Formation of Triacylglycerol Core Aldehydes During Rapid Oxidation of Corn and Sunflower Oils with *tert*-Butyl Hydroperoxide/Fe^{2+}

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ABSTRACT: The lipid ester core aldehydes formed during a rapid oxidation (7.8 M *tert*-butyl hydroperoxide, 90 min at 37°C) of the triacylglycerols of purified corn and sunflower oils were isolated as dinitrophenylhydrazones by preparative thin-layer chromatography and identified by reversed-phase high-performance liquid chromatography with on-line electrospray ionization mass spectrometry and by reference to standards. A total of 113 species of triacylglycerol core aldehydes were specifically identified, accounting for 32–53% of the 2,4-dinitrophenylhydrazine (DNPH)-reactive material of high molecular weight representing 25–33% of the total oxidation products. The major core aldehyde species (50–60% of total triacylglycerol core aldehydes) were the mono(9-oxo)nonanoyl- and mono(12-oxo)-9,10-epoxy dodecenoyl- or (12-oxo)-9-hydroxy-10,11-dodecenoyl-diacylglycerols. A significant proportion of the total (9-oxo)nonanoyl and epoxidized (12-oxo)-9,10-dodecenoyl core aldehydes was found in complex combinations with hydroperoxy or hydroxy fatty acyl groups (6–10% of total triacylglycerol core aldehydes). Identified were also di(9-oxo)nonanoylmonoacylglycerols (0.5% of total) and tri(9-oxo)nonanoylglycerols (trace). The identification of the oxoacylglycerols was consistent with the products anticipated from *tert*-butyl hydroperoxide oxidation of the major species of corn and sunflower oil triacylglycerols (mainly linoleoyl esters). However, the anticipated (13-oxo)-9,11-tridecadienoyl aldehyde-containing acylglycerols were absent because of further oxidation of the dienoic core aldehyde. A significant proportion of the unsaturated triacylglycerol core aldehydes contained *tert*-butyl groups linked to the unsaturated fatty chains *via* peroxide bridges (2–9%). The study demonstrates that rapid peroxidation with *tert*-butyl hydroperoxide constitutes an effective method for enriching natural oils and fats in triacylglycerol core aldehydes for biochemical and metabolic testing.

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Autoxidation of unsaturated triacylglycerols yields hydroperoxides as primary oxidation products. In the presence of divalent cations, the hydroperoxides readily undergo chain cleavage, yielding both volatile and nonvolatile carbonyl compounds of a large variety of structural types (1,2). Whereas the volatile carbonyl compounds have been extensively studied owing to their contribution to off-flavors, color, and aroma of stored food products (3,4), the nonvolatile acylglycerol aldehyde molecules have largely been ignored (5,6). Extensive research has shown that volatile carbonyls form adducts with proteins (7–9) and some of them precipitate adverse physiological effects (9–11). The chain cleavage products remaining associated with the acylglycerol molecules, although not directly contributing to off-flavors, color, and aroma, can serve as reservoirs of potentially toxic oxidation materials as well as complexing agents. Furthermore, polar glycerolipids containing an aldehyde group are likely to remain associated with cell membranes (12) and affect their structure and function, including receptor activity. Because the nonvolatile aldehydes have been shown (13,14) to react with amino compounds as readily as the volatile aldehydes and because hydroperoxides of dietary linoleic acid esters are converted to aldehydes in the stomach before being absorbed into the body (15,16), it is essential that acylglycerol core aldehydes be available for studies on the gastrointestinal fate and metabolic consequences of ingestion of the secondary products of triacylglycerol oxidation.

As synthetic lipid ester core aldehydes are not commercially available, previous biochemical and nutritional studies have been performed with the small amounts of products generated by autoxidation of unsaturated fats and oils (17,18). Because of low yields of oxoacylglycerols, direct identification of the potential dietary precursors and the absorbed materials has been difficult, and analysts have relied on indirect tests based on various color reactions (17,18). We have previously isolated the C_5 and C_9 core aldehydes of cholesteryl esters (19,20), glycerophospholipids (19), and standard triacylglycerols (21,22) following treatment with *tert*-butyl hydroperoxide (TBHP) and have identified them by reversed-phase high-performance liquid chromatography (HPLC) with on-line electrospray ionization mass spectrometry (LC/ESI/MS) after preparation of the 2,4-dinitrophenylhydrazones (13,22). In the present study, we have used this analytical method to identify over 113 core aldehydes and their derivatives generated from corn and sunflower oil triacylglycerols by a rapid oxidation with TBHP and ferrous ions.

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Abbreviations: ALD, aldehyde; CI, chemical ionization; CID, collision-induced dissociation; DLI, direct liquid inlet; DNPH, 2,4-dinitrophenylhydrazine; EI, electron impact; ESI, electrospray ionization; HPLC, high-performance liquid chromatography; LC, liquid chromatography; M, molecular ion; MS, mass spectrometry; Rt, retention time; TBHP, *tert*-butyl hydroperoxide; TCN, theoretical carbon number; TLC, thin-layer chromatography.

MATERIALS AND METHODS

Materials. Corn oil (Mazola; Best Foods Canada Inc., Etobicoke, Ontario, Canada) was purchased from a local grocery store, and sunflower seed oil (Kultasula) was obtained from a Finnish manufacturer (Raision Margariini Oy, Toijala, Finland). The triacylglycerols were purified by thin-layer chromatography (TLC) as previously described (22). Reference triacylglycerols, hydroperoxytriacylglycerols, and core aldehydes were available from a previous study (22), including the oxidation products of 16:0/18:1/18:2 (21).

Oxidation. The oxidation was accelerated by adding 1 mL of 70% TBHP in water (7.8 M) to 10 mg of purified triacylglycerols in the presence of 10 μM $FeSO_4$ and 100 μL of 0.2% taurocholic acid (16). The mixture was incubated on a mechanical agitator in the dark for 1.5 h at 37°C. The reaction was stopped by diluting with 5 mL of chloroform/methanol (2:1, vol/vol) and by washing three times with water (3 × 1 mL). The solvent was evaporated under nitrogen at 38°C, and the lipid residue was subjected to preparation of derivatives of 2,4-dinitrophenylhydrazine (DNPH).

Preparation of DNPH derivatives. The DNPH derivatives of triacylglycerol core aldehydes were obtained by adding 2 mL of freshly prepared DNPH in 1 N HCl (3.6 mg/mL) to a 6–9-mg dry sample (6). The mixture was shaken vigorously and kept in the dark at room temperature for 4 h and then overnight at 4°C. The lipids were extracted with 5 mL of chloroform/methanol (2:1, vol/vol), the chloroform phase was blown down under nitrogen, and the residue was taken up in an appropriate solvent for chromatography and MS as described below.

TLC. Normal-phase TLC was used to purify triacylglycerols and their oxidized derivatives. Silica gel H (E. Merck & Co., Darmstadt, Germany) plates were prepared in the laboratory, and heptane/isopropyl ether/acetic acid (60:40:4, by vol) solution was used as the mobile phase (22). The DNPH derivatives of the core aldehydes were seen as yellow bands on the chromatoplates (in daylight). The compounds were recovered from the silica gel scrapings by extraction with chloroform/methanol (2:1, vol/vol). Extracts were washed with distilled water, dried with anhydrous Na_2SO_4, and saved in 2-propanol.

HPLC, LC/ESI/MS, and LC/ESI/CID/MS. Procedures for HPLC, LC/ESI/MS, and LC with on-line ESI/collision-induced dissociation/MS (LC/ESI/CID/MS) of triacylglycerols were as described previously (21,22). The reversed-phase HPLC profiles of native corn and sunflower oil triacylglycerols were recorded with the use of a direct liquid inlet (DLI) chemical ionization (CI) mass spectrometer (Hewlett-Packard, Palo Alto, CA) as a detector, as previously described (23). For ESI/CID/MS, the capillary exit voltage was raised to 300 V to obtain fragment ions from any clearly resolved components (pseudo MS/MS) (24,25). The triacylglycerol samples were dissolved in isopropanol by heating for 2–3 min at 80°C prior to injection. Addition of chloroform to assist the dissolution of triacylglycerols was avoided as it caused distortion of peak shape and variation in retention time.

GC/MS. GC/MS analyses were performed using an HP5890A gas chromatograph (Hewlett-Packard, San Diego, CA) coupled to the single-quadrupole mass spectrometer described above. A fused-silica capillary column (15 m × 0.32 mm, i.d.) of the DB-1 bonded phase type (J&W Scientific, Rancho Cordova, CA) was used for the analysis with temperature programming from 200 to 300°C at 5°C/min. Spectra were recorded under electron impact (EI) (70 eV) ionization conditions.

Peak identification and quantification. The structures of the resolved oxoacylglycerols were established on the basis of their behavior on normal-phase TLC, reversed-phase HPLC, and LC/ESI/MS as follows. The tentative identities obtained by chromatography in relation to standards were confirmed by LC/ESI/MS, which provided high-intensity $[M + 1]^+$ and low-intensity $[M - RCOOH]^+$ ions for the underivatized oxoacylglycerols. The DNPH derivatives of the TBHP oxidation products of the oils gave $[M - 1]^-$ as the major ions on LC/ESI/MS in the negative mode. The DNPH derivatives of oxidized 16:0/18:1/18:2 were characterized by LC/ESI/CID/MS, which permitted the detection of greatly increased yields of the [M − RCOOH] ions. The data obtained with the TBHP oxidation products of 16:0/18:1/18:2 were used to assign the structures of the TBHP oxidation products of corn and sunflower oil triacylglycerols. The abundances of the major molecular ions of the core aldehydes in the TLC bands provided an estimate of the relative quantities of each component of each homologous series, which could be summed to match the estimates for the major oxoacylglycerol core aldehydes in the total negative ion profile.

RESULTS

Analysis of seed oils. The molecular species composition of native corn and sunflower oil triacylglycerols was determined by reversed-phase HPLC and was found to be similar to that reported previously for commercial corn (23) and sunflower (26) seed oils. Figure 1 shows the triacylglycerol profiles of the two oils before oxidation as obtained by LC/DLI/CI/MS. Table 1 compares molecular species of triacylglycerols of corn and sunflower seed oils before oxidation. The major molecular species of triacylglycerols in both oils were 18:2/18:2/18:2 (20–22%), 18:1/18:2/18:2 (17–18%), 18:1/18:1/18:2 (12–21%), 16:0/18:2/18:2 (7–10%), 16:0/18:1/18:2 (4–9%), 18:1/18:1/18:1 (9–14%), and 18:0/18:1/18:1 (3–7%). The sunflower oil also contained minor amounts (1–5%) of 18:0/18:1/18:1, 22:1/18:1/18:2, and 22:0/18:1/18:2. The overall fatty acid composition of the original sunflower and corn oil triacylglycerols was similar to that reported previously (23,26), with linoleic (18:2), oleic (18:1), and palmitic (16:0) acids accounting for over 90% of the total, as shown in Table 2.

Analysis of total oxidation mixtures. Figure 2 compares the total reversed-phase HPLC elution profiles, as detected by light scattering, of corn and sunflower seed oil triacylglycerols following the TBHP treatment. The peaks corresponding to unoxidized triacylglycerols are eluted toward the end of the chromatograms and are identified by their theoretical

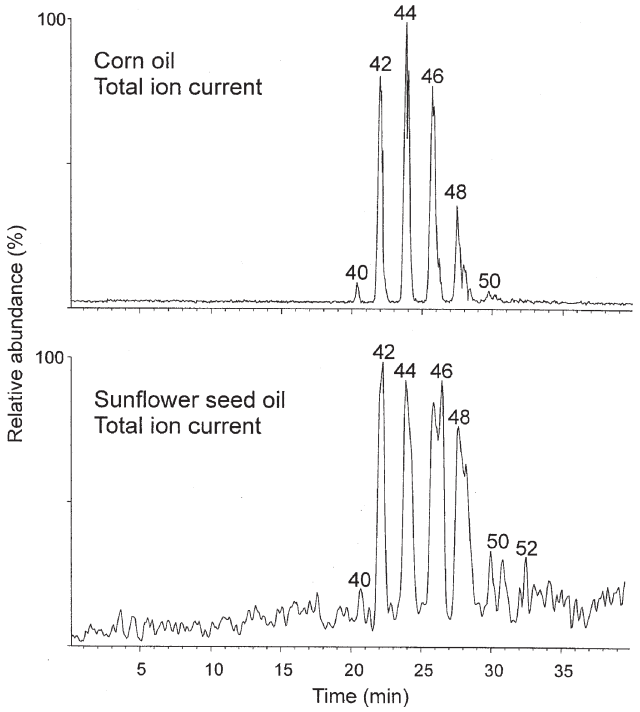

FIG. 1. Total positive ion current profiles of triacylglycerols of corn and sunflower seed oils before oxidation. Peak numbering refers to the partition number. Triacylglycerols were detected as the ammonium adducts $[M + NH_4]$ in the positive ion mode. Conditions and instrumentation of reversed-phase high-performance liquid chromatography (HPLC) and mass spectrometry (MS) with direct liquid inlet (DLI) chemical ionization (CI) are described in the Materials and Methods section. Solvent gradient: 20–80% 2-propanol in methanol (0.85 mL/min) in 30 min.

TABLE 1
Major Molecular Species of Triacylglycerols of Native Corn (Mazola, Canada) and Sunflower (Kultasula, Finland) Oils[a]

ACN/DB[b]	$[MH]^+$	Molecular species	Corn oil (mol%)	Sunflower oil (mol%)
54:7	894	18:2/18:2/18:3	4.2 ± 0.1	0.05 ± 0.1
54:6	896	18:2/18:2/18:2	22.3 ± 2.0	20.3 ± 2.1
52:5	870	16:0/18:2/18:3	0.9 ± 0.1	0.0 ± 0.0
54:5	898	18:1/18:2/18:2	18.0 ± 2.0	17.3 ± 2
52:4	872	16:0/18:2/18:2	9.9 ± 1.0	6.8 ± 1
54:4	900	18:1/18:1/18:2	12.0 ± 1.0	21.5 ± 1.0
52:3	873	16:0/18:1/18:2	8.6 ± 1.0	4.0 ± 1.0
50:2	848	16:0/16:0/18:2	3.2 ± 0.5	0.05 ± 0.1
54:3	902	18:1/18:1/18:1	9.0 ± 0.5	13.7 ± 1.1
52:2	876	16:0/18:1/18:1	6.0 ± 0.5	0.7 ± 0.1
50:1	850	16:0/16:0/18:1	2.3 ± 0.2	0.2 ± 0.1
48:0	824	16:0/16:0/16:0	0.4 ± 0.2	0.1 ± 0.5
54:2	904	18:0/18:1/18:1	2.8 ± 0.2	7.4 ± 0.2
52:1	878	16:0/18:0/18:1	0.9 ± 0.2	1.1 ± 0.2
58:4	956	18:1/18:2/22:1	—	4.8 ± 0.5
54:1	906	18:0/18:0/18:1	—	0.9 ± 0.5
58:3	958	18:1/18:2/22:0	—	1.2 ± 0.5

[a]Analysis by liquid chromatography with the use of a direct liquid inlet mass spectrometer as detector (LC/DLI/CI/MS) and using a linear gradient of 30–90% propionitrile in acetonitrile. Values are means ± range/2.
[b]ACN, acyl carbon number; DB, double bond.

TABLE 2
Fatty Acid Composition of Corn (Mazola, Canada) and Sunflower (Kultasula, Finland) Oils[a]

Fatty acid	Corn oil (mol%)	Sunflower oil (mol%)
16:0	10.96 ± 0.8	6.4 ± 1.0
16:1n-7	0.09 ± 0.1	0.2 ± 0.2
18:0	1.85 ± 0.2	3.9 ± 0.8
18:1n-9	28.83 ± 1.7	34.0 ± 3.2
18:2n-6	56.59 ± 2.5	49.4 ± 4.0
18:3n-3	0.70 ± 0.3	0.3 ± 0.2
20:0	0.46 ± 0.1	1.2 ± 0.8
20:1n-9	0.40 ± 0.1	1.0 ± 0.1
22:0	0.11 ± 0.1	0.5 ± 0.2
22:1n-11	Trace	1.5 ± 0.5

[a]Gas–liquid chromatographic analysis (in duplicate) on a polar capillary column (21,22). Values are means ± range/2.

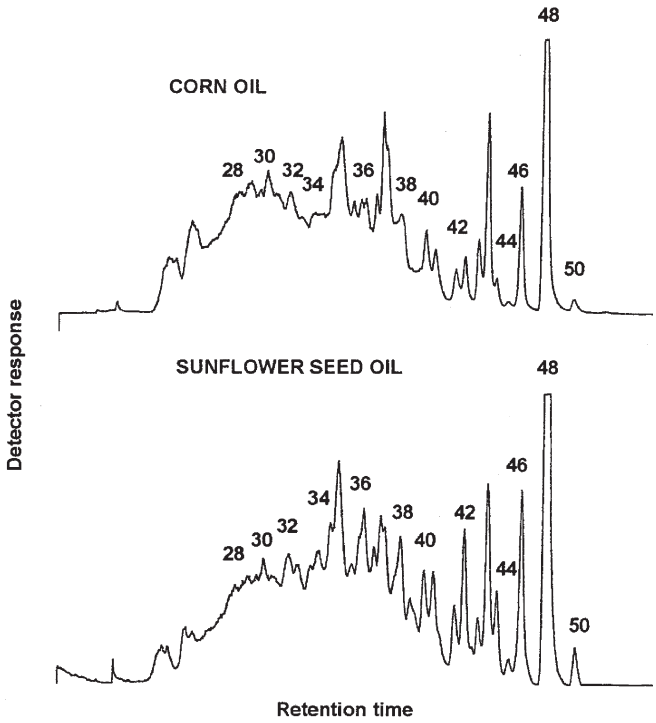

FIG. 2. Comparison of reversed-phase HPLC elution profiles of the corn and sunflower seed oils following oxidation with *tert*-butyl hydroperoxide (TBHP) and ferrous iron. Peak numbering for residual triacylglycerols is based on partition number. Conditions of HPLC separation and light-scattering detection as in the Materials and Methods section. Solvent gradient as in Figure 1. See Figure 1 for abbreviation.

carbon number (TCN). The oxidized triacylglycerols are eluted earlier with retention times corresponding to those recorded for synthetic triacylglycerols containing epoxy, hydroperoxy, and hydroxy fatty acids in combination with unmodified fatty acids or the core aldehydes as reported previously (22). On the basis of total peak area, up to 75–80% of the original oil has been oxidized. An examination of the total oxidation mixtures by reversed-phase LC/ESI/MS in the positive ion mode gave elution profiles similar to those obtained

by HPLC with light-scattering detection (chromatograms not shown). Although the residual triacylglycerol species were readily recognized, the parts of the chromatograms containing the oxygenated triacylglycerols were too complex for identification of the molecular species of the core aldehydes. Further reversed-phase HPLC analyses of the oxidized oils were performed following conversion of the aldehyde-containing triacylglycerols into DNPH derivatives.

Figure 3 (upper panel) shows the elution profile of the DNPH derivatives of oxidized sunflower oil triacylglycerols as recorded by reversed-phase LC/ESI/MS in the negative ion mode, along with the full mass spectrum averaged over the core aldehyde elution range (4–25 min). In the negative mode, only the DNPH derivatives and the presumed DNPH polymers (0–5 min) were ionized and detected. The negative ions recorded in the early part of the chromatogram (1.5–4.6 min) were of much lower molecular mass (*m/z* 403–513) and were attributed to the DNPH derivatives of the short-chain aldehydes and presumed DNPH derivatives produced during the oxidation of the sunflower oil triacylglycerols and were not further investigated. Similar results were obtained for the negative ion mass chromatograms of oxidized corn oil.

The major ions in the core aldehyde elution range (Fig. 3, lower panel) extend from *m/z* 926 to *m/z* 1142, the higher masses clearly exceeding those anticipated for the DNPH derivatives of the simple C_9–$C_{12:1}$ core aldehydes of sunflower oil triacylglycerols. The major peaks or peak clusters are numbered 1–9 for reference. The major ions are tentatively identified and assigned to specific peak clusters by determining the full mass spectra associated with each peak cluster as given in Table 3. The peak clusters correspond to the polyoxygenated (clusters 1–3), dioxygenated (clusters 4 and 5), monooxygenated (cluster 6), and simple core aldehydes (clusters 7, 8, and 9) in the oxidized sunflower oil sample. The peaks were identified and quantified by ion extraction from the total negative ion current profile by the computer. All ions represent the DNPH derivatives of triacylglycerols containing at least one core aldehyde group per molecule. The peaks possess symmetrical shapes but are resolved into two or more components based on the relative proportions of the regio- and geometric isomers in the sample. The peaks belong to homologous series and may be aligned according to their TCN. Many more ions could be similarly extracted and identified on the basis of the relative retention times observed for standards or extrapolated from them.

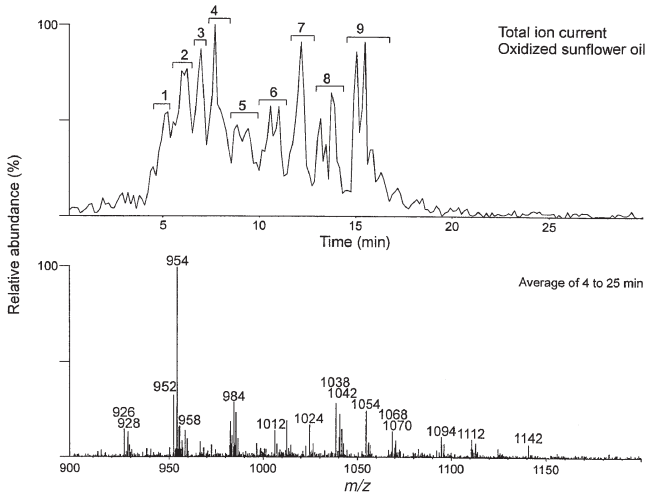

FIG. 3. Total negative ion elution profile of the 2,4-dinitrophenylhydrazine (DNPH) derivatives of oxidized sunflower oil triacylglycerols and full mass spectrum averaged over the acylglycerol elution range. Peaks are numbered as clusters in the elution profile and identified on the basis of subsequent analyses as follows: cluster 1, trialdehydes; cluster 2, core aldehyde trihydroxides or epoxydihydroxides; cluster 3, core aldehyde dihydroxides or epoxyhydroxides; cluster 4, core aldehyde hyroperoxides or epoxyhydroxides; cluster 5, core aldehyde hydroperoxides; cluster 6, TBHP adducts of core aldehyde epoxides; cluster 7, TBHP adducts of core aldehydes; cluster 8, core aldehydes; cluster 9, core aldehydes. For identification of ions, see Tables 4–6. Conditions for liquid chromatography with on-line electrospray ionization mass spectrometry (LC/ESI/MS) as in the Materials and Methods section. See Figure 2 for abbreviations.

TABLE 3
The Major Ions [as 2,4-dinitrophenylhydrazine (DNPH) derivatives] in Peak Clusters of Total Ion Current Profile of *tert*-Butyl Hydroperoxide (TBHP)-Oxidized Sunflower Seed Oil[a]

Peak cluster	Time range (min)	*m/z*	Molecular structure[a]
1	4.66–5.52	996	6:0ALD/8:0ALD/8:0ALD
		1012	18:2/18:3/9:0ALD, diOOH
		1052	18:2/18:3/9:0ALD, OH, TBHP
		1128	9:0ALD/9:0ALD/9:0ALD
		1140	18:1/18:2/9:0ALD, OH, diTBHP
		1142	18:1/18:1/9:0ALD, OH, diTBHP
2	5.66–6.53	1054	18:2/18:2/9:0, OH, TBHP
		1070	18:2/18:2/9:0ALD, OOH, TBHP
3	6.63–7.25	982	18:2/18:2/9:0ALD, OOH
		1012	16:0/18:3/12:1ALD, OOH, epoxy
		1038	18:2/18:2/12:1ALD, OOH, epoxy
		1056	18:1/18:2/9:0ALD, OH, TBHP
4	7.25–8.54	958	16:0/18:2/9:0ALD, OOH
		984	18:1/18:2/9:0ALD, OOH
5	8.54–9.84	940	16:0/9:0ALD/18:3, OH
		966	18:2/9:0ALD/18:2, OH
		984	18:1/18:2/9:0ALD, OOH
		1032	16:0/18:1/9:0 ALD, OH, TBHP
		1092	18:2/12:1ALD/18:3, TBHP, epoxy
		1112	18:2/18:2/8:0ALD, diTBHP
6	9.98–11.27	996	16:1/18:3/8:0ALD, TBHP
		1022	18:2/18:3/8:0, TBHP
		1038	18:2/18:2/9:0ALD, TBHP
		1068	16:0/18:3/12:1ALD, epoxy, TBHP
		1070	16:0/12:1ALD/18:2, epoxy, TBHP
		1094	18:2/18:2/12:1ALD, epoxy, TBHP
7	11.56–12.57	1006	18:2/12:1ALD/18:2, epoxy
		1024	18:2/18:2/8:0ALD, TBHP
		1042	18:2/9:0ALD/22:0, OOH
8	12.71–14.58	926	16:0/18:2/9:0ALD
		952	18:1/18:2/9:0ALD
		982	16:0/18:2/12:1ALD, epoxy
9	14.72–16.01	954	18:1/18:1/9:0ALD
		928	16:0/18:1/9:0ALD

[a]Ions were derivatized by DNPH. ALD, aldehyde.

TLC of DNPH derivatives. To confirm the identification of the triacylglycerol core aldehydes, the 2,4-DNPH-treated reaction mixture was subjected to TLC prefractionation. Figure 4 shows the TLC separation of the DNPH derivatives of oxidized sunflower seed oil. The complex mixture was resolved into a total of nine yellow bands and a residual triacylglycerol band (TLC band 10), which did not absorb in daylight but possessed a weak ultraviolet absorption. The oxotriacylglycerols were resolved on the basis of overall polarity and regio- and geometric configuration of the DNPH derivatives as subsequently established by reversed-phase LC/ESI/MS of the individual TLC bands in comparison to standards.

TLC/LC/ESI/MS of DNPH derivatives. The structures of the triacylglycerol core aldehydes were further confirmed and the core aldehyde content quantified by examining each TLC band by reversed-phase LC/ESI/MS.

TLC band 10. LC/ESI/MS with positive ionization showed that TLC band 10 was made up of residual triacylglycerols along with their TBHP adducts (27).

TLC bands 9 and 8. The DNPH derivatives recovered from TLC bands 9 and 8 showed mainly ions with masses below 600, which suggested that the major components of those bands were DNPH derivatives of the volatile short-chain aldehydes also produced by peroxidation of the sunflower oil triacylglycerols, although artifacts arising from polymerization of DNPH also were present. A typical low-molecular-weight aldehyde, hexanal, as the DNPH derivative (*m/z* 273), was found to migrate between residual triacylglycerols and the simple core aldehyde acylglycerols in our TLC system.

TLC bands 7 and 6. Figure 5 shows the total ion current profile (upper panel) of TLC band 7 from oxidized sunflower seed oil, along with the full mass spectrum (*m/z* 850–1150; lower panel) averaged over the oxotriacylglycerol elution range (2–23 min.). The complex profile of the chromatogram (upper panel of Fig. 5) is deceptive in view of the relative simplicity of the total mass spectrum (lower panel of Fig. 5), which shows major ions at *m/z* 928, 952, 954, and 956, with minor ions at *m/z* 876, 1014, and 1040. The peak complexity is due to the chromatographic resolution of the regio- and geometric isomers of the DNPH derivatives of the core aldehydes, which possess identical molecular masses, as shown by the mass chromatograms recorded in Figure 6 for simple core aldehyde peaks eluted over the period of 14.2–18.8 min. These peaks represent combinations of 9:0ALD (where ALD is aldehyde) with the common sunflower oil fatty acids. The major species are: 18:1/9:0ALD/18:2 (*m/z* 952), 18:1/18:1/9:0ALD (*m/z* 954), 16:0/18:2/9:0ALD (*m/z* 926), 18:0/9:0ALD/18:1 (*m/z* 956), and 16:0/18:0/9:0ALD (*m/z* 930), and there are many minor ones. These peaks clearly represent derivatives of homologous series of sunflower oil triacylglycerols. However, some fatty acids of the core aldehydes are more saturated than the fatty acids of the typical native

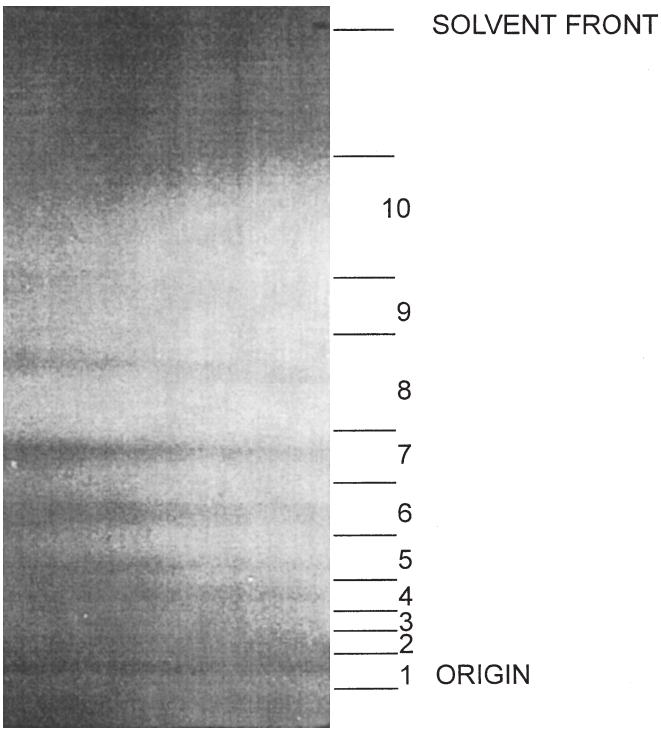

FIG. 4. Normal-phase thin-layer chromatography (TLC) separation of the total mixture of oxidized sunflower oil triacylglycerols following derivatization with 2,4-dinitrophenylhydrazine. TLC band 10, residual triacylglycerols; yellow TLC bands (in daylight) represent, in descending order, the following major components: 9 and 8, short-chain aldehydes; 7 and 6, simple triacylglycerol core aldehydes; 5 and 4, dioxygenated core aldehydes; 3 and 2, polyoxygenated core aldehydes; 1, trioxygenated core aldehydes. Heptane/isopropyl ether/acetic acid (60:40:4, by vol) solution was used as a mobile phase. The compounds were recovered by extraction with chloroform/methanol 2:1 (by vol) from silica gel 60 H plates. Procedures and conditions are given in the Materials and Methods section.

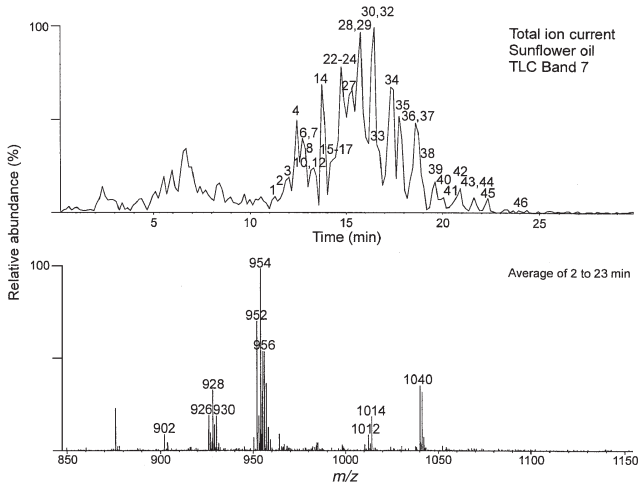

FIG. 5. Total negative ion current profile of TLC band 7 of the DNPH derivatives of oxidized sunflower seed oil (upper panel) and the full mass spectrum (lower panel) averaged over the core aldehyde elution range (2–23 min). Peak identification and ion assignment are given in Table 4. Earlier eluted ions are attributed to hydrazones of short-chain aldehydes and artifacts from polymerization of DNPH. LC/ESI/MS conditions are as given in the Materials and Methods section. See Figures 3 and 4 for abbreviations.

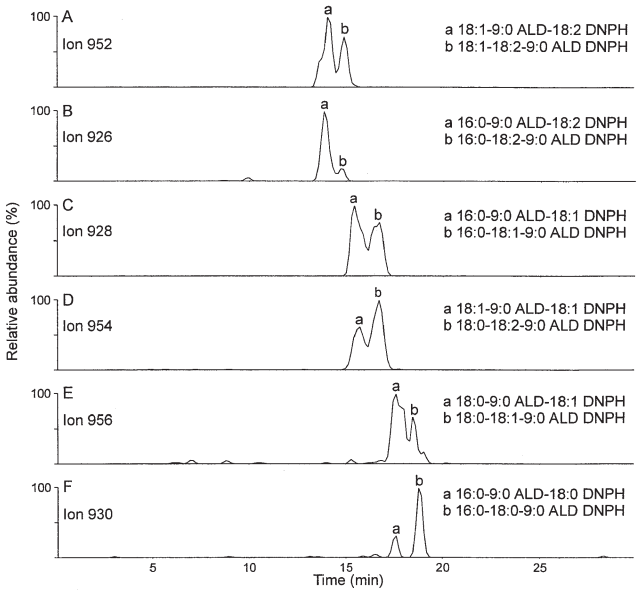

FIG. 6. Mass chromatograms of major ions of (9-oxo)nonanoyl acylglycerols as DNPH derivatives from TLC band 7 of oxidized sunflower oil. Ion identification is given in Table 4. LC/ESI/MS conditions are as described in the Materials and Methods section. ALD, aldehyde; see Figures 3 and 4 for other abbreviations.

sunflower oil. In addition to 9:0 core aldehydes, a series of compounds was found to correspond to 8:0 core aldehydes. However, there were no masses corresponding to the 13:2 and 12:1 core aldehydes in TLC band 7 nor in TLC bands 8 and 9, which were examined in case these core aldehydes had migrated ahead of the other 9:0 core aldehydes. The peaks eluted over the range of 11–13.5 min, and clearly ahead of the simple core aldehydes, represented the TBHP adducts of the unsaturated core aldehydes. The major adduct species were: 18:1/18:2/9:0ALD, TBHP (*m/z* 1042), 18:1/18:1/9:0ALD, TBHP (*m/z* 1040), and 16:0/18:2/9:0ALD, TBHP (*m/z* 1014). No adducts were seen for the saturated triacylglycerol core aldehyde species. TLC band 7 contained the bulk of the triacylglycerol core aldehydes (mainly mono-9:0) with minor amounts found also in TLC band 6 (*m/z* 928, *m/z* 952, and *m/z* 956) as listed in Table 4. A total of 32 9:0 and 14 8:0 core aldehyde-containing triacylglycerols, including the TBHP adducts, were identified in both corn and sunflower oils. The identifications were consistent with the ion masses, the reversed-phase HPLC retention times of standards, as well as the TCN, and the relative proportions of the parent triacylglycerols in the original oils. The abundance of the molecular ions in the major TLC bands provided an uncalibrated quantification of the various members of each homologous series. The major TBHP adduct species also corresponded to the major species of the triacylglycerol core aldehydes: 18:1/18:2TBHP/9:0ALD DNPH (*m/z* 1040), 18:2/18:2TBHP/9:0ALD DNPH (*m/z* 1038), 16:0/18:2TBHP/9:0ALD DNPH (*m/z* 1014), and others. The masses of the TBHP adducts were consistent with the presence of peroxide bridging at the sites expected to be occupied by hydroperoxyl groups, the retention times on the reversed-phase column, and TCN, as well as with the structures of the major molecular species of residual triacylglycerols in both corn and sunflower oils. There were no significant ions corresponding to the TBHP adducts of the 12:1 core aldehydes in these bands. A similar approach was used in examining other TLC bands.

TLC bands 5 and 4. TLC band 5 contained the bulk of the DNPH-derivatized material. The major ions were found at *m/z* 956, 966, 982, and 984, with minor ions at *m/z* 1006, 1008, 1068, 1012, 1024, and 1094, indicating the presence of core aldehydes in combination with two full-length normal or oxidized-chain fatty acids in the same triacylglycerol molecule. There were indications of the presence of several homologous series of the core aldehydes. The minor peaks eluted over the time period 7.5–11.7 min represented mostly the hydroperoxides of the 9:0 core aldehydes with *m/z* values of 982, 958, 984, 986, 988, and 1042. In the hydroperoxides of 9:0 core aldehydes, the hydroperoxyl group was located on one of two unsaturated fatty acids bound to the triacylglycerol molecule containing the aldehyde group. The peaks eluted over the time period 11.8–13.9 min included ions with *m/z* values of 1000 and 1026, which could be attributed to TBHP adducts of 12:1 core aldehydes. The peaks eluted over the time period 9.3–12.7 min represent the TBHP adducts of the 12:1 core aldehyde monoepoxides with *m/z* values of 1092, 1094, 1068, 1070, 1096, and 1098. The peaks emerging over the time period 12.3–20.3 min represent an orderly elution sequence of the monoepoxides of 12:1 core aldehydes with *m/z* values of 1006, 980, 956, 1008, 982, and 1010. The sudden appearance of the 12:1 aldehydes in the epoxy triacylglycerols suggests that the epoxy group is located on the 12:1 aldehyde chain. The monoepoxides of 12:1 core aldehydes have two fatty acids and the epoxidized 12:1 aldehyde chain esterified to a parent triacylglycerol. However, the structures of epoxy compounds are shown in this paper without the loss of double bonds to indicate the original composition of core aldehydes. TLC band 4 contained the regioisomers of the epoxy core aldehydes in TLC band 5 along with the corresponding TBHP adducts (mass chromatograms not shown). The chromatographic properties and the relative quantities of the epoxides of the 12:1 core aldehydes and the corresponding TBHP adducts in TLC bands 5 and 4 are given in Table 5 in order of the reversed-phase HPLC retention times. In all instances, the relative proportions of the aldehyde derivatives corresponded to the relative proportions of the major triacylglycerols in the original oils. In the case of corn oil, some of the core aldehyde hydroperoxides were found, in part, in TLC bands 6 and 4 and in TLC bands 4 and 3. The identification of all major aldehyde hydroperoxides was consistent with ion masses and relative retention times of standards, as well as the TCN and the total acyl carbon and double-bond numbers. The molecular-ion abundances in the major TLC bands provide uncorrected estimates of the relative quantities of the various members of each homologous series.

TLC bands 3, 2, and 1. These minor TLC fractions represent the core aldehydes of the more highly oxygenated tri-

TABLE 4
Composition and Structure of Triacylglycerol Core Aldehydes and Peroxide Adducts in TBHP-Oxidized Corn and Sunflower Seed Oils as Determined by Reversed-Phase Liquid Chromatography with On-line Electrospray Ionization Mass Spectrometry (LC/ESI/MS) (TLC bands 6 and 7)[a]

No	Avg Rt	TCN	ACN/DB	Mass[b]	Abundance[c]		Molecular structure[d]
					Corn	Sunflower	
1	11.23	31.33	45:4	1038	500	11400	18:2/9:0ALD/18:2, TBHP
2	11.43	30.99	42:2	1000	400	500	16:1/8:0ALD/18:1, TBHP
3	12.03	31.67	45:4	1038	6500		18:2/18:2/9:0ALD, TBHP
4	12.45	33.17	43:2	1014	2900	4900	16:0/9:0ALD/18:2, TBHP
5	12.59	32.51	42:2	1000	400		16:0/18:2/8:0ALD, TBHP
6	12.63	32.66	45:3	1040	8400	14500	18:1/9:0ALD/18:2, TBHP
7	12.79	33.51	43:2	1014	5400	3300	16:0/18:2/9:0ALD, TBHP
8	12.97	33.27	45:4	950	400	1900	18:2/9:0ALD/18:2
9	13.32	33.60	44:3	938	400		18:1/8:0ALD/18:2
10	13.37	33.00	45:3	1040	3500	5500	18:1/18:2/9:0ALD, TBHP
11	13.58	34.05	44:3	938	200		18:1/18:2/8:0ALD
12	13.63	33.72	45:4	950	300	2000	18:2/18:2/9:0ALD
13	13.94	33.06	44:3	1026	500		18:0/18:3/8:0ALD, TBHP
14	14.20	34.60	45:3	952	8100	18100	18:1/9:0ALD/18:2
15	14.20	35.11	43:2	926	6100	5900	16:0/9:0ALD/18:2
16	14.30	34.66	44:3	938	100		18:0/8:0ALD/18:3
17	14.58	34.21	45:2	1042	4100		18:1/9:0ALD/18:1, TBHP
18	14.70	35.15	44:2	940	500	1500	18:1/8:0ALD/18:1
19	14.70	35.89	40:0	888	200	500	16:0/8:0ALD/16:0
20	14.73	35.11	44:3	938	400		18:0/18:3/8:0ALD
21	14.79	35.38	42:1	914	500	1300	16:0/8:0ALD/18:1
22	14.80	35.56	43:2	926	1200	9100	16:0/18:2/9:0ALD
23	15.03	35.83	42:1	914	900	300	16:0/18:1/8:0ALD
24	15.03	36.11	45:3	952	4300	15500	18:1/18:2/9:0ALD
25	15.11	35.60	44:2	940	1600	2400	18:1/18:1/8:0ALD
26	15.36	36.34	40:0	888	100	200	16:0/16:0/8:0ALD
27	15.48	36.89	41:0	902	5900	2300	16:0/9:0ALD/16:0
28	15.52	34.44	45:2	1042	3200		18:1/18:1/9:0ALD, TBHP
29	15.76	36.38	43:1	928	16700	10600	16:0/9:0ALD/18:1
30	15.83	36.15	45:2	954	9500	14300	18:1/9:0ALD/18:1
31	16.41	37.38	44:1	942	400		18:0/8:0ALD/18:1
32	16.56	37.34	41:0	902	3500	1800	16:0/16:0/9:0ALD
33	16.61	36.83	43:1	928	13000	10200	16:0/18:1/9:0ALD
34	16.65	37.56	45:2	954	15300	12300	18:0/18:2/9:0ALD
35	17.60	38.89	43:0	930	2000	5300	16:0/9:0ALD/18:0
36	17.75	38.38	45:1	956	5500	10900	18:0/9:0ALD/18:1
37	18.75	38.83	45:1	956	5400	8100	18:0/18:1/9:0ALD
38	18.77	39.34	43:0	930	5800	11200	16:0/18:0/9:0ALD
39	19.71	40.89	45:0	958	5300	4100	18:0/9:0ALD/18:0
40	20.11	40.38	47:1	984	4100	1800	20:0/9:0ALD/18:1
41	20.58	41.11	49:2	1010	700		18:2/9:0ALD/22:0
42	21.13	41.34	45:0	958	1000	3600	18:0/18:0/9:0ALD
43	21.65	41.56	49:2	1010	700		22:0/18:2/9:0ALD
44	21.82	42.38	49:1	1012	400	700	18:1/9:0ALD/22:0
45	22.73	42.83	49:1	1012	100	2600	22:0/18:1/9:0ALD
46	24.19	44.89	49:0	1014	100	400	18:0/9:0ALD/22:0

[a]Abbreviations: Avg Rt, average retention time; TCN, theoretical carbon number; ACN, acyl carbon number; DB, double bond; TLC, thin-layer chromatography. See Table 3 for other abbreviations.
[b][M + DNPH].
[c]Abundance of ion in the major TLC band.
[d]Core aldehyde regioisomers consistent with chromatographic and MS properties.

acylglycerols possessing complex elution profiles (total ion currents not shown). The tentative peak identifications again were based on the TLC/HPLC behavior of synthetic standards, the specificity of the negative ion response to the DNPH derivatives, and knowledge of the general nature of the products of TBHP oxidation of standard triacylglycerols. As an example, Figure 7 shows the single ion mass chromatograms of major hydroxy (9-oxo)nonanoyl acylglycerols extracted from TLC band 2. Table 6 summarizes the chromatographic properties of the identified peaks along with the ion abundance recorded for each molecular species in TLC bands 3 and 2 and in the most polar minor TLC band 1. There remained significant ion abundance at *m/z* 1040, 1056, 958, 980, 960, 982, 986, 1008, and 1012 in TLC band 3 and at *m/z*

Table 5
Composition and Structure of Triacylglycerol Core Aldehydes and Peroxide Adducts in TBHP-Oxidized Corn and Sunflower Seed Oils as Determined by Reversed-Phase LC/ESI/MS (TLC bands 4 and 5)[a]

No	Avg Rt	TCN	ACN/DB[b]	Mass[c]	Abundance[d]		Molecular structure[b,e]
					Corn	Sunflower	
1	7.52	24.87	45 : 4	982		3300	18:2/9:0ALD/18:2, OOH
2	8.20	26.71	43 : 2	958	9600	2900	16:0/9:0ALD/18:2, OOH
3	8.44	26.20	45 : 3	984	9500	8400	18:1/9:0ALD/18:2, OOH
4	9.23	27.28	45 : 2	986	7000	3100	18:1/9:0ALD/18:1, OOH
5	9.25	27.51	43 : 1	960		600	16:0/9:0ALD/18:1, OOH
6	9.32	25.81	48 : 6	1092		9500	18:2/12:1ALD/18:3, epoxy, TBHP
7	9.91	26.22	48 : 6	1092	1700	9200	18:2/18:3/12:1ALD, epoxy, TBHP
8	10.08	29.51	45 : 1	988		800	18:0/9:0ALD/18:1, OOH
9	10.77	27.20	48 : 5	1094		17500	18:2/12:1ALD/18:2, epoxy, TBHP
10	10.86	27.59	46 : 4	1068	4100	12200	16:0/2:1ALD/18:3, epoxy, TBHP
11	11.06	29.04	46 : 3	1070		1100	16:0/12:1ALD/18:2, epoxy, TBHP
12	11.24	27.72	48 : 5	1094	6400	5200	18:2/18:2/12:1ALD, epoxy, TBHP
13	11.31	28.81	48 : 4	1096	700	4000	18:1/12:1ALD/18:2, epoxy, TBHP
14	11.43	28.00	46 : 4	1068	7000	3200	16:0/18:3/12:1ALD, epoxy, TBHP
15	11.63	32.71	49 : 2	1042	1600	600	18:2/9:0ALD/22:0, OOH
16	11.68	33.51	49 : 1	1044	400	300	22:0/9:0ALD/18:1, OOH
17	11.81	31.44	44 : 3	1026		800	18:1/8:0ALD/18:2, TBHP
18	12.27	29.53	46 : 4	980	5100	8400	16:0/12:1ALD/18:3, epoxy
19	12.27	31.66	44 : 3	1026	3500	1800	18:1/8:0ALD/18:2, TBHP
20	12.32	29.14	48 : 5	1006	6300	13700	18:2/12:1ALD/18:2, epoxy
21	12.32	32.17	42 : 2	1000	900		16:0/8:0ALD/18:2, TBHP
22	12.44	29.22	48 : 4	1096		800	18:1/18:2/12:1ALD, epoxy, TBHP
23	12.55	29.66	48 : 5	1006	2700	8700	18:2/18:2/12:1ALD, epoxy
24	12.59	32.51	42 : 2	1000	500		16:0/18:2/8:0ALD, TBHP
25	12.65	30.05	46 : 4	980	3600	8700	16:0/18:3/12:1ALD, epoxy
26	12.66	32.00	44 : 3	1026	4100	2000	18:1/18:2/8:0ALD, TBHP
27	12.71	31.08	48 : 3	1098		6800	18:1/18:1/12:1ALD, epoxy, TBHP
28	13.45	30.53	44 : 2	956	8000	4100	16:0/12:1ALD/16:1, epoxy
29	13.54	30.75	48 : 4	1008	6400	11300	18:1/12:1ALD/18:2, epoxy
30	13.59	30.98	46 : 3	982	29700	17100	16:0/12:1ALD-18:2, epoxy
31	13.75	31.05	44 : 2	956	11000	5800	16:0-16:1/12:1ALD, epoxy
32	13.92	31.27	48 : 4	1008	19400	25800	18:1/18:2/12:1ALD, epoxy
33	13.94	33.06	44 : 3	1026	900		18:0/18:3/8:0ALD, TBHP
34	13.98	32.72	48 : 3	1010	3300	3500	18:1/12:1ALD/18:1, epoxy
35	14.04	31.50	46 : 3	982	16000	12200	16:0/18:2/12:1ALD, epoxy
36	15.57	32.53	46 : 2	984	1700	7300	16:0/12:1ALD/18:1, epoxy
37	15.63	33.24	48 : 3	1010	3800	8600	18:1/18:1/12:1ALD, epoxy
38	17.67	34.53	48 : 2	1012		1800	18:0/12:1ALD/18:1, epoxy
39	19.61	36.98	52 : 3	1066		1300	22:0/12:1ALD/18:2, epoxy
40	20.25	37.50	52 : 3	1066		800	22:0/18:2/12:1ALD, epoxy

[a]See Tables 3 and 4 for abbreviations.
[b]ACN/DB values of molecular structure are presented without loss of double bonds due to epoxidation.
[c][M + DNPH].
[d]Abundance of ion in the major TLC band.
[e]Core aldehyde regioisomers consistent with chromatographic and MS properties.

902, 984 1054, 1142, 1026, 944, 970, 1194, 986, 982, and 1012 in TLC band 2**,** for which identities consistent with the retention time, TCN, acyl carbon and double-bond numbers, and *m/z* values could not be immediately suggested.

Table 7 gives the estimated yields of the identified oxidation products for both seed oils. Depending on the mass range over which the total ion current is integrated, the overall yields of the identified aldehyde-containing triacylglycerols range from 32 to 43% of DNPH derivatives in the total oxidation mixture. The major product was the mixed acid acylglycerol containing the 9:0 monoaldehyde (44–60% of identified core aldehydes) followed by hydroperoxy (13–16% of the identified species) and monoepoxy aldehydes (11–12% of the identified species) and hydroperoxyepoxy aldehydes (up to 10% of the identified species). More of the TBHP adduct was obtained during the oxidation of the sunflower oil. The major ion masses correspond to the monoaldehyde esters of the 16:0/18:1/18:2 (16:0/18:1/9:0ALD DNPH, *m/z* 928), 16:0/18:2/18:0 (16:0/9:0ALD/18:0DNPH, *m/z* 930), 18:1/18:1/18:2 (18:1/18:1/9:0ALD DNPH, *m/z* 954), 18:1/18:2/18:2 (18:1/18:2/9:0ALD DNPH, *m/z* 952), and 18:0/18:1/18:2 (18:0/18:1/9:0ALD DNPH, *m/z* 956) species, which are the major triacylglycerols in corn and sunflower oils. Apparently, the linoleic acid residue in each instance was converted to the (9-oxo)nonanoate and, to a lesser extent, the

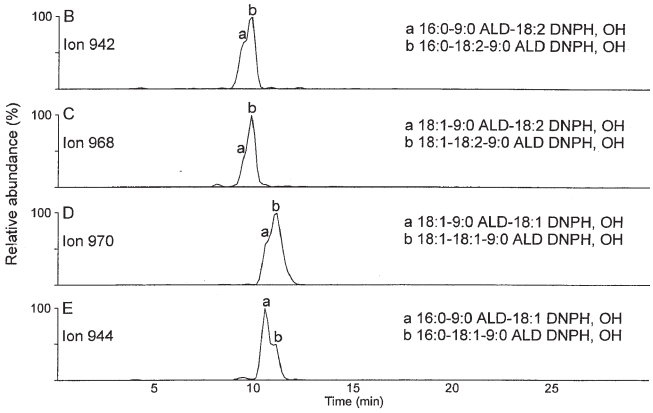

FIG. 7. Mass chromatograms of major ions of hydroxy (9-oxo)nonanoyl acylglycerols as DNPH derivatives from TLC band 2 of oxidized sunflower oil. Ion identification is given in Table 6. LC/ESI/MS conditions are as described in the Materials and Methods section. See Figures 3, 4, and 6 for abbreviations.

TABLE 7
Proportion of Identified Core Aldehydes in Total TBHP Oxidation Mixture[a]

Type of aldehyde	Corn oil[b] (mol%)	Sunflower oil[b] (mol%)
Mixed acid mono aldehydes (8:0)	0.3 ± 0.1	0.2 ± 0.1
Mixed acid mono aldehydes (9:0)	19.2 ± 3.2	18.9 ± 3.8
Hydroperoxy aldehydes (9:0)	5.0 ± 1.0	5.4 ± 1.1
Hydroxy aldehydes (9:0)	0.4 ± 0.2	0.5 ± 0.1
Epoxy aldehydes (12:1)	3.5 ± 0.7	4.9 ± 1.0
Hydroperoxyepoxy aldehydes (12:1)	0.1 ± 0.1	4.1 ± 0.8
Diepoxy aldehydes (12:1)	0.8 ± 0.3	0.3 ± 0.1
Dialdehydes (9:0)	0.5 ± 0.1	0.4 ± 0.2
TBHP adduct aldehydes (8:0)	0.3 ± 0.1	0.3 ± 0.2
TBHP adduct aldehydes (9:0)	0.6 ± 0.2	4.2 ± 1.0
TBHP adduct epoxy aldehydes (12:1)	1.5 ± 0.4	4.1 ± 0.9
Total	32.2 ± 6.4	43.1 ± 9.3

[a]See Table 3 for abbreviation.
[b]Proportion of integrated areas of ion current (mass range 750–1250) of total oxidation mixture.

(12-oxo)-9,10-dodecenoate, whereas most of the anticipated (13-oxo)-9,11-tridecadienoyl core aldehydes were oxidized further to the (9-oxo)nonanoyl core aldehydes.

The most polar oxotriacylglycerols were these with two or more functional groups, e.g., epoxy aldehyde esters and hydroperoxy aldehyde esters (mainly TLC bands 2 and 3). Oxo-

TABLE 6
Composition and Structure of Triacylglycerol Core Aldehydes and Peroxide Adducts in TBHP-Oxidized Corn and Sunflower Seed Oils as Determined by Reversed-Phase LC/ESI/MS (TLC bands 1, 2, and 3)[a]

No	Avg Rt	TCN	ACN/DB[b]	Mass[c]	Abundance[d] Corn oil	Abundance[d] Sunflower	Molecular structure[b,e]
1	5.99	19.35	48:6	1036	700		18:2/12:1ALD/18:3, OOH, epoxy
2	6.41	24.33	36:3	1020	900	1700	18:3/9:0ALD/9:0ALD
3	6.67	20.74	48:5	1038	8200	6500	18:2/12:1ALD/18:2, OOH, epoxy
4	6.67	21.13	46:4	1012	4900	4000	16:0/12:1ALD/18:3, OOH, epoxy
5	6.85	25.42	46:4	996		2800	16:0/12:1ALD/18:3, diepoxy
6	7.13	22.58	46:3	1014	2700		16:0/12:1ALD/18:2, OOH, epoxy
7	7.24	25.78	36:2	1022	1600	4400	18:2/9:0ALD/9:0ALD
8	7.29	25.87	46:3	996		2400	16:0/18:3/12:1ALD, diepoxy
9	7.55	22.35	48:4	1040	1400	4000	18:1/12:1ALD/18:2, OOH, epoxy
10	7.95	27.56	34:0	998	1300	5300	16:0/9:0ALD/9:0ALD
11	8.05	27.05	36:1	1024	3300	3500	18:1/9:0ALD/9:0ALD
12	8.37	25.10	45:4	966		300	18:2/9:0ALD/18:2, OH
13	8.40	27.09	48:4	1024	2200		18:1/18:2/12:1ALD, diepoxy
14	8.65	29.56	36:0	1026		2000	18:0/9:0ALD/9:0ALD
15	8.78	25.55	45:4	966		7700	18:2/18:2/9:0ALD, OH
16	9.57	26.94	43:2	942	3500	8800	16:0/9:0ALD/18:2, OH
17	9.59	26.43	45:3	968	4900	12400	18:1/9:0ALD/18:2, OH
18	9.99	26.88	45:3	968	5600	35700	18:1/18:2/9:0ALD, OH
19	10.01	27.20	43:2	942	8700	13400	16:0/18:2/9:0ALD, OH
20	10.79	27.69	45:2	970	11000	32100	18:1/9:0ALD/18:1, OH
21	10.83	27.92	43:1	944	23900	36600	16:0/9:0ALD/18:1, OH
22	11.30	28.14	45:2	970	32500	45400	18:1/18:1/9:0ALD, OH
23	11.34	28.12	43:1	944	18300	18200	16:0/18:1/9:0ALD, OH
24	11.99	28.61	48:3	1026		5200	18:1/12:1ALD/18:1, diepoxy
25	12.50	29.92	45:1	972	500	17000	18:0/9:0ALD/18:1, OH
26	13.11	30.12	45:1	972	1400	14600	18:0/18:1/9:0ALD, OH
27	16.41	33.92	49:1	1028		6000	22:0/9:0ALD/18:1, OH
28	17.03	34.12	49:1	1028		1100	22:0/18:1/9:0ALD, OH

[a]See Tables 3 and 4 for abbreviations.
[b]ACN/DB values of molecular structure are presented without loss of double bonds due to epoxidation.
[c][M + DNPH].
[d]Abundance of ion in the major TLC band.
[e]Core aldehyde regioisomers consistent with chromatographic and MS properties.

triacylglycerols of intermediate polarity were represented by triacylglycerols containing two core aldehyde groups (mainly TLC bands 4 and 5), whereas the least polar were the oxotriacylglycerols with a single core aldehyde group (TLC bands 6 and 7). There was partial resolution of triacylglycerols containing a single 9:0 or 12:1 core aldehyde in the primary and the secondary position. Further complications arose from a conversion of some of the aldehyde groups into acids by further oxidation. These oxidation products were seen as DNPH derivatives only when combined with another aldehyde or keto group in the same molecule (data not shown).

DISCUSSION

TBHP is a synthetic organic hydroperoxide that is commonly employed to accelerate lipid peroxidation *in vitro*. The decomposition of the hydroperoxide to alkoxy or peroxy radicals stimulates the chain reaction of lipid peroxidation. The decomposition is aided by metal ions such as Fe^{2+} and their complexes (28). In order to increase the yield of the secondary oxidation products (e.g., core aldehydes) in the present study, the triacylglycerol peroxidation and hydroperoxide decomposition were further stimulated by elevated temperature (37°C) and the inclusion of bile salts as emulsifiers. As a result of oxidation, it was possible to obtain within 1–2 h a controlled oxidative destruction of corn and sunflower oil triacylglycerols equivalent to many months of autoxidation by thin-film exposure to air. About 90% destruction of 18:2 had occurred with relatively little loss of 18:1 as judged from the ratio of the unsaturated fatty acids to palmitic acid in the oxidized oil. The proportion of the saturated fatty acids (16:0, 18:0, 20:0, and 22:0) had proportionally increased in the oxidized corn and sunflower oil triacylglycerols along with the appearance of hydroperoxy and epoxy fatty acids (data not shown). It was, therefore, anticipated that the major triacylglycerol core aldehydes would have arisen largely from the oxidation of 18:2 and would be found in combination with palmitic and oleic acids as the DNPH derivatives of the oxotriacylglycerols.

The main mechanism for the formation of aldehydes from lipid hydroperoxides is homolytic scission β-cleavage) of the two C–C bonds on either side of the hydroperoxy group (6,29) as shown in Figure 8. This reaction proceeds *via* the lipid alkoxy radical. Applying this mechanism to triacylglycerols, it would be anticipated that the cleavage of the carbon–carbon bond would result in aldehydes derived from the methyl termini of the fatty acid chains and of aldehydes still bound to the parent lipid molecule. Hydroperoxides also undergo heterolytic reactions yielding the same products as homolytic reactions. Another possible mechanism for the formation of aldehydes is the Hock–Criegee rearrangement (29,30). This acid-catalyzed carbon-to-oxygen rearrangement of hydroperoxides would be expected to yield products from linoleic acid hydroperoxide cleavage similar to those arising from homolytic scission (29).

Oxidation of methyl oleate (1–3,6), has shown that the 9- and 10-hydroperoxides are formed in amounts approximately similar to those of the 8- and 11-hydroperoxides. Therefore, the oleic acid-containing triacylglycerols would be expected to yield (8-oxo)octanoyl and (11-oxo)-9,10-undecenoyl acylglycerols, as well as (9-oxo)nonanoyl and (10-oxo)-8,9-decenoyl acylglycerols. Indeed, small amounts of (8-oxo)-octanoyl-containing acylglycerols were observed. Specific masses corresponding to (10-oxo)-Δ^8-decenoyl and (11-oxo)-9,10-undecenoyl acylglycerols were not found, however. It is possible that the ions arising from the minor (11-oxo)-9,10-undecenoyl acylglycerols overlapped with those arising from the major (9-oxo)nonanoyl acylglycerols.

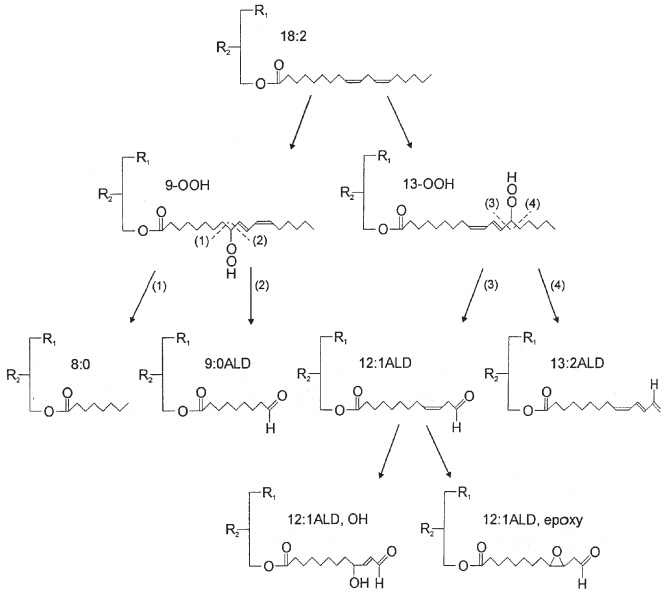

FIG. 8. Postulated formation of triacylglycerol core aldehydes from linoleic acid-containing triacylglycerol. R_1 and R_2 are fatty acids esterified to a parent triacylglycerol. Positions of cleavage of the fatty acid chain of hydroperoxides are numbered 1 to 3. In oxidation by TBHP, the 9,10-epoxy derivative of the (12-oxo)dodecenoyl core aldehyde is assumed to be formed in preference to 9-hydroxy-10,11-dodecenoyl core aldehydes. Formation of the core aldehyde can take place on any *sn*-position of acylglycerol. See Figures 2 and 6 for abbreviations.

The major hydroperoxides arising from the oxidation of methyl linoleate are the 9-hydroperoxy and 13-hydroperoxy isomers in equal amounts (2,31). Recently, the 11-hydroperoxide of linoleic acid has been recognized as one of the primary oxidation products (32). It made up 5–10% of the abundance of 9- and 13-hydroperoxide in vitamin E-controlled autoxidation. However, in the absence of vitamin E, 11-peroxyl radicals are not stable and 9- and 13-hydroperoxides predominate (32). The core aldehydes derived from 9- and 13-hydroperoxides of linoleic acid-containing acylglycerols are expected to be 8-nonanoic acid and 9-oxononanoyl-, (12-oxo)-9,10-dodecenoyl, and (13-oxo)-9,11-tridecadienoyl acylglycerols (6,29,33,34). Figure 8 shows the proposed formation of the major core aldehydes from linoleic acid attributable to autoxidation and oxidation by TBHP. According to Frankel (33), the homolytic cleavage of the oxygen–oxygen

bond is the first step in the decomposition of unsaturated hydroperoxides (33). Decomposition proceeds with cleavage of the fatty acid chain on either side of the hydroperoxide group or by cleavage of the oxygen–carbon bond, leading to positional isomerization of the unsaturated hydroperoxides. Simple β-cleavage to 9- and 13-hydroperoxide would produce 9-oxo- and 12-oxo-derivatives as major core aldehydes. The formation of the (12-oxo)-9,10-dodecenoyl acylglycerols would involve another mechanism linked closely with the β-cleavage reaction and would depend upon an alkenyl radical formation. This 1-olefin radical could combine with a hydroxy radical to form an enol which would rearrange to an aldehyde (6,33). Esterbauer *et al.* (6) have pointed out still another possibility provided by the reaction with oxygen to form a hydroperoxide that can decompose again to the enol and then form the aldehyde. If this radical abstracted a hydrogen, it would become a core alkene, which would not be detected as the DNPH derivative unless another aldehyde group was present in the acylglycerol molecule. In the present study, there was no evidence for the presence of core aldehydes carrying short alkene chains in the acylglycerol molecules.

Acid-catalyzed cleavage of the fatty acid chain between the hydroperoxide group and the α-olefinic carbon leads also to formation of the (12-oxo)-9,10-dodecenoyl acylglycerol from the 13-hydroperoxide of oxidized linoleic acid (29,30). Whereas the 9-oxononanoyl and (12-oxo)-9,10-dodecenoyl acylglycerols were readily detected, those corresponding to the (13-oxo)-9,11-tridecadienoyl acylglycerols were not found. Apparently, this unsaturated core aldehyde did not survive the oxidation and/or workup conditions. In plants, enzymatic processes resembling radical cleavage reactions take place. Gardner (34) has categorized two types of hydroperoxide lyases as heterolytic and homolytic types, found in higher plants and mushrooms, respectively. The heterolytic type of lyase catalyzes the cleavage of the fatty acid chain between the hydroperoxide group and the α-olefinic carbon, resembling acid-catalyzed cleavage of the hydroperoxide. As a result of decomposition of the 13-hydroperoxide, (13-oxo)-9,11-tridecadienoyl is formed when homolytic lyase activity is present, mimicking β-scission.

The absence of the 12:1 aldehydes from the simple core aldehyde fractions and their appearance in the epoxy triacylglycerol fractions suggest that the epoxy group is associated with the 12:1 aldehyde chain. According to Frankel *et al*. (35) and Frankel (1,3), epoxy esters of 18:1 can arise from its reaction with hydroperoxides or from the hydroperoxides themselves. Gardner *et al.* (36) have discussed the enzymatic and nonenzymatic decomposition mechanisms for linoleic acid hydroperoxides leading to epoxide formation. The major pathways postulated for decomposition in the presence of Fe^{3+}/cysteine catalyst involved formation of an alkoxy radical by loss of OH, cyclization of the alkoxy radical to the α-unsaturation, and reaction of the epoxy allylic radical either with O_2 to form an epoxyhydroperoxy monoene or with OH to form the epoxyhydroxymonoene, as well as other reactions. Noordermeer *et al*. (37) have identified 9-hydroxy-(12-oxo)-(10E)- and 11-hydroxy-(12-oxo)-(9Z)-dodecenoic acids in incubations of (12-oxo)-(9Z)-dodecenoic acid formed by a nonenzymatic process. Our results are in accordance with the findings of Noordermeer *et al*. (37), which showed that (12-oxo)dodecenoyl acylglycerols are readily further oxidized to the corresponding hydroxy, hydroperoxy, or epoxy compounds. It is possible that epoxy compounds may be formed more readily than hydroperoxy or hydroxy derivatives during TBHP oxidation. Indeed, TBHP has been employed as the oxidant for epoxidation of oleic, linoleic, linolenic, and arachidonic acids in aqueous buffers containing surfactant or in organic solvents (38). In the present study, tentative identifications were made of the diepoxides based on their migration on TLC.

The identity of the hydroperoxy linoleic and oleic acid components of the oxoacylglycerols was determined by GC–MS analysis following collection of individual HPLC peaks, hydrogenation (platinum oxide catalyst), and transmethylation with sodium methoxide. Samples were analyzed as their trimethylsilyl ether derivatives as described by Hughes *et al*. (39). In each case, the position of hydroxylation was determined from the major fragment ions formed on either side of the oxygen trimethylsilyl group. The *cis/trans* configuration of bonds was assigned on the basis of previous work. Porter *et al.* (40) had shown that the *cis/trans* isomers eluted earlier than the corresponding *trans/trans* isomers when normal-phase HPLC systems were used. According to Park *et al.* (41), the *cis/trans* isomer of the 9-hydroperoxy derivative of trilinoleoylglycerol eluted ahead of the 9-hydroperoxy *trans/trans* isomer from a reversed-phase column. However, the *trans/trans* isomer of the 13-hydroperoxy trilinoleoylglycerol eluted ahead of the corresponding *cis/trans* isomer.

In addition to core aldehydes, ketones were expected to be found among DNPH derivatives of TBHP-oxidized seed oil triacylglycerols. Because a homologous series of appropriate ions was not found, it was not possible to conclude that any isolated masses corresponding to hypothetical DNPH derivatives of ketotriacylglycrols were actually present. Individual keto compounds may have been present in our preparations, but specific identifications were not made owing to a lack of appropriate reference compounds. Possible ketotriacylglycerols could be represented by such ions as *m/z* 1092, *m/z* 1094, and *m/z* 1096, which were tentatively assigned to the TBHP adducts of epoxy 12:1 aldehydes on the basis of their chromatographic behavior.

Many of the triacylglycerol core aldehydes appeared as double peaks, which were attributed to regioisomers. On the basis of previous work with standard oxotriacylglycerols (22,42), the earlier eluted peak was attributed to an aldehyde in the secondary position and the later eluted peak to an aldehyde in the primary position of the oxidized triacylglycerol molecule. A functional group in the *sn*-2 position is known to exert higher polarity than a similar group in the *sn*-1 or *sn*-3 position of the triacylglycerol molecule (22,43,44). Furthermore, each regioisomer possessed a double peak, which was

attributed to a partial resolution of the *syn/anti* isomers of the DNPH derivatives. Previous studies with DNPH derivatives of core aldehydes of known triacylglycerol structure (22) also had given two peaks, the main peak, representing 75–85% of the total, emerging ahead of the minor one, accounting for 15–25%, as estimated by reversed-phase HPLC with ultraviolet detection at 358 nm. However, a clear separation of the geometric isomers was not always obtained for the mixed triacylglycerols of the oxidized seed oils. Identification of regioisomers was complicated due to *syn/anti* isomerism of dinitrophenylhydrazones. Double peaks of DNPH derivatives have been reported to occur also in other analyses of DNPH derivatized carbonyl compounds. Kuklev *et al.* (45) have shown that the 12,13-epoxide runs ahead of the 9,10-epoxide of linoleate on adsorption TLC; this order would be reversed on reversed-phase HPLC. Likewise, 13-keto linoleate migrated ahead of 9-keto linoleate on TLC; again, the order would be reversed on reversed-phase HPLC. The nature of the solvent is known to affect the order of HPLC elution as well as the isomer ratios of these compounds (46).

The only unusual structures, although not unexpected (47), were the TBHP adducts of the unsaturated triacylglycerol core aldehydes. These compounds could have been formed in a termination reaction between a *tert*-butylhydroperoxy radical and an alkenyl radical or between a lipid hydroperoxy radical and the *tert*-butyl radical. The formation of the *tert*-butyl derivatives of the lipid hydroperoxides has a precedent in the existence of di-*tert*-butyl peroxide (40), as well as in the formation of cyclic peroxides during decomposition of linoleate hydroperoxides (31,48) and tocopherol adducts (42). Furthermore, Miyashita *et al*. (31) have demonstrated that oxygenation of methyl linoleate hydroperoxides yields dimers composed of octadecadienoate and octadecenoate moieties cross-linked through either ether or peroxy linkages across the 9- or 13-positions. Yamauchi *et al*. (49) have shown the formation of α-tocopherol adducts with phosphatidylcholine peroxy radicals.

Finally, the absence of the (13-oxo)tridecadienoyl core aldehydes as oxidation products seems to be consistent with the absence of this core aldehyde also among the autoxidation products of cholesteryl linoleate (20,50) and of linoleate-containing glycerophospholipids (51). Very little work has been done on the triacylglycerol core aldehydes (21,22,42), and their structures have been assigned on the basis of the known routes of degradation of the hydroperoxides of methyl oleate and linoleate (1–3).

In conclusion, the present study demonstrates the formation of a large variety of triacylglycerol core aldehydes during a brief treatment of corn and sunflower oils, with TBHP. The identification of the high-molecular-weight aldehydes was simplified by derivatization with DNPH, normal-phase TLC isolation, and reversed-phase HPLC separation with on-line negative ion MS, which specifically detects the DNPH derivatives. By the combined application of the criteria of chemical derivatization, chromatographic, and MS behavior, it was possible to eliminate many of the uncertainties posed by coincidental peaks and ions. The chromatographic and MS methods developed here for the characterization of the triacylglycerol core aldehydes arising from *tert*-butyl hydroperoxidation should be suitable for the identification of the core aldehydes among the autoxidation and enzymatic oxidation products of tissue triacylglycerols. This technology may also have applications in atherosclerosis research by permitting identification of aldehydic products formed during the autoxidation of low density lipoprotein (LDL), in which cholesteryl esters predominate. The study shows that rapid peroxidation with TBHP provides an effective method for enriching unsaturated vegetable oils in triacylglycerol core aldehydes for dietary and metabolic testing.

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