG composition and structure for 20 different soybean varieties and the results are presented in Table 3. The ΔPV 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.

TABLE 1

Fatty Acid Composition, Oxidizability and Stability of Soybean Oil Triacylglycerols

Sample number	Fatty acid composition				
	0	L	Ln	OX[a]	ΔPV[b]
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

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

[b]Slope obtained by linear regression plot of PV *versus* time.

Correlations developed for PV and for ΔPV 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 ΔPV showed strong positive correlations with L and Ln located on the internal carbon. A low correlation value for ΔPV 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 ΔPV 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 ΔPV had a significant, positive correlation with those TAG molecular species containing Ln. As indicated by the correlation of ΔPV 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

TABLE 2

Fatty Acid Ratios at Glyceride Carbons[a], Oxidizability and Stability of Soybean Oil Triacylglycerols

Sample number	Glyceride carbon number: 2 L/O	2 L/Ln	1,3 L/O	1,3 L/Ln	1,2;1,3 L/O	1,2;1,3 L/Ln	OX[b]	ΔPV[c]
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

[a]Composition at glyceride carbon atoms determined by lipolysis procedures (43).

[b]Oxidizability = (0.02 [0%] + [L%] + 2 [Ln%])/100 (46).

[c]Slope obtained by linear regression plot of PV *versus* time.

TABLE 3

Correlation Coefficients[a] of ΔPV[b] 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	PV 48 hr	PV 72 hr
Oxidizability[c]	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[d]	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

[a]Procedure (44).

[b]Slope obtained by linear regression plot of PV *versus* time.

[c]Oxidizability = (0.02 [0%] + [L%] + 2 [Ln%])/100 (46).

[d]NS = not significant, (P>0.05; >95% confidence level).

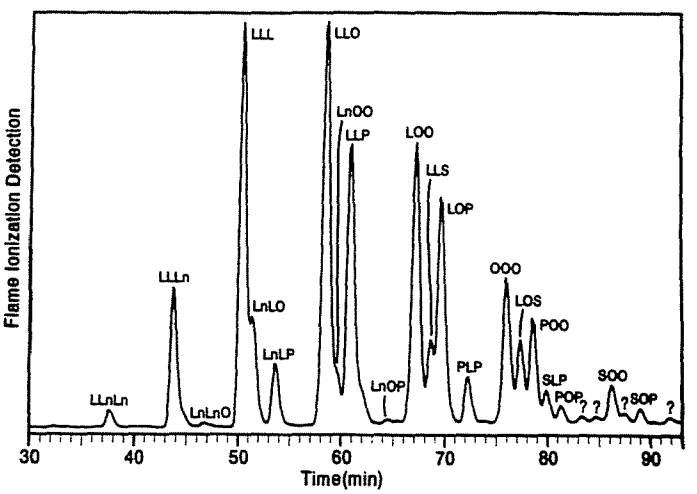

FIG. 1. RP-HPLC analysis of purified soybean oil triacylglycerols. Sample (#8, Table 1): 0.5–1.0 mg; 5 μ C-18 column (0.49 × 50 cm); 120 min solvent gradient acetonitrile:methylene chloride (70:30 to 40:60 v/v); flow rate, 0.8 mL/min; FID detector. Column cleaned with methylene chloride after analysis.

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 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

TABLE 4

Correlation of ΔPV and ΔTV with Major Soybean Oil Triacylglycerol Molecular Species

TAGMS	Content[a] (%)	Correlation coefficients[b] ΔOOH[c]	ΔPV[d]	ΔTV[e]
LnLL	1.5 - 11.6	0.023	0.85	0.60
LLL	11.7 - 26.5	0.015	0.38 NS[f]	0.54
LnLO	1.6 - 5.6	ND[g]	0.74	0.40 NS
LnLP	1.0 - 5.6	ND	0.88	0.50
LLO	12.9 - 20.8	0.006	−0.65	−0.16 NS
LLP	9.6 - 16.9	−0.002	0.79	0.62
LOO	3.9 - 14.9	0.013	−0.82	−0.63
LOP	6.1 - 13.3	0.004	−0.60	−0.50
OOO	1.0 - 9.5	ND	−0.76	−0.66
OOP	0.9 - 5.5	ND	−0.80	−0.67

[a]Range of content of triacylglycerol molecular species in 20 soybean varieties.

[b]Procedure (44).

[c]Slope of linear regression plot of area % of hydroperoxides formed with oxidation time.

[d]Slope obtained by linear regression plot of PV *versus* time.

[e]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.

[f]NS = not significant.

[g]ND = not determined.

TABLE 5

Rate of Volatile Formation[a] and Oxidizability of Soybean Oil Triacylglycerols

Sample number	Volatiles ΔTV[b]	ΔHX[c]	ΔPR[d]	OX[e]	ΔPV[f]
1	0.251	0.084	0.065	0.536	0.135
2	0.393	0.127	0.125	0.553	0.152
3	0.254	0.069	0.064	0.579	0.156
5	0.624	0.204	0.099	0.617	0.147
6	0.854	0.256	0.292	0.613	0.130
7	0.706	0.173	0.240	0.618	0.229
8	0.482	0.154	0.144	0.620	0.221
9	0.668	0.160	0.157	0.628	0.181
13	2.491	0.711	0.568	0.755	0.490
14	2.214	0.535	0.504	0.760	0.281
16	0.991	0.317	0.320	0.771	0.213
17	1.810	0.539	0.492	0.780	0.444
18	1.498	0.419	0.459	0.805	0.480
20	2.254	0.843	0.949	0.810	0.370

[a]Slope obtained by linear regression of peak area *versus* time.

[b]Procedure (44).

[c]Linear regression of FID peak areas for hexanal *vs* time.

[d]Linear regression of FID peak areas for propanal *vs* time.

[e]Oxidizability = (0.02 [0%] + [L%] + 2 [Ln%])/100 (46).

[f]Slope obtained by linear regression plot of PV *versus* time.

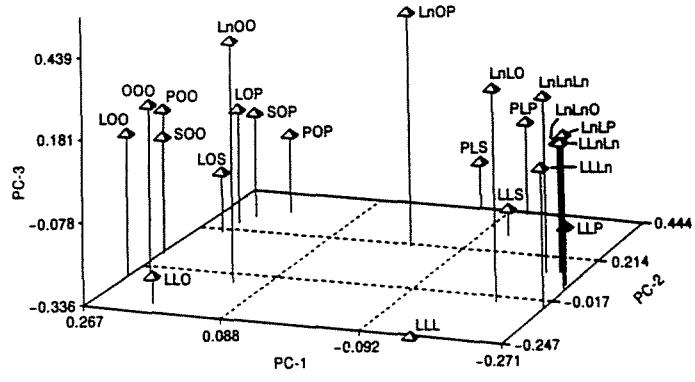

FIG. 2. Principal-component analysis (PC) (45) of influence on ΔPV from interaction of 22 individual triacylglycerol molecular species from 20 soybean varieties. PC-1, assignment to each molecular species with most obvious positive and negative correlations of oxidative stability with other molecular species, with regard to oxidizability, average number of double bonds, etc., accounts for 57% of similarities and differences among the molecular species; PC-2, assignment of correlations left over after effects of PC-1 considered; 19% of total variation explained; PC-3, assignment of effects not considered by PC-1 and -2; 16% of total variation explained. Numbers assigned to each molecular species by the 3 PCs are shown. Triacylglycerol composition by RP-HPLC, see Figure 1.

general, is a method of simultaneously considering correlation among several variables and of grouping variables that tend to react in a consistent manner with regard to one another. Each principal component is defined in Figure 2. In this experiment, as the ΔPV changed, those TAG that changed in similar patterns were grouped together. Figure 2 shows that LLLn, LnLO, LLnLn, LnLP, LnLnO, LnLnLn, LLP and LLS, grouped to the rear right side of the graph, tended to be oxidized similarly when PV changed. The LnOO, POP, SOP, LOS and LOP can be considered as another loosely structured group, while SOO, OOO, POO and LOO can be considered as a second group. LnOP, PLS and LLO do not appear to fit any group. Plots such as Figure 2 cannot be used quantitatively, but are useful for showing oxidative relationships.

Oxidative stability studies by correlation of volatiles with TAG composition and structure. Soybean oil produces volatiles at low oxidation levels (<1%, PV = 5-20) (4,5,15). Mechanisms for this deterioration are not well understood and are controversial. Soybean oil TAGs are potential precursors to 14 positionally isomeric hydroperoxides formed upon oxidation of O, L and Ln (5). Data for ΔTV, ΔPR, and ΔHX for TAGs from 14 soybean samples are presented in Table 5. The ΔTV, ΔPR, and ΔHX are correlated with calculated oxidizability, degree of unsaturation, and TAG unsaturated fatty acid content and position in the glyceride. The correlation coefficients are presented in Table 6. The ΔTV had a high correlation with calculated oxidizability and with degree of unsaturation. These results are in agreement with the correlation for ΔPV with oxidizability and degree of unsaturation presented in Table 3. As expected, there is a high positive correlation of ΔTV with L and Ln and a high negative correlation with oleic acid content. Rate of change of total volatiles showed a slight negative correlation with oxidative stability when oleic acid was on carbon 2 compared to carbons 1,3. This agrees with the correlations determined for ΔPV with oleic acid (Table 3). Also, oxidative stability may be slightly improved with L on glycerol carbons 1 and 3 compared to carbon 2, which agrees with correlations with ΔPV. Further, oxidative stability may be slightly reduced with Ln on carbons 1 and 3 compared to carbon 2.

TABLE 6

Correlation of ΔTV, ΔHX and ΔPR with Oxidizability, Degree of Unsaturation, and Soybean Oil Triacylglycerol Unsaturated Fatty Acid Content and Position at Glyceride Carbons

Factor	Correlation coefficients[a]		
	ΔTV[b]	ΔHX[c]	ΔPR[d]
Oxidizability[e]	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

[a]Procedure (44).

[b]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.

[c]Linear regression of FID peak areas for hexanal *vs* time.

[d]Linear regression of FID peak areas for propanal *vs* time.

[e]Oxidizability = (0.02 [0%] + [L%] + 2 (Ln%])/100 (46).

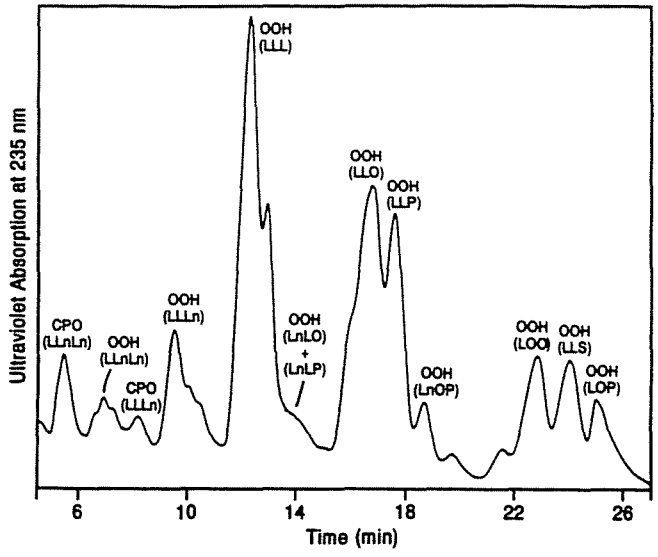

FIG. 3. RP-HPLC resolution of oxidized soybean oil triacylglycerols (PV = 16). Sample: 5–10 mg; 3 μ C-18 column (0.62 × 8.0 cm); solvent: acetonitrile/methylene chloride (80:20, v/v), isocratic elution at 1.0 mL/min; UV detector at 235 nm. Column cleaned with methylene chloride to remove unoxidized triacylglycerols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

Correlations for ΔHX, from L, and ΔPR, from Ln, are also presented in Table 6. Both ΔHX and ΔPR 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 ΔHX and ΔPR are reduced with oleic acid content and increased with L and Ln content. L at carbon 2 compared to carbons 1,3 increased ΔHX and ΔPR, while Ln on carbons 1,3 slightly increased ΔHX and ΔPR 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 peroxides (CPO) formed from major TAGs were identified by procedures given in the experimental section.

As presented in Table 4, the ΔOOH 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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