

Photooxidation of Soybean Oils as Affected by Triacylglycerol Composition and Structure¹

W.E. Neff*, T.L. Mounts, W.M. Rinsch and H. Konishi²

Food Quality and Safety Research, National Center for Agricultural Utilization Research, Agricultural Research Service, United States Department of Agriculture, Peoria, Illinois 61604

The photooxidation of soybean oil was determined and correlated with triacylglycerol composition and structure. Purified triacylglycerols were photooxidized at room temperature under fluorescent light. Rates of peroxide formation and total headspace volatiles were related positively ($P < 0.5$ significance) to oxidizability ($r = 0.75$, $r = 0.76$); content of linolenic acid ($r = 0.80$, $r = 0.85$) and linoleic acid ($r = 0.61$, $r = 0.57$); linoleic acid on carbon 2 ($r = 0.64$, $r = 0.64$); and average number of double bonds ($r = 0.76$, $r = 0.76$). Negative correlations were observed with respect to oleic acid ($r = -0.70$, $r = -0.70$). Soybean oil stability was decreased by linolenic acid-containing triacylglycerols and increased by oleic acid-containing triacylglycerols. Trilinoleoylglycerol and dilinoleoyl-oleoylglycerol were the most important oxidation product precursors. However, for high-linolenic acid soybean oil, dilinoleoyl-linolenoylglycerol and trilinoleoylglycerol were the most important oxidation product precursors. The most abundant volatile produced from thermal decomposition at 140°C of photooxidized triacylglycerols was 2-heptenal, except for high-linolenic acid oils, where the most abundant volatile was propanal. The photooxidative stability of soybean oil triacylglycerols with respect to composition and structure is of interest for the development of soybean varieties with oils of improved odor and flavor stability.

KEY WORDS: Fluorescent light, oxidative stability, peroxide value, photooxidative stability, soybean oil photooxidation, stereospecific analysis, total volatiles, triacylglycerols.

Oxidative deterioration of fats and oils can be initiated by metals, heat, light and prooxidation sensitizers, which can be activated by light (1,2). The resulting oxidation reactions produce free radicals that react with oxygen to form oxidation products, which include hydroperoxides and cyclic peroxides. The oxidation products can decompose under the influence of metals, heat, light and free radicals, to produce a variety of volatile products. These products can produce undesirable flavors and odors in oils, fats and fat products (1-5). How a fat resists oxidation depends on the triacylglycerol (TAG) composition and positional structure (6) and the non-TAG components like tocopherols, carotenoids, chlorophyll, free fatty acids and sterols, which may promote or retard oxidation (1,2,7-11).

We previously investigated the oxidative stability of purified TAG from soybean oils (SBO) oxidized in air in the dark at 60°C (6), and we showed that oxidative stability in the dark correlated positively with increased oleic (O) and decreased linoleic (L) and linolenic (Ln) acid

contents of SBO TAG (6). There is continuing interest in how light influences oxidation of vegetable and animal fats. A recent review by Bekbölet on light-influenced catalyzed changes in dairy, meat, fat and oil products cited 176 references (12). No previous studies reported the photooxidative stability of purified SBO TAG as reported here.

EXPERIMENTAL PROCEDURES

Materials. Soybeans [*Glycine max* (L.) Merr.] were commercial cultivars obtained from the U.S. Department of Agriculture Germplasm Collection (University of Illinois, Urbana, IL), which included plant introductions selected on the basis of fatty acid composition and experimental varieties obtained from the U.S. Department of Agriculture Germplasm Collection (North Carolina State University, Raleigh, NC). All other required materials were described previously (6).

Methods. Oil extraction, TAG purification, peroxide value (PV), static headspace-gas chromatography, TAG composition by reversed-phase high-performance liquid chromatography (HPLC), TAG structure by lipolysis, fatty acid analysis by gas chromatography (GC) and statistical methods were described previously (6).

Photooxidation of purified TAG. Four purified TAG samples (225 mg each) were placed in capped vials with oxygen in the headspace, including three samples from the same SBO variety—one wrapped with aluminum foil to exclude light, plus one control SBO and photooxidized at $25.0 \pm 1.0^\circ\text{C}$ in a photochemical reactor (Model RPR-100, The Southern New England Ultraviolet Co., Hamden, CT). Two fluorescent lamps (Sylvania Cool White F8T5/CW, 8 watts, GTE Sylvania Inc., Danvers, MA) were placed 180° apart in the reactor. Light intensity was 545 foot candles (FC) at the surface of the sample vials. Vials were placed on an electrically driven turntable (5 revolutions per min) to allow equal irradiation of all samples.

The aluminum foil-wrapped vial was included to determine the differences between photooxidation and autoxidation for the same SBO variety at the same temperature (25°C). Analytical samples were removed from each TAG at 24, 48 and 72 h of reaction, *i.e.*, three 15-mg samples for PV determination by the colorimetric ferric thiocyanate method, two 50-mg samples for volatile headspace analysis (HS-GC) and one 50-mg sample for oxidation product analysis by reverse-phase high-performance liquid chromatography (RP-HPLC) and, after derivatization to methyl esters, by capillary GC (6).

TAG oxidation product analysis. RP-HPLC of the oxidized TAGs (TAG-OOH) was modified from the previous procedure (6). HPLC conditions were: 5 μ Vydac ODS column, 25 \times 0.46 cm (The Separations Group, Hesperia, CA); mobile phase was methylene chloride/methanol/acetonitrile (5:5:90, vol/vol/vol) at a flow rate of 0.8 mL/min; ultraviolet (UV) detector was set at 235 nm for conjugated diene functionality of oxidized fatty acid (FA) with

¹ Presented at the 20th ISF World Congress 83rd Annual American Oil Chemists' Society Meeting, May 10-14, 1992, Toronto, Canada.

*To whom correspondence should be addressed at 1815 N. University St., Peoria, IL 61604.

² Visiting Scientist from Technical Research Institute, Snow Brand Milk Products Co., Ltd., Saitama, Japan.

sensitivity set at 0.4 absorbance units. Sample size was 10 μ L of a solution of 52 mg TAG-OOH per 100 μ L hexane. The formation of oxidation products from Ln₂L, LnL₂, L₃, LnLO, LnLP (P, palmitic acid), L₂O, L₂P, LO₂, L₂S (S, stearic acid) and LOP was monitored. Less polar TAG-OOH were not eluted by the isocratic mobile phase and were removed by methylene chloride stripping of the column before the next injection. The TAG-OOH that were eluted were derived from about 90% of the oxidation precursor TAG in the SBO. The remaining 10% TAG are highly saturated, *i.e.*, PLP or LOS, each less than 1.0% of the total TAG mixture and do not contribute significantly to TAG-OOH. Identification of TAG-OOH was made by matching HPLC retention times with UV detection at 235 nm with standard TAG-OOH as described previously (6).

RESULTS AND DISCUSSION

The oxidation apparatus described above was employed for light-influenced oxidation of purified TAG to levels typical of flavor deterioration of SBO under mild storage conditions while exposed to fluorescent light (5,12). Oxidation levels for TAG oxidized under fluorescent light (545 FC), measured by PV, ranged from 2.8 to 18.9 at 24 h, 4.9 to 32.7 at 48 h and 6.9 to 58.7 meq/kg at 72 h depending on oil composition. Oxidation was definitely light-promoted, because the same oils at 25°C in the dark in the same apparatus showed little oxidation, *i.e.*, PVs ranged from 0.5 to 2.4 at 24 h, 0.9 to 3.3 at 48 h and 1.0 to 4.7 meq/kg at 72 h depending on oil composition.

Correlation of PV and Δ PV with TAG structure. The unsaturated fatty acid composition and location at the glycerol carbons of the TAG, isolated from seven soybean varieties, are presented in Table 1. Significant differences in the composition of O, L and Ln permitted valid comparison of oil oxidative stability with respect to FA composition. Samples are listed in increasing order of calculated oxidizability (OX), which is based on unsaturated FA composition (13).

Plots of PV with oxidation time are presented in Figure 1 for the photooxidation of seven different purified

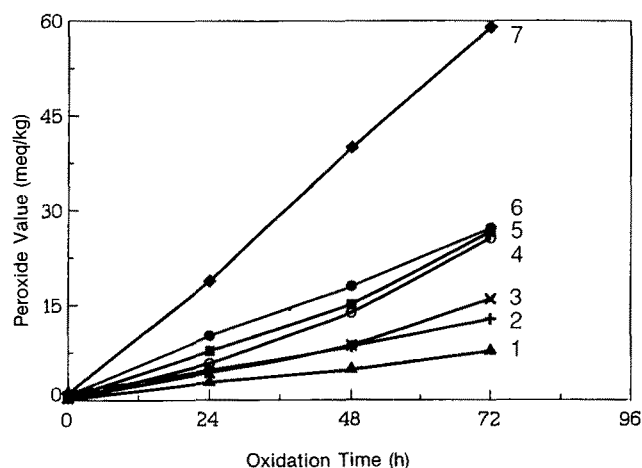


FIG. 1. Photooxidation of soybean oil triacylglycerols. Samples 1-7 as per Table 1.

SBO TAG samples. The slopes, obtained by linear regression analysis (6), of the PV *vs.* time plots are a measure of the oil oxidative stability or experimental OX (Δ PV) and are also presented in Table 1. The Δ PV for photooxidation increased in the same order as OX.

Peroxide values determined at 24, 48 and 72 h and Δ PV for each of the TAG studied are correlated by statistical procedures (6) with FA composition and glycerol position in Table 2. The Δ PV and PV at each oxidation time correlated positively with OX and degree of unsaturation for each TAG. The negative correlation of Δ PV and PV with O content showed that O strongly improved oxidative stability. On the other hand, Ln content showed a strong positive correlation, indicating that Ln decreased oxidative stability. Content of L, compared to Ln, showed less of a positive correlation for reduction of TAG stability.

Correlation with glycerol position indicated that the positioning of O or Ln on the internal, compared to the external, carbons apparently has little influence on Δ PV. However, the difference in the correlation coefficients for L positioned on the internal glycerol carbon is significant

TABLE 1

Fatty Acid Composition, Composition at Glycerol Carbons^a of Triacylglycerol, Oxidizability (OX) and Photooxidative Stability of Soybean Oil Triacylglycerols

Sample number	Variety ^b number	Fatty acid composition (%)									OX ^d	Δ PV ^c Light ^e
		Fatty acid composition (%)			2-position			1,3-position				
		O	L	Ln	O	L	Ln	O	L	Ln		
1	N 85-2176	50.5	34.4	2.8	51.9	44.6	2.6	49.8	29.3	2.9	0.4101	0.1032
2	N 89-2009	30.2	51.7	3.6	29.7	65.0	3.4	30.4	45.1	3.8	0.5950	0.1591
3	N 83-375	28.1	50.2	6.4	29.1	64.7	5.6	27.9	42.6	6.9	0.6360	0.2133
4	Hardin-90	24.5	53.6	8.0	24.3	67.9	6.5	24.6	46.5	8.7	0.7009	0.3494
5	326.580	16.5	55.4	11.7	15.2	73.8	10.2	17.1	46.2	12.5	0.7913	0.3584
6	291.277	15.3	56.8	11.9	11.6	76.5	11.2	17.1	47.0	12.3	0.8090	0.3628
7	401.418	15.4	56.2	12.5	14.9	73.9	10.4	15.7	46.7	13.1	0.8151	0.7982

^a Composition of glyceride carbon atoms determined by lipolysis procedure (6). O, oleic; L, linoleic; Ln, linolenic; PV, peroxide value.

^b Sample varieties 1-3 and 4-7 from germplasm collections at North Carolina State University and University of Illinois, respectively.

^c Slope obtained by linear regression plot of peroxide value *vs.* time.

^d OX = (0.02 [O%] + [L%] + 2 [Ln%])/100 (13).

^e Triacylglycerols oxidized at 25°C in oxygen under 545 foot candles fluorescent light.

PHOTOOXIDATION OF TRIACYLGLYCEROLS

TABLE 2

Correlation of Δ Peroxide Value (Δ PV)^a and PV^b with Oxidizability,^c Degree of Unsaturation, Unsaturated Fatty Acid Content and Position at Glyceride Carbons^d

Factor	Correlation coefficients ^e			
	Δ PV	PV		
		24 h	48 h	72 h
Oxidizability	0.75	0.73	0.72	0.75
Degree of unsaturation	0.76	0.73	0.72	0.76
Oleic acid	-0.70	-0.68	-0.67	-0.71
Linoleic acid	0.61	0.58	0.58	0.62
Linolenic acid	0.80	0.78	0.77	0.80
Oleic acid/carbon 2	-0.68	-0.67	-0.65	-0.68
Oleic acid/carbon 1,3	-0.72	-0.69	-0.68	-0.72
Linoleic acid/carbon 2	0.64	0.63	0.61	0.64
Linoleic acid/carbon 1,3	0.55	0.51	0.51	0.55
Linolenic acid/carbon 2	0.74	0.75	0.72	0.74
Linolenic acid/carbon 1,3	0.80	0.77	0.76	0.79

^aSlope obtained by linear regression plot of PV vs. time.

^bPV procedure (6).

^cOxidizability = (0.02 [0%] + [L%] + 2 [Ln%])/100 (13); O = oleic acid; L, linoleic acid; LN, linolenic acid.

^dComposition at glyceride carbon atoms determined by lipolysis procedure (6).

^eStatistical procedure (6).

and indicates that L positioned on the internal glycerol carbon reduced oxidative stability.

Correlation of PV and Δ PV with TAG species. Quantitative analyses of SBO TAG as determined by RP-HPLC are presented in Table 3. These analyses detected 18 to 23 TAG species, in agreement with previous studies (6,14). The 15 predominant TAG species are listed in Table 3. The major TAG species present in most SBO are LnLL, LLL, LLO, LLP, LOO and LOP (6,15,16). Sample 1, however, also contains OOO as a major TAG species. The range in composition of the major TAG species allowed correlation of oil oxidative stabilities with TAG species composition.

Correlations of the percent compositions of TAG species with Δ PV are presented in Table 3. Correlation of Δ PV is strongly positive for most L- and Ln-containing TAG. While no significant correlation of Δ PV with LLL was observed, replacement of one L of LLL with O, as in LLO, increased oxidative stability, i.e., negative correlation (-0.57) with Δ PV. Replacement of 2 L of LLL with 2O, as in LOO, increased stability somewhat compared to LLO. Unexpectedly, replacement of one L of LLL with P decreased oxidative stability with respect to LLO. Moreover, a greater decrease in oxidative stability was observed for replacement of one L of LLL with S compared to LLP ($r = 0.65$ to 0.80). Oxidative stability increased sequentially with TAG species OOO, SOO, POO, LOP, LOO and LOS ($r = -0.58$ to -0.79).

A three-principal component analysis (PC) (6) was performed on the correlations of Δ PV to evaluate the interaction of TAG species with respect to photooxidative stability. The PC analysis shown in Figure 2 illustrates TAG groupings clustered in similar patterns with respect to Δ PV. It was found that the LLnLn, LLLn, LnLO, LnLP, LLP and LLS species had reduced photooxidative stability (i.e., greater Δ PV). On the other hand, OOO, LOO, POO, SOO, LOS, LOP had increased photooxidative stability

TABLE 3

Correlation of Δ Peroxide Value (Δ PV)^a and Total Selected Volatiles (TV)^b with Triacylglycerol Molecular Species (TAG-MS)^c

TAG-MS	Content ^d		Correlation coefficients ^e	
	Average (%)	Range (%)	Δ PV	Δ TV
LnLnL	—	0.0-2.4	0.77	0.91
LnLL	7.2	1.5-12.0	0.79	0.87
LLL	15.4	7.2-21.1	0.48, NS ^f	0.49, NS
LnLO	4.7	2.6-7.2	0.67	0.67
LnLP	3.8	0.7-6.8	0.81	0.86
LLO	14.9	11.7-19.5	-0.57	-0.68
LLP	13.1	7.5-17.9	0.65	0.71
LOO	8.6	3.6-16.5	-0.73	-0.80
LLS	3.5	2.2-4.5	0.80	0.77
LOP	8.8	6.6-10.2	-0.70	-0.91
PLP	2.1	0.9-2.9	0.73	0.72
OOO	4.8	0.8-18.3	-0.58	-0.53
LOS	2.8	1.6-4.2	-0.79	-0.89
POO	3.3	0.8-8.9	-0.67	-0.65
SOO	1.1	0.2-2.9	-0.65	-0.65

^aSlope obtained by linear regression plot of PV vs. time.

^bSlope of linear regression plot of TV vs. time.

^cComposition obtained by reversed-phase high-performance liquid chromatography with flame-ionization detection (6). Individual triglyceride, i.e., LnL₂ triglyceride with one linolenic and two linoleic acids. Abbreviations as in Table 2, footnote c.

^dRange of content of TAG-MS in the seven soybean varieties.

^eStatistical procedure (6).

^fNS = not significant; ($P > 0.05$; <95% confidence level).

(i.e., lower Δ PV). The TAG species LLL did not fit any group with respect to Δ PV.

Correlation of volatiles with TAG composition and structure. A GC chromatogram of volatiles generated by thermal decomposition of photooxidized TAG (PV 26.4) is presented in Figure 3. Pentane, propanal, pentanal, hexanal, 2-heptenal and 2,4-heptadienal were the major volatiles. These have been identified previously by GC and GC-mass spectrometry (MS) of oxidized SBO and related to retention and mass spectra of authentic compounds (17-19). Other volatiles in Figure 3 were 2-pentenal, octanal, nonanal, 2-decenal and 2-pentylfuran.

The composition of the major volatiles generated from photooxidized TAG are in Table 4. The relative composition of each volatile did not change significantly with oxidation time; therefore, the values presented are an average of the composition of the 24-, 48- and 72- h samples. The Ln-derived volatiles, propanal and 2,4-heptadienal, in general, increased with increased Ln content of the TAG. However, L-derived volatiles, hexanal and 2-heptenal, decreased with increased L content. The ratio of L/Ln decreased from sample 2 through 7 (Table 1). Sample 1 had a lower L/Ln ratio than sample 2, due to low L (34.4%) content. Therefore, sample 1 had a greater ratio of total selected Ln volatiles to L volatiles than did sample 2. However, the Ln/L volatile ratio increased with increased Ln and decreased L/Ln ratio for samples 2 to 7. With the exception of high Ln (12%) containing SBO samples, which had propanal as the major volatile, 2-heptenal was the major volatile for photooxidized TAG.

Plots of total selected volatiles generated from photooxidized TAG vs. oxidation time are presented in Figure 4.

PHOTOOXIDATION OF TRIACYLGLYCEROLS

TABLE 4

Selected Volatiles^a from Thermal (140°C) Decomposition of Photooxidized Triacylglycerols (TAGs)^b

Sample number	Selected volatiles ^c						Volatile ratio ^d Ln/L
	Area percent [average of oxidized soybean oil (SBO) at 24, 48, 72 h]						
	Pentane	Propanal	Pentanal	Light hexanal	Light 2-heptenal	Light 2,4-heptadienal	
1	1.3	18.2	11.9	27.6	34.8	6.3	0.324
2	3.2	19.4	12.2	25.3	37.7	2.2	0.276
3	3.5	23.0	12.1	23.2	33.9	4.3	0.376
4	11.4	22.8	12.0	14.4	29.9	9.6	0.480
5	5.6	28.0	10.3	20.8	27.2	8.2	0.565
7	15.5	27.8	9.0	15.0	20.0	12.7	0.688

^aComposition obtained by gas-chromatography-mass spectrometry analysis (6).^bTAGs oxidized neat in oxygen at 25°C under fluorescent light (545 foot candles).^cThese were the major SBO volatiles detected.^dVolatile ratio = Ln-derived volatiles: (propanal + 2,4-heptadienal) to L-derived volatiles: (pentane + pentanal + hexanal + 2-heptenal). Ln, linolenic acid; L, linoleic acid.

TABLE 5

Correlation of ΔTV ,^a ΔHX , ΔPR , ΔHT , $\Delta PANE$, ΔPAL and $\Delta DIENAL$ ^b with Oxidizability, Degree of Unsaturation, Unsaturated Fatty Acid Content and Position at Glyceride Carbons

Factor	Correlation coefficients ^c						
	ΔTV	ΔHX	ΔPR	ΔHT	$\Delta PANE$	ΔPAL	$\Delta DIENAL$
Oxidizability ^d	0.76	ND ^e	ND	ND	ND	ND	ND
Degree of unsaturation	0.76	0.77	0.73	0.89	0.60	0.87	0.68
Oleic acid	-0.70	-0.72	-0.67	-0.88	-0.54	-0.86	-0.62
Linoleic acid (L)	0.57	0.58	.53	0.75	0.43 N.S.	0.73	0.49 N.S.
Linolenic acid (Ln)	0.85	0.86	0.83	0.94	0.69	0.92	0.79
Oleic acid/carbon 2	-0.70	-0.71	-0.67	-0.88	-0.53	-0.86	-0.62
Oleic acid/carbon 1,3	-0.71	-0.72	-0.68	-0.88	-0.55	-0.86	-0.63
Linoleic acid/carbon 2	0.64	0.65	0.60	0.83	0.48 N.S.	0.82	0.55
Linoleic acid/carbon 1,3	0.47 N.S.	0.48 N.S.	0.44 N.S.	0.66	0.35 N.S.	0.65	0.40 N.S.
Linolenic acid/carbon 2	0.84	0.86	0.82	0.96	0.67	0.95	0.77
Linolenic acid/carbon 1,3	0.83	0.85	0.81	0.94	0.67	0.93	0.77

^aSlope of linear regression plot of sum of selected volatiles vs. oxidation time.^bSlopes of linear regression of gas-liquid chromatography peak areas for ΔHX (hexanal), ΔPR (propanal), ΔHT (2-heptenal), $\Delta PANE$ (pentane), ΔPAL (pentanal) and $\Delta DIENAL$ (2,4-heptadienal) with oxidation time.^cStatistical procedure (6) N.S. = not significant $P > 0.05$; <95% confidence level.^dOxidizability = $(0.2 [0\%] + [L\%] + 2 [Ln\%])/100$ (13); O = oleic acid.^eND = not determined.

Therefore, it can be concluded that the same interactive effects of FA of a TAG on oxidative stability as measured by ΔPV were operative for oxidative stability as measured by ΔTV . A PC analysis of TAG interaction (Fig. 5) showed the same TAG that acted together to change ΔPV (Fig. 2) also acting together to increase or decrease ΔTV . Oleate-containing TAG tended to interact as a group to reduce ΔTV . Conversely, Ln-containing TAG species tended to interact to increase ΔTV , while LLL was inclined to act alone and to have a less significant effect on ΔTV .

Hydroperoxide formation during photooxidation of SBO TAG. The selected TAG-OOH compositions, as determined by RP-HPLC, of the samples after 72-h photooxidation are presented in Table 6. The most important TAG-OOH precursor is LLL, followed in a generally decreasing order by LLO, LLP, LOL and LOP for samples 1-4. However, with samples 5-7, which have high Ln (11-12%) composition (Table 1), LnLL was as important as LLL as

a TAG-OOH precursor. This agreed with the increase in the Ln-derived volatiles, propanal and 2,4-heptadienals, compared to the L-derived volatile, hexanal, for samples 5 and 6 (Table 4).

As OX increased, ΔPV , ΔTV and $\Delta TAG-OOH$ increased (Tables 1,5,6). Thus, as the amount of polyunsaturated FA increase in the TAG, the greater were the rates of oxidation as measured by ΔPV and $\Delta TAG-OOH$. As expected, as $\Delta TAG-OOH$ (volatile precursors) increased, the greater became the ΔTV . As the L- and Ln-containing TAG increased in content (sample 7), TAG-OOH were produced 12 times faster than for low L and Ln and high O (sample 1). The ΔTV also was accelerated by $\Delta TAG-OOH$, i.e., ΔTV was about 50 times greater for the higher Ln-containing sample (sample 7) than for the low-Ln and high O (sample 1).

The oxidative-stability results presented here for purified SBO TAG oxidation under light with regard to

