Oxidative Stability of Purified Canola Oil Triacylglycerols with Altered Fatty Acid Compositions as Affected by Triacylglycerol Composition and Structure[1]

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Canola oil triacylglycerols from genetically modified canola lines (InterMountain Canola Co., Cinnaminson, NJ) have been evaluated for their photooxidative and autoxidative stabilities, as influenced by their fatty acid compositions and their triacylglycerol compositions and structures. Purified canola oil triacylglycerols were oxidized in duplicate in fluorescent light at 25°C and in the dark at 60°C under oxygen, and their oxidative deterioration with time was monitored by determining colorimetric peroxide values. Also monitored with time, oxidation products were determined by reversed-phase high-performance liquid chromatography with ultraviolet absorbance detection. Total volatiles, generated by thermal decomposition of the oxidized triacylglycerols, were quantitated by static-headspace gas chromatography. These experimental parameters were statistically correlated with predicted oxidizability, fatty acid composition, position of fatty acids on glycerol carbons and triacylglycerol composition. Oxidative deterioration of canola triacylglycerols correlated negatively with oleic acid composition, with oleic acid content at carbon 2 and with trioleoylglycerol content of the oil. Deterioration was positively correlated with the amount of linolenic acid on nonspecific locations on glycerol carbons 1,2 and 3, the amount of linoleic acid on glycerol carbon 2 and with *sn*-oleoyllinoleoyllinolenoyl glycerol content. Differences in character or quantity of volatile product and triacylglycerol hydroperoxides were low, whether generated during autoxidation or photooxidation of the canola triacylglycerols.

KEY WORDS: Autoxidation, canola oil, oxidation products, oxidative stability, peroxide value, photooxidation, stereospecific analysis, triacylglycerols, volatiles.

Investigations of vegetable oil oxidative stability have generally considered the effect of nontriacylglycerol components of oils, such as tocopherols, carotenoids, free fatty acids and sterols (1-7). However, autoxidative and photooxidative stabilities of oils are also affected by their fatty acid composition, triacylglycerol (TAG) composition and positions of the fatty acids (FA) on the TAGs (8-10).

Previous studies concerning the oxidative stability of canola oil (CNO) have involved mostly refined, bleached and deodorized oils (11-16) that still contained non-TAG compounds. These compounds affect the course of oxidation or oxidative stability (1,11,12,17). Thus, the real effect on oxidative stability of FA composition, TAG composition and fatty acid distribution cannot be determined unless the CNO is free of all non-TAG components.

Much research has been directed toward improving stability of vegetable oils by altering FA composition, such as canola (or low-erucic acid rapeseed) (15,16,18-20), soybean (8,9, 21-23) and peanut oils (24). These altered oils with improved oxidative stability (16,22-24) have been developed by decreasing the content of linolenic (Ln; 18:3) and linoleic acids (L; 18:2) and increasing the content of oleic acid (O; 18:1).

Continuing along the lines of our previous work (8,9) with soybean oil, we report studies of the autoxidation and photooxidation of TAG from various CNO with altered FA composition.

EXPERIMENTAL PROCEDURES

Materials. Canola seed from 11 experimental varieties were obtained from InterMountain Canola Company (IMC) (Cinnaminson, NJ). Variety identifications with IMC numbers are: 100 IMC 01, commercially-grown low α-linolenic acid (ALA) spring canola variety, protected by the Plant Variety Protection Act (PVPA), utility patent pending; 200 IMC 129, commercially-grown high-oleic acid spring canola variety, protected by PVPA, utility patent pending; 300 IMC 144, commercially-grown low-saturates spring canola variety, protected by PVPA, utility patent pending; 400 Westar, commercially-grown generic spring canola cultivar, developed by Agriculture Canada Saskatoon (Saskatchewan, Canada); 500 Legend, commercially-grown generic spring canola cultivar, developed by Svalöf (PVPA No. 8800075; Malmoehus, Sweden); 600 Hyola 41, commercially-grown generic spring canola hybrid cultivar, developed by Zeneca Seeds (Wilmington, DE); 700 Stellar, commercially-grown low-ALA spring canola cultivar, developed by University of Manitoba (Winnipeg, Manitoba, Canada); 800 F116, experimental IMC low-ALA breeding germplasm; 900 MM3200, experimental IMC low-ALA breeding germplasm; 1000, Westar; 1100 89A, experimental IMC low-ALA breeding germplasm.

All other required materials were described previously (8,9). These varieties were selected to provide oils with a gradation of TAG compositions suitable for statistical studies of oxidation results.

Methods. Procedures were based on those described previously (8,9) from soybean oil oxidative stability studies for oil extraction, for TAG purification by solid-phase extraction (SE), TAG composition by reversed-phase high-performance liquid chromatography (RP-HPLC) with flame-ionization detection, FA analysis by capillary gas chromatography (GC), calculated oxidizability (25), TAG structure by lipolysis-GC analysis, dark and light oxidation under a static oxygen atmosphere, determination of peroxide values (PV), total selected volatiles by static-headspace-GC, total selected TAG oxidation products by RP-HPLC with ultraviolet (UV) detection and statistical methods. These methods, with modifications for canola, are given in the next paragraphs.

Extraction. Crude CNO (4.5-6.0 g) was obtained by extraction of 15 g canola seeds in duplicate. Fifteen grams

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of seeds 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 seed mixture was cooled in ice to room temperature. This mixture was filtered over 0.6 g Celite filter aid and 1.2 g charcoal. The filter cake was rinsed five 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 75 min on a roto-evaporator with bath temperature at 25°C. Recovered crude oil was stored neat, under helium, at −20°C.

SE chromatography. The crude CNO was stripped of non-TAG components by SE chromatography. Before chromatography, each oil (1.2 g) was mixed with 840 mg of activated carbon and soaked in 2 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 adsorbent under helium pressure. The oil was eluted with 2.5 mL hexane to give fraction 1 (nonpolar material), 14 mL chromatographed diethyl ether/hexane (10:90, vol/vol) to give fraction 2 (TAG) and 15 mL methanol to give fraction 3 (polar material). Solvent was removed with a roto-evaporator [50 min, water bath temperature 25°C at 0.1 mm (Hg), mechanical vacuum pump].

Analysis of purified TAG. Purified TAG was evaluated by PV analysis of triplicate samples (15 mg each) by the ferric thiocyanate method (8). FA composition was determined by GC of the methyl esters. A 10-mg sample was transmethylated by reaction with 3 mL of 0.5 N KOH in methanol at 50°C for 15 min. The reaction mixture was neutralized to pH 7 with dilute hydrochloric acid and extracted once with 10 mL petroleum ether/diethyl ether (1:1, vol/vol) and dried with 5 mL acetone azeotrope. Fatty acid methyl ester (FAME) samples were analyzed by direct-injection capillary GC with SP2380 column (Supelco, Inc., Bellefonte, PA), 30 m × 0.25 mm i.d. with 0.2 μm film thickness in a Varian gas chromatograph, Star 3400, equipped with a flame-ionization detector (Walnut Creek, CA). The column was operated at 150°C for 35 min, then at 3°C/min to 210°C with a helium head pressure of 10 psi. Injector and detector temperatures were 240 and 280°C, respectively. The procedure was quantitated by analysis of standard FAME mixture 15A (Nu-Chek-Prep, Inc., Elysian, MN).

TAG molecular species (TAGMS) analysis was performed, in duplicate, by using 10-mg samples for (RP-HPLC) with flame-ionization detection and two C-18 (5 μ, 0.49 × 50 cm, Zorbex; Dupont, Inc., Wilmington, DE) 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 (vol/vol) 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 of 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 (8).

Stereospecific analysis. Stereospecific analysis by lipolysis (8) was performed with a 30-mg sample of purified TAG.

Autoxidation 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). Samples (225 mg each) were weighed into 20-mL vials, flushed with oxygen and placed in a 150-mL beaker. Two TAG samples per variety were prepared for each oxidation time (24, 48 and 72 h). Three 15-mg samples were removed from each TAG per time period for PV determination by the colorimetric ferric thiocyanate method in triplicate; two 50-mg samples for volatile headspace analysis in duplicate; and one 50-mg sample for hydroperoxide analysis by RP-HPLC. The formation rate of peroxides (ΔPV) was determined from linear regression of the plot of PV vs. time and was used as a measure of oxidative stability.

Photooxidation of purified TAG. Three purified TAG samples (225 mg each) were placed in capped vials with oxygen in the headspace: with one wrapped in aluminum foil to exclude light, the latter sample to verify photooxidation, and photooxidized at 25 ± 1°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 degrees apart in the reactor. Light intensity was 545 foot candles 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. Analytical samples were removed from each TAG at 24, 48 and 72 h of reaction and analyzed by the same procedures used for dark studies.

TAG oxidation product analysis. RP-HPLC of the oxidized TAGs (TAG-OX) (monohydroperoxides) was modified from the previous procedure. HPLC conditions were: 5 μ Vydac ODS column, 25 × 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; UV detector was set at 235 nm for conjugated diene functionality of oxidized FA with sensitivity set at 0.4 absorbance units. Sample size was 10 μL of a solution of 52 mg TAG-OX per 100 μL hexane. The formation of oxidation products from LnLnO, LnLL, LLL, LnLO, LnLP (P = palmitic), LLO, LNOO, LOO and LOP was monitored. Less polar TAG-OX were not eluted by the isocratic mobile phase and were removed by methylene chloride stripping of the column before the next injection. Identification of TAG-OX was made by matching HPLC retention times with UV detection at 235 nm with standard TAG monohydroperoxide (TAG-OOH). The sum of the peak areas of the TAG-OX, with respect to oxidation time (ΔTAG-OX), was used as a second measure of oxidative stability.

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 a flame-ionization detector (Norwalk, CT). Detector temperature was 180°C. A 50-mg sample of TAG was sealed in a 20-mL vial, heated to 140°C and held for 20 min in the magazine of the headspace sampler. The headspace sampler was placed into the injection position and pressurized for 0.5 min. Volatiles generated were automatically transferred onto a Durabond DB 1701 capillary column (30 m × 0.32 mm i.d. with 1 μm film thickness; J&W

Scientific, Folsom, CA) after the helium flow was interrupted. Helium velocity for the column was 24 cm/s. The GC oven was held at 50°C for 1 min and temperature-programmed to 100°C at 10°C/min, then to 250°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. One sample was analyzed every 30 min with this procedure. Formation of 14 volatiles was monitored as a measure of TAG oxidation. The flame-ionization detector response was monitored by a real-time computer programmed to calculate peak areas. The sum of the peak areas of these selected volatiles (TV), with respect to oxidation time, was used as a third measure of experimental oxidizability (ΔTV).

The methyl hydroxy unsaturated esters of the hydroperoxy TAG-OX mixtures were prepared by transmethylation and sodium borohydride reduction (8) and analyzed by capillary GC. Capillary GC conditions were: SP2380 30 m × 0.25 mm i.d. column with a 0.20-μm film (Supelco, Inc., Bellefonte, PA), temperature-programmed from 180°C, hold for 12 min, then at 5°C/min to 220°C with temperature hold for 20 min. Injector and detector temperatures were 240 and 280°C, respectively. Helium inlet pressure was 15 psi. The contribution of O, L and Ln as precursors to CNO monohydroperoxides was determined by the GC flame-ionization detector response to the respective O, L and Ln monohydroperoxide derivatives, methyl hydroxy oleate (enols), methyl hydroxy linoleate (dienols) and methyl hydroxy linolenate (trienols), respectively (26,27).

Statistical methods. Correlation coefficients ($P < 0.05$) were calculated for oxidizability (25), O, L, Ln fatty acid composition, location of O, L and Ln on TAG glycerol carbons 1, 2, 3 and for TAG molecular species composition with respect to ΔPV, ΔTAG-OX and ΔTV (8,28).

RESULTS AND DISCUSSION

Compositional analyses for 11 canola TAGs are presented in Table 1 (FA composition) and Table 2 (TAG composition). The calculated oxidizability (25) of each sample based on its FA composition is also reported in Table 1. Variety IMC 600 had the highest calculated oxidizability and, therefore, would be expected to have the lowest oxidative stability, whereas variety IMC 200 should have the highest oxidative stability because it has the lowest calculated oxidizability. The recurrent selection breeding experiments showed considerable modification of the Ln content, which was lowered from 9 (IMC 600) to 2% (IMC 700). However, IMC 200 was predicted to be the most stable due to its high O (81%) and lower L and Ln content and high content of OOO (49.5%). Oxidative deterioration is directly related to the polyunsaturated FA content of the oil. As shown in Table 1, the oxidizable FAs found in highest concentration were O (60–81%), L (6–23%) and Ln (2–9%). Other unsaturated FAs (16:1, 20:1 and 22:1) were less than 2% for each canola variety.

Two TAG, OOO (22–50%) and LOO (13–28%), predominated in all samples (Table 2, Fig. 1). Depending on the variety, these compounds comprised 45 (IMC 600) to 65% (IMC 1100) of the total TAG. In variety 600, the contents of LOO and OOO were approximately the same, whereas in the other CNO varieties, the content of OOO was consistently higher. Other important TAG were LnOO (3–10%), LLO (1–11%), LnLO (1–8%), LOP (2–6%), POO (4–8%) and SOO (2–5%). The remaining 11 TAG occurred at concentrations of 2% or less (Table 2). Varieties 200 and 1100 contained the highest contents of OOO, with lower contents of Ln- and L-containing TAG, such as LnLO, LLO and LOO. It will be demonstrated that the high OOO content of 200 and 1100 has a strong positive impact on oil stability. TAG composition was statistically correlated with oxidative stability of the oils.

TAG structures were identified by analysis of the FA composition at the internal (carbon 2 and external [carbon 1(3)]) positions of the glycerol moiety as presented in Table 3 (carbon 2) and Table 4 [carbon 1(3)]. Contents of unsaturated FA positioned at carbon 2 ranged from 3 to 14% for Ln, from 11 to 36% for L and from 50 to 81% for O (Table 3). At carbon 1(3), Ln ranged from 2 to 7%, L from 4 to 18% and O from 65 to 81% (Table 4). Higher contents of Ln and L were positioned at carbon 2, while O content was slightly greater on carbon 1(3), except for varieties 200 and 300 with the highest O, where O concentrations at carbon 2 and carbon 1(3) were about the same. The minor unsaturated FA, 20:1, was predominantly concentrated at carbon 1(3). As expected, saturated FA were concentrated at carbon 1(3) for each oil.

TABLE 1

Fatty Acid Composition, Calculated Oxidizability and Ratios of Linoleic and Linolenic Acids to Oleic Acid of Canola Oil Triacylglycerols[a]

IMC[b] variety	Oxidizability[c]	Fatty acid composition (gas chromatography/flame-ionization detector area %)												Ratios[d]	
		14:0	16:0	16:1	18:0	18:1	18:2	20:0	18:3	20:1	22:0	22:1	24:0	L/O	Ln/O
600	0.412	0.2	3.9	0.1	2.2	60.0	22.4	0.7	8.8	1.2	0.3	0.0	0.3	0.37	0.14
500	0.351	0.0	3.5	0.0	2.0	65.2	18.7	0.8	7.6	1.4	0.4	0.0	0.3	0.29	0.12
1000	0.331	0.1	3.8	0.2	2.0	66.4	18.0	0.6	6.9	1.4	0.3	0.2	0.2	0.27	0.10
400	0.328	0.0	3.7	0.2	1.8	67.2	18.2	0.6	6.6	1.4	0.3	0.2	0.2	0.27	0.10
300	0.299	0.0	2.7	0.0	2.5	68.1	17.7	1.0	5.4	1.9	0.4	0.0	0.4	0.27	0.09
100	0.289	0.0	3.9	0.0	2.5	66.3	20.4	0.8	3.6	1.1	0.8	0.0	0.5	0.31	0.05
700	0.286	0.0	3.7	0.2	2.3	66.3	23.1	0.7	2.1	1.1	0.3	0.0	0.2	0.35	0.03
800	0.267	0.0	3.3	0.2	2.2	69.7	18.7	0.8	3.3	1.2	0.4	0.0	0.3	0.27	0.05
900	0.267	0.1	3.8	0.2	2.3	68.5	20.4	0.7	2.4	1.2	0.3	0.0	0.2	0.30	0.04
1100	0.205	0.0	3.4	0.2	2.5	77.7	8.1	0.7	5.4	1.4	0.4	0.0	0.2	0.10	0.07
200	0.163	0.1	3.2	0.1	2.5	81.3	6.5	0.7	4.1	1.5	0.2	0.0	0.0	0.08	0.05

[a]Fatty acid analysis was performed according to the procedure in the Experimental Procedures section.
[b]InterMountain Canola Co. (Cinnaminson, NJ) (see Experimental Procedures section for variety identification).
[c]Oxidizability: = (0.02 [18:2%] + [18:2%] + 2 [18:3%] + 2 [18:3%])/100 (Ref. 25).
[d]L, linoleic; Ln, linolenic; O, oleic.

TABLE 2

Triacylglycerol Composition (HPLC-FID area%) of Canola Oils[a]

Triacylglycerol[b]	InterMountain Canola Company (IMC) variety[c]										
	600	500	1000	400	300	100	700	800	900	1100	200
LnLnLn	0.2	0.1	0.1	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0
LnLnL	0.6	0.4	0.2	0.3	0.3	0.5	0.0	0.1	0.2	0.1	0.5
LnLL	1.4	0.9	0.5	0.6	0.7	0.3	0.0	0.5	0.0	0.1	0.3
LnLnO	1.7	1.3	0.9	1.1	1.0	1.1	0.4	0.4	0.3	0.1	0.1
LnLnP	0.2	0.1	0.1	0.1	0.1	0.0	0.2	0.1	0.2	0.0	0.0
LLL	1.3	0.7	0.5	0.6	0.8	1.1	1.6	0.9	1.0	0.1	0.2
LnLO	7.6	5.3	4.4	4.9	4.2	1.8	1.7	3.2	1.1	1.6	1.5
LnLP	0.9	0.6	0.5	0.9	0.6	0.4	0.3	0.6	0.7	0.2	0.5
LLO	8.6	6.3	5.9	6.5	6.5	8.3	11.0	7.4	7.8	1.2	1.1
LnOO	10.4	10.2	8.9	8.7	8.1	4.9	2.6	5.5	2.7	10.1	8.6
LLP	1.4	0.9	0.9	1.2	1.0	1.2	1.1	1.2	0.9	0.3	0.8
LnOP	2.1	1.5	1.1	1.6	1.3	0.8	0.5	0.8	0.3	0.8	1.1
LOO	22.5	21.8	23.8	20.8	22.4	24.9	28.4	21.8	26.6	14.6	12.7
LLS	0.3	0.3	0.3	0.6	0.7	0.7	0.6	0.4	0.6	0.3	0.4
LOP	5.7	4.6	3.4	5.4	3.6	4.6	4.2	4.8	3.7	2.2	2.2
PLP	0.3	0.0	0.2	0.3	0.3	0.3	0.3	0.2	0.5	0.2	0.2
OOO	22.4	30.1	36.2	29.1	31.5	31.7	32.8	31.9	37.9	50.4	49.5
LOS	1.6	1.7	1.3	2.7	2.1	2.3	1.9	1.8	2.2	1.3	1.0
POO	4.6	5.3	4.2	6.4	4.3	6.0	4.8	6.8	5.3	6.9	7.7
SLP	0.2	0.2	0.2	0.4	0.2	0.0	0.2	0.3	0.0	0.0	0.4
POP	0.2	0.2	0.1	0.2	0.0	0.0	0.2	0.3	0.0	2.1	0.3
PPP	0.1	1.7	0.1	1.5	2.5	1.5	1.4	2.3	1.5	0.4	2.8
SOO	2.6	2.6	1.9	2.8	2.9	3.1	2.4	4.0	2.7	3.9	5.0
SLS	0.4	0.1	0.2	0.2	0.1	0.0	0.2	0.2	0.0	0.0	0.4
SOP	0.1	0.3	0.2	0.3	0.2	0.3	0.2	0.2	0.1	0.2	0.3
PPS	0.5	0.6	0.6	0.1	0.9	0.8	0.6	1.3	0.2	0.9	0.6
SPS	0.2	0.3	0.0	0.1	0.5	0.5	0.3	0.6	0.9	0.2	0.8
SSS	0.1	0.1	0.1	0.2	0.3	0.3	0.2	0.4	0.3	0.0	0.1
Unidentified	2.2	2.9	1.9	3.0	3.5	3.4	2.2	2.6	2.3	0.4	1.1

[a]Reversed-phase high-performance liquid chromatography with flame-ionization detection (HPLC-FID). HPLC conditions and chromatogram peak identification given in Figure 1 and in the Experimental Procedures section. [b]Ln, linolenic; L, linoleic; O, oleic; P, palmitic; and S, stearic. [c]See Experimental Procedures section for complete variety identification.

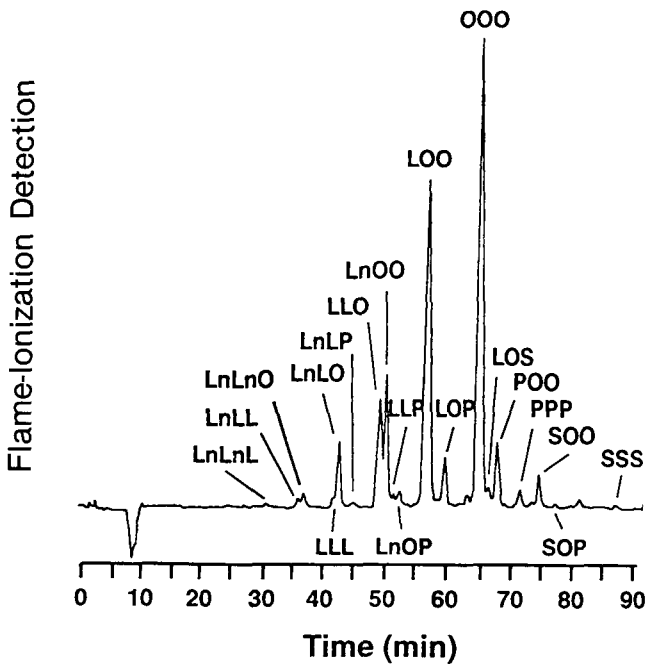

FIG. 1. Reversed-phase high-performance liquid chromatographic analysis of purified canola oil triacylglycerols (IMC 1000, InterMountain Canola Co., Cinnaminson, NJ; see Experimental Procedures section). Analysis conditions: 0.5–1.0 mg sample, 5 μ (C-18 column, 0.49 × 50 cm); 120-min solvent gradient of acetonitrile/methylene chloride (70:30 to 40:60, vol/vol); flow rate 0.8 mL/min; flame-ionization detector, triacylglycerol fatty acids: S, stearic; P, palmitic; O, oleic; L, linoleic; Ln, Linolenic.

Correlation of PV and ΔPV with TAG structure and composition. Linear regression plots of PV vs. oxidation time for each sample gave the rates of oxidation (ΔPV = slope) presented in Table 5. Differences in ΔPV determined for light and dark oxidation of the same variety probably reflect differences in input energy under the two reaction conditions. TAG hydroperoxide products and their volatiles were formed in similar amounts for both light and dark oxidation of CNO.

The ΔPV and PV, determined for CNO, oxidized to 24, 48 and 72 h were correlated by statistical procedures (8) with oxidizability, unsaturated FA, FA content of glycerol carbons and selected TAG (Table 6). Strong positive correlations of ΔPV and PV with calculated oxidizabilities indicate that photooxidation and autoxidation reactions follow the predicted deterioration processes quite closely. There was a strong negative correlation of ΔPV and PV with overall O content, but a slightly greater negative correlation for O on glycerol carbon 2 as compared to carbon 1 (3). These results confirm that CNO oxidative stability increases with O content in the oil and, especially at levels where O also occupies the carbon 2 position. In contrast, ΔPV and PV were positively correlated with L and Ln for both light and dark oxidation. For PV, there was a greater positive correlation for L at glycerol carbon 2 as compared to carbon 1(3). Therefore, increased L and Ln content and also increased content of L positioned at carbon 2 reduce CNO oxidative stability. Both ΔPV and PV had strong negative correlations with trioleoylglycerol

TABLE 3

Fatty Acid Composition (%) and Ratios of Linoleic and Linolenic Acid to Oleic Acid Occupying Glycerol Carbon 2 for Canola Oil Triacylglycerols[a,b]

IMC[c] variety	Fatty acid composition (gas chromatography/flame-ionization area %)												Ratios	
	14:0	16:0	16:1	18:0	18:1	18:2	20:0	18:3	20:1	22:0	22:1	24:0	L/O	Ln/O
600	0.0	0.0	0.0	0.0	50.2	36.2	0.0	13.6	0.0	0.0	0.0	0.0	0.72	0.27
500	0.0	0.0	0.0	0.0	61.4	28.5	0.0	10.1	0.0	0.0	0.0	0.0	0.46	0.17
1000	0.0	0.2	0.1	0.5	61.0	28.3	0.1	9.8	0.0	0.0	0.0	0.0	0.46	0.16
400	0.0	0.2	0.1	0.2	60.8	28.9	0.0	9.8	0.0	0.0	0.0	0.0	0.48	0.16
300	0.0	0.0	0.0	0.0	67.7	25.7	0.0	6.6	0.0	0.0	0.0	0.0	0.38	0.10
100	0.0	0.0	0.0	0.0	62.5	30.4	0.0	4.8	0.0	1.3	0.0	1.0	0.49	0.08
700	0.0	0.2	0.0	0.7	61.6	34.3	0.3	2.6	0.3	0.0	0.0	0.0	0.56	0.04
800	0.0	0.2	0.1	0.3	66.0	28.5	0.0	4.9	0.0	0.0	0.0	0.0	0.43	0.07
900	0.1	0.3	0.2	0.3	64.4	30.8	0.3	3.1	0.3	0.1	0.0	0.1	0.48	0.05
1100	0.0	0.3	0.2	1.0	76.7	13.7	0.0	7.9	0.0	0.0	0.0	0.2	0.18	0.10
200	0.2	0.4	0.1	0.9	81.2	10.7	0.0	6.5	0.0	0.0	0.0	0.0	0.13	0.08

[a]Fatty acid analysis at glycerol carbon 2 according to Neff *et al.* (8).
[b]L, linoleic; Ln, linolenic; O, oleic.
[c]As in Table 2.

TABLE 4

Fatty Acid Composition and Ratios of Linoleic and Linolenic Acid to Oleic Acid on Glycerol Carbon 1 (3) for Canola Oil Triacylglycerols[a,b]

IMC[c] variety	Fatty acid composition (gas chromatography/flame-ionization area %)												Ratios	
	14:0	16:0	16:1	18:0	18:1	18:2	20:0	18:3	20:1	22:0	22:1	24:0	L/O	Ln/O
600	0.3	5.8	0.2	3.4	64.9	15.5	1.1	6.5	1.8	0.4	0.0	0.5	0.24	0.10
500	0.0	5.3	0.0	3.0	67.1	13.8	1.2	6.3	2.1	0.7	0.0	0.5	0.21	0.09
1000	0.1	5.5	0.2	2.8	69.2	12.9	0.9	5.5	2.1	0.4	0.3	0.3	0.19	0.08
400	0.0	5.5	0.3	2.6	70.4	12.9	0.9	5.0	2.1	0.5	0.3	0.3	0.19	0.07
300	0.0	4.0	0.0	3.7	68.3	13.7	1.5	4.8	2.9	0.6	0.0	0.6	0.20	0.07
100	0.0	5.8	0.0	3.8	68.2	15.2	1.4	2.9	1.7	1.3	0.0	0.8	0.23	0.04
700	0.0	5.5	0.2	3.1	68.7	17.5	0.9	1.8	1.5	0.5	0.0	0.3	0.26	0.03
800	0.0	4.9	0.2	3.1	71.7	13.6	1.1	2.5	1.8	0.6	0.0	0.5	0.19	0.04
900	0.1	5.5	0.3	3.3	70.3	15.2	0.9	2.1	1.7	0.5	0.0	0.3	0.22	0.03
1100	0.0	5.0	0.2	3.3	78.2	5.2	1.1	4.2	2.2	0.5	0.0	0.2	0.07	0.05
200	0.1	4.6	0.1	3.3	81.4	4.4	1.1	2.9	2.3	0.3	0.0	0.0	0.06	0.04

[a]Fatty acid analysis at glycerol carbon 1 (3) was performed according to Neff *et al.* (8).
[b]L, linoleic; Ln, linolenic; O, oleic.
[c]As in Table 2.

TABLE 5

Experimental Oxidative Stability of Canola Oil Triacylglycerols as Measured by ΔPV[a], ΔTV[b] and ΔTAG-OX[c]

IMC variety[d]	Experimental oxidative stability					
	Light oxidation[e]			Dark oxidation[f]		
	ΔPV	ΔTV	ΔTAG-OX	ΔPV	ΔTV	ΔTAG-OX
600	0.440	1.535	13.613	0.254	1.155	6.214
500	0.171	0.786	3.914	0.139	0.911	4.240
1000	0.151	0.799	4.478	0.106	0.610	1.590
400	0.198	0.669	4.511	0.089	0.645	1.491
300	0.188	0.509	3.523	0.088	0.445	1.266
100	0.195	0.522	3.954	0.090	0.299	1.076
700	0.174	0.244	3.788	0.063	0.134	0.867
800	0.172	0.321	3.628	0.081	0.235	1.785
900	0.163	0.375	3.468	0.060	0.257	1.310
1100	0.141	0.351	1.778	0.034	0.266	0.335
200	0.094	0.309	0.820	0.017	0.108	0.203

[a]Slope, linear regression plot of peroxide value (PV) vs. time; PV procedure (8).
[b]Slope, linear regression plot of total selected volatiles (TV) (Fig. 2) vs. time; TV procedure (see Reference 8).
[c]Slope, linear regression plot of total selected triacylglycerol oxidation products (TAG-OX) (Fig. 3) vs. time; TAG-OX procedure (see Experimental Procedures section).
[d]As footnote c in Table 2.
[e]Sample oxidized under fluorescent light (545 foot candles) in oxygen.
[f]Sample oxidized in the dark at 60°C under oxygen.

TABLE 6

Correlation of Δ Peroxide Value (ΔPV)[a] and PV[b] with Oxidizability[c], Unsaturated Fatty Acid Content and Position at Triacylglycerol Glycerol Carbons[d] and Triacylglycerol Composition[e] for Light[f] and Dark[g] Oxidations

	Correlation coefficients[h]							
	Light oxidation PV at				Dark oxidation PV at			
Factor	ΔPV	24 h	48 h	72 h	ΔPV	24 h	48 h	72 h
Oxidizability	0.76	.80	.82	.76	0.90	.92	.92	.90
Oleic acid	−.71	−.80	−.77	−.71	−.81	−.89	−.85	−.81
Linoleic acid	NS[i]	.68	.60	.55	.58	.72	.64	.58
Linolenic acid	NS	NS	.56	NS	.71	.57	.68	.72
Oleic acid/carbon 2	−.77	−.83	−.81	−.77	−.84	−.89	−.87	−.84
Oleic acid/carbon 1,3	−.63	−.75	−.71	−.63	−.75	−.86	−.81	−.75
Linoleic acid/carbon 2	.61	.73	.67	.62	.65	.77	.70	.65
Linoleic acid/carbon 1,3	NS	.62	NS	NS	NS	.67	.58	NS
Linolenic acid/carbon 2	.58	NS	.57	.57	.71	NS	.66	.72
Linolenic acid/carbon 1,3	NS	NS	NS	NS	.70	.58	.67	.70
Triolein	−.71	−.79	−.76	−.71	−.79	−.85	−.82	−.78
Oleoyllinoleoyllinolenoylglycerol	.76	.71	.77	.75	.91	.82	.89	.90

[a]Slope, linear regression plot of PV vs. time.
[b]PV procedure (Ref. 8).
[c]Oxidizability (see Table 1) (Ref. 25).
[d]Composition at triacylglycerol glycerol carbons determined by lipolysis procedure (Ref. 8).
[e]Triacylglycerol composition determined by reversed-phase high-performance liquid chromatography with flame-ionization detection (as in Fig. 1).
[f]Oxidation conducted on neet oil at 25°C in oxygen under fluorescent light (545 foot candles).
[g]Oxidation conducted on neet oil at 60°C in oxygen in the dark.
[h]$P < .05$, statistical procedure (Ref. 8).
[i]NS, not significant.

(OOO) and positive correlations with oleoyllinoleoyllinolenoyl glycerol (LnLO). For both light and dark oxidation, CNO oxidative stability increased with OOO content and decreased with LnLO content.

Correlation of volatiles with TAG structure and composition. A GC chromatogram of volatiles generated by thermal decomposition of a typical sample of oxidized TAG is presented in Figure 2. Volatiles identified on the chromatogram are those summed to give the value of total selected volatiles (TV).

Volatile composition data for the oxidized CNO investigated are presented in Table 7. Propanal, which ranged from 9–28%, was generated in the greatest concentration by thermal decomposition of TAG oxidized in the light or in the dark. Other major volatiles generated from autoxidized CNO TAG were (decreasing order): 2,4-heptadienal (9–21%), 2-heptenal (4–17%), pentanal (6–11%), pentane (1–9%), hexanal (3–10%) and nonanal (3–7%). The same order and similar concentration ranges were observed for photooxidized TAG. The volatile data show that although the content of Ln is much less than either O and L (Table 1), Ln is the most important volatile-generating precursor in CNO's oxidized TAG. CNO varieties 200 and 1100, which had the highest concentrations of O (Table 1) and OOO (Table 2), generated the most nonanal by thermal decomposition of their oxidized TAG.

Analysis of TAG hydroperoxides, which are the oxidative precursors to volatiles, is presented in Table 8 for 24- and 48-h oxidation periods. These data show that O hydroperoxides were present at concentrations of less than 15%, and L and Ln hydroperoxides were at about equal concentrations. The Ln hydroperoxides may be more easily decomposed than the L hydroperoxides, which may account for the observation that Ln hydroperoxides were the most important volatiles precursors. As observed with the volatile data, TAG hydroperoxide concentration showed little difference between light and dark oxidation. Among the CNO for which TAG-OX composition was determined, there was a slight increase in O hydroperoxides (Table 8) as the O content increased (Table 1). The distribution of CNO TAG hydroperoxides is consistent with that previously reported for the oxidation of mixtures of methyl esters of O, L and Ln (26).

A second measure of experimental oxidative stability (8,9), rate of change of volatile formation with oxidation (ΔTV), as determined by the slope of linear regression plots of TV vs. oxidation times, are presented in Table 5. Correlations of ΔTV with oxidizability, unsaturated FA content, TAG structure and TAG composition are presented in Table 9. The ΔTV had strong negative correlations with both O and OOO contents, and a slightly higher negative correlation for O content on glycerol carbon 2 as compared to carbon 1(3). These results indicate that higher O content and, therefore, higher OOO content (varieties 200 and 1100) and more O content on glycerol carbon 2 increases oxidative stability. Both overall Ln and LnLO contents showed strong positive correlations with ΔTV, indicative of low CNO oxidative stability. LnOO content, with a slightly positive correlation with ΔTV, is indicative of greater oxidative stability imparted by O to this TAG. As would be expected, specific volatiles were correlated with the content of their precursor TAG and FA. Finally,

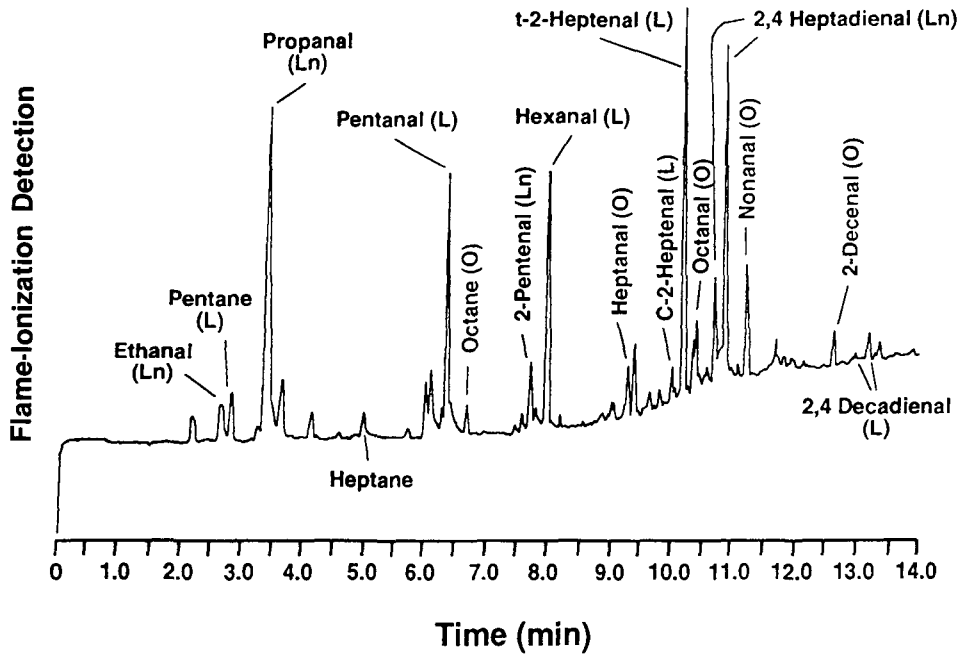

FIG. 2. Resolution of volatiles by capillary gas chromatography from canola oil triacylglycerols thermally decomposed in the static-headspace analyzer (140°C, N_2, 20 min). Gas-liquid chromatography and headspace conditions given previously (Ref. 8). Abbreviations for the fatty acid volatile precursors as in Figure 1.

TABLE 7

Volatile Formation[a] Under Light and Dark Oxidation[b] of Canola Oil Triacylglycerols

Selected volatiles	Volatile fatty acid precursor[c]	GC/flame-ionization detector response (area%)	
		Light oxidation (MIN – MAX)[d]	Dark oxidation (MIN – MAX)
Ethanal	Ln	0.5 – 2.6	1.4 – 2.5
Propanal	Ln	8.8 – 27.7	9.7 – 25.3
2-Pentenal	Ln	0.3 – 1.6	0.3 – 2.2
2,4-Heptadienal	Ln	5.6 – 21.1	8.5 – 20.8
Pentane	L	3.5 – 10.3	0.7 – 8.7
Pentanal	L	4.5 – 10.1	6.0 – 10.8
Hexanal	L	3.7 – 9.4	3.1 – 10.1
2-Heptenal	L	5.8 – 17.2	4.4 – 17.1
2,4-Decadienal	L	1.4 – 3.0	0.8 – 2.5
Heptane	O	0.9 – 1.8	0.2 – 1.4
Octane	O	1.2 – 2.4	0.7 – 1.8
Octanal	O	0.4 – 2.4	0.4 – 5.5
Nonanal	O	2.3 – 9.0	2.7 – 7.0
2-Decenal	O	0.7 – 2.1	0.7 – 3.0

[a]See Figure 2 and Reference 8 for volatile analysis by static-headspace capillary (GC) method.
[b]See Table 6 for oxidation conditions.
[c]Ln, linolenic; L, linoleic; O, oleic.
[d]MIN, minimum; MAX, maximum.

predicted oxidative stability, (i.e., oxidizability) showed a strong positive correlation with the experimentally measured parameter ΔTV.

An RP-HPLC chromatogram of CNO TAG oxidation products (TAG-OX) is presented in Figure 3. The UV detector system with this RP-HPLC technique does not detect oxidized O (monoene hydroperoxides), only conjugated diene systems from oxidized L and Ln. However, as observed in Table 8, L and Ln account for more than 80% of the oxidation products at the low oxidation levels studied. TAG-OX composition data are presented in Table 10. The same abundance order and similar concentration ranges were observed for CNO oxidation products under either light or dark conditions. LOO-OX (i.e., LOO-OOH, Fig. 3) was the most abundant TAG-OX (35–76%). Other TAG-OX (monohydroperoxides) in decreasing abundance order were: LnOO (3–27%), LOP (4–16%), LnLO (3–12%), LLO (3–11%), LLL (3–9%) and LnLL (2–3%). Thus, results

TABLE 8

Contribution of Oleic, Linoleic and Linolenic Acids as Precursors to Hydroperoxides Formed During Canola Oil Oxidation[a]

IMC variety	Oxidation		GC/FID Response (area%) reduced hydroperoxide products			
	Conditions	Time (h)	PV	Oleate enols	Linoleate dienols	Linolenate trienols
500	Light	24	4.7	8.9	44.6	46.5
1000	Light	24	3.5	8.6	46.8	44.6
400	Dark	24	1.1	9.5	46.6	43.9
300	Light	24	4.4	11.8	43.6	44.6
500	Light	48	11.2	9.5	45.8	44.7
1000	Light	48	7.0	8.9	45.4	45.6
400	Dark	48	2.2	11.0	46.1	42.9
300	Light	48	8.6	14.6	41.9	43.5

[a]Analysis by capillary gas chromatography (GC). The oxidized triacylglycerol hydroperoxides were reduced with sodium borohydride and then transmethylated to give mixtures of unsaturated hydroxy methyl esters, which were categorized and analyzed as enols (from oleic acid products), dienols (from linoleic acid products) and trienols (from linolenic acid products). FID, flame-ionization dectetor; PV, peroxide value.
[b]As footnote c in Table 2.

TABLE 9

Correlations of Rate of Formation of Triacylglycerol Oxidation Products (ΔTAG-OX)[a] and Δ Total Gas Chromatography Volatile Area (ΔTV)[b] with Oxidizability, Fatty Acid Content and Position at Glycerol Carbons, and Triacylglycerol Composition

Factor[c]	Correlation coefficients[d]			
	ΔTAG-OX		ΔTV	
	Light[e]	Dark	Light	Dark
Oxidizability	0.81	0.82	0.82	0.87
O acid	−.75	−.71	−.66	−.68
L acid	.57	NS	NS	NS
Ln acid	.58	.71	.85	.91
O acid carbon 2	−.82	−.75	−.72	−.71
O acid carbon 1,3	−.66	−.65	−.58	−.62
L acid carbon 2	.65	.56	NS	NS
L acid carbon 1,3	NS	NS	NS	NS
Ln acid carbon 2	.62	.70	.86	.88
Ln acid carbon 1,3	NS	.69	.81	.90
OOO	−.73	−.71	.62	−.66
LOO	NS	NS	NS	NS
LnOO	NS	NS	.57	.63
LLO	NS	NS	NS	NS
LnLO	0.80	0.85	.91	.94

[a]Slope, linear regression plot of peak areas vs. time of TAG-OX products shown in Figure 3.
[b]Slope, linear regression plot of sum of areas of selected volatiles (TV) vs. time.
[c]L, linoleic; Ln, linolenic; O, oleic.
[d]$P < 0.05$, statistical procedure (Ref. 8), NS, not significant.
[e]Oxidation conditions given in Table 6.

support the volatile data in Table 7, which indicated little apparent difference between light and dark oxidation product formation.

A third measure of experimental oxidative stability (8,9) is ΔTAG-OX (rate of formation of oxidation products) as determined from the slope of the linear regression plot of total TAG-OX vs. oxidation time. The ΔTAG-OX are also presented in Table 5. Correlations of ΔTAG-OX with oxidizability, unsaturated FA content, TAG structure and TAG composition are presented in Table 9. The ΔTAG OX

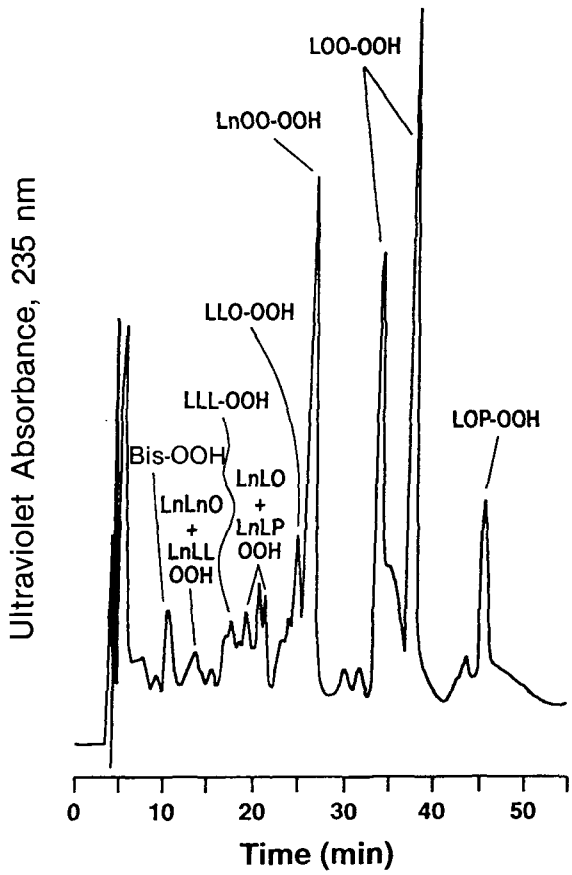

FIG. 3. Reversed-phase high-performance liquid chromatography (HPLC) resolution of canola oil triacylglycerol oxidation products (TAG-OX). HPLC conditions: 5.2 mg sample, 5 μ (C18 column, 0.46 × 25 cm), mobile phase, methylene chloride/methanol/acetonitrile (5:5:90, vol/vol/vol), flow rate, 0.8 mL/min; ultraviolet absorption detector at 235 nm for conjugated-diene functionality of oxidized linoleic and linolenic acids of the TAG-OX (Ref. 9). TAG-OX fatty acids are: P, palmitic; O, oleic; L, linoleic; Ln, linolenic. Bis-OOH are TAG-OX that contain two hydroperoxy-bearing fatty acids.

TABLE 10

Triacylglycerol Hydroperoxide (TAG-OX) Formation with Respect to Dark and Light Oxidation of Chromatographed Canola Oils[a]

Selected TAG-OX[a]	Chromatogram area percent[b]	
	Light oxidation[c] (Minimum – Maximum)	Dark oxidation (Minimum – Maximum)
LnLL + LnLnO	1.6 – 3.1	1.9 – 3.4
LLL	3.0 – 8.0	2.9 – 9.0
LnLO + LnLP	1.6 – 11.7	3.3 – 11.8
LLO	7.0 – 10.8	2.9 – 11.3
LnOO	5.0 – 21.3	2.7 – 26.6
LOO	34.6 – 73.6	39.8 – 75.8
LOP	4.0 – 16.0	3.5 – 15.0

[a]See Figure 3 and Reference 9 for reversed-phase HPLC analysis conditions. Abbreviations as in Tables 2, 7 and 9.
[b]Ultraviolet HPLC detector absorption at 236 nm.
[c]Oxidation conditions given in Table 6.

had strong negative correlations with overall O content and with O on carbon 2 as compared to carbon 1(3). Thus, as observed for ΔPV and ΔTV, ΔTAG-OX relationships also implicate increased content of O with increased oxidative stability. Other relationships (as shown in Table 9) and product characterizations (Table 10, Fig. 3) affirm the fact that increased contents of L and Ln in CNO lead to reduced oxidative stability.

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