Soybean Oil Triacylglycerol Analysis by Reversed-Phase High-Performance Liquid Chromatography Coupled with Atmospheric Pressure Chemical Ionization Mass Spectrometry

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ABSTRACT: Soybean oil triacylglycerols from genetically modified soybean lines were conclusively identified by reversed-phase high-performance liquid chromatography coupled with mass spectrometry with atmospheric pressure chemical ionization. Atmospheric pressure chemical ionization is a soft ionization technique which gives simple spectra for triacylglycerols. Spectral identification of the triacylglycerols was based on the molecular $[M + 1]^+$ ion and the 1(2)-, 2(3)- and 1(3)-diacylglycerol fragments. Triacylglycerols identified in high-stearic and high-palmitic soybean varieties were quantitated by reversed-phase high-performance liquid chromatography with flame-ionization detection. There was excellent agreement between the fatty acid composition calculated from the triacylglycerol composition and the fatty acid composition obtained by gas chromatography of the transmethylated oils. The oils of the modified soybean varieties, compared to typical soybean oil, contained increased content of triacylglycerols known to be more oxidatively stable, such as linoleoyloleoylstearoyl, linoleoylpalmitoylstearoyl, and linoleoyldipalmitoyl glycerols, and less triacylglycerols like trilinoleoylglycerol, known to decrease oxidative stability. This study showed that the atmospheric pressure chemical ionization technique is suitable for mass spectral identification of neutral molecules, such as triacylglycerols, which do not contain a chargeable functional group.
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KEY WORDS: Atmospheric pressure chemical ionization, evaporative light-scattering detector, flame-ionization detector, high-palmitic soybean oil, high-performance liquid chromatography, high-stearic soybean oil, mass spectrometry, reversed-phase high-performance liquid chromatography, soybean oil, triacylglycerol analysis, triacylglycerols.

Recently, research has been directed toward the improvement of the functional properties of soybean oil (SBO) for food uses (i.e., frying oils, margarine basestocks) by altering the fatty acid (FA) composition and the triacylglycerol (TAG) composition (1–6). Furthermore, the oxidative stability of SBO partly depends on the TAG composition and structure (7,8).

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Identification of SBO TAG has involved various techniques which included collection of fractions resolved by reversed-phase high-performance liquid chromatography (RP-HPLC) for subsequent gas chromatography (GC) identification of the TAG methyl esters after transmethylation or by matching HPLC retention times or volumes with TAG equivalent carbon numbers with respect to standard TAG (9–23). Also, SBO TAG have been identified by mass spectrometry (MS) of isolated HPLC fractions, or by HPLC–MS *via* direct liquid inlet or thermospray interfaces (16,24–33). These MS and HPLC–MS procedures have relied mainly on electron impact (EI) or chemical ionization (CI) with ammonia or the mobile phase solvent to give molecular fragments, molecular ions (M), and molecular ion adducts (16,24–33). However, these procedures are not without limitations for TAG identification. EI–MS produced complicated fragmentation with variable M formation for TAG (24,29). CI–MS, with ammonia as reagent gas, produced simple spectra which conclusively identified TAG (16,25–28, 30–32). However, the interface for CI–MS between HPLC and MS involved the introduction of HPLC eluants into the MS by either thermospray (30,31) or the direct injection of a portion of the HPLC eluant (25–28). The thermospray technique, which uses heat to remove the HPLC solvent, may alter TAG with unsaturated FA (16,19). Direct injection or introduction of the HPLC stream into the MS utilizes 1/100 of the HPLC stream (25), and may result in MS sensitivity problems for complex natural TAG mixtures like SBO, which have many TAG components (7,8,28).

In recent work at our laboratory, an HPLC–MS interface technique, using atmospheric pressure chemical ionization (APCI), has been employed for the quadrapole MS analysis of a mixture of homogeneous triglycerides (34). APCI is a soft ionization technique. Coupled with HPLC, eluant is constantly introduced into the APCI ion source where reactions promoted by a high voltage corona produce protonated TAG molecular ions $[M + 1]^+$. The protons are derived from trace water in the nitrogen sheath gas in the APCI source. The resultant simple MS spectra contain only the $[M + 1]^+$ adduct ion and diacylglycerol fragments to conclusively identify

TAG (16). The $[M + 1]^+$ TAG and diacylglycerol ions also are produced in sufficient quantity for good sensitivity detection and mass analysis by a quadrapole mass spectrometer (33–35). We report here the use of the coupled RP-HPLC–MS with APCI technique in an analysis scheme for the identification and quantitation of TAG in the oils from new soybean lines bred to yield high palmitic (Hi-P-SBO) and high stearic (Hi-S-SBO) acid oils.

EXPERIMENTAL PROCEDURES

Materials. Two experimental soybean varieties were obtained from Pioneer Hi-Bred International, Inc. (Waterloo, IA), identified as A90-143073, Hi-S-SBO and A90-214040, Hi-P-SBO varieties. A refined, bleached, and deodorized reference SBO was obtained from Riceland Foods (Stuttgart, AR). All solvents were purchased from Aldrich Chemical Company (Milwaukee, WI). Solvents were HPLC quality and were used without further purification.

Sample preparation. Crude oils were obtained by hexane extraction of the soybean varieties and refined, bleached, and deodorized by laboratory procedures, as previously reported (36).

HPLC–MS. The components of the system used were an HPLC pump, an evaporative light-scattering detector (ELSD), and a quadrapole mass spectrometer. The HPLC (LDC 4100 MS; Thermo Separation Products, Schaumburg, IL) quaternary pump system with a membrane degasser had two columns in series which were used for analysis of the oil samples: an Adsorbosphere UHS C18 (Alltech Associates, Deerfield, IL) 25 cm × 4.6 mm, 10 μm (30% carbon load) and a 25 cm × 4.6 mm, 5 μm normal Adsorbosphere C18 (12% carbon load). A gradient solvent program with propionitrile (PrCN) and hexane (Hex) was used to accomplish the separations as follows: 100% PrCN for 65 min, linear from 65–75 min to 75% PrCN and 25% Hex, held for 15 min. The flow rate was 1 mL/min. The ELSD (ELSD MKIII; Varex, Burtonsville, MD) was operated as follows: drift tube temperature of 120°C; 2.0 standard liters per minute (SLPM) gas flow, high purity nitrogen nebulizer gas. The quadrapole mass spectrometer (Finnigan MAT SSQ 710C; Finnigan-MAT, San Jose, CA) was fitted with an APCI source; a vaporizer temperature of 225–240°C, capillary heater temperature of 240°C, corona current of 5 μA, high purity nitrogen sheath gas at 55 psi and auxiliary gases at 5 mL/min. A mass defect of 0 mmu at 0 amu to 500 mmu at 1000 amu was subtracted from decimal masses to give nominal masses.

HPLC effluent was split using a tee and needle valve to provide a flow of ~400 μL/min into the APCI source, with ~600 μL/min being diverted to the Varex detector. After initial scans did not show any substantial fragments below 400 amu, scans were obtained from 400 to 1100 amu, with a scan time of 0.65 s.

RP-HLPC coupled with flame-ionization detector (FID). Quantitative analysis of TAG molecular species was performed in triplicate. [Sample: 10 mg TAG (0.5 mg in 5–10 μL methylene chloride); two C-18 Zorbex columns (5 μ, 0.49 × 50 cm; Dupont, Inc., Wilmington, DE) placed in series; 120 min linear gradient, 70:30 to 40:60 acetonitrile/methylene chloride (vol/vol); flow rate, 0.8 mL/min.] The columns were cleaned between analyses with 100% methylene chloride. The FID (Tracor Model 945 HPLC detector; Tremetrics, Austin,

TABLE 1
Normal Soybean Oil Mass Spectral Results Determined by Reversed-Phase High-Performance Liquid Chromatography (HPLC) Coupled with Quadrapole Mass Spectrometer *via* Atmospheric Pressure Chemical Ionization[a]

TAG name[b,c]	Mol. wt	DG1 Mass[d]/Int.%[e]	DG2 Mass/Int.%	DG3 Mass/Int.%	[TAG + 1] + Mass[f]/Int.%	Ret. times[g]	% Total[h]
LnLnL	874	595 (3.06)	597 (7.84)		875 (100)	20:12	1.0
LLLn	876	597 (7.20)	599 (4.79)		877 (100)	25:03	10.6
LLL	878	599(16.48)			879 (100)	31:18	22.7
LnLO	878	597 (8.28)	599(19.44)	601 (6.65)	879 (100)	32:34	0.3
PLLn	852	573 (4.78)	575 (6.38)	597(14.26)	853 (100)	34:46	2.7
LLO	880	599(17.16)	601(19.27)		881 (100)	40:59	21.5
LLP	854	575(19.43)	599(28.18)		855 (100)	44:06	14.2
OOL	882	601(94.65)	603(38.11)		883 (100)	54:25	7.8
LLS	882	599(20.06)	603(25.66)		883 (100)	56:34	0.5
POL	856	575(42.87)	577(34.45)	601 (100)	857(79.12)	58:38	6.5
PPL	830	551(27.38)	575 (100)		831 (5.19)	1:03:29	1.0
OOO	884	603 (100)			885(18.59)	1:13:01	1.7
SOL	884	601 (100)	603(96.08)	605(39.09)	885(78.66)	1:15:46	1.0
OOP	858	577 (100)	603(75.33)		859(15.67)	1:18:56	1.1
PSL	858	575(59.81)	579(27.01)	603 (100)	859(12.22)	1:20:43	0.9
OOS	886	603(77.64)	605 (100)		887(17.36)	1:24:21	2.8

[a]See Experimental Procedures section for HPLC–mass spectrometry conditions.
[b]Reversed-phase HPLC light-scattering detector chromatogram peaks (see Fig. 1A).
[c]TAG, triacylglycerol. TAG fatty acids: S, stearic; P, palmitic; O, oleic; L, linoleic; Ln, linolenic.
[d]DG1, DG2, and DG3 are diacylglycerol fragments remaining after loss of one fatty acid residue from the TAG during mass spectrometry.
[e]Int.% is the abundance of a particular ion with respect to the most abundant ion formed during mass spectrometry.
[f]TAG + 1 is the protonated TAG molecular ion formed during mass spectrometry.
[g]Reversed-phase HPLC light-scattering detector TAG retention times.
[h]Area percent of the TAG resolved by reversed-phase HPLC light-scattering detector (not quantitative as response factors were not used).

TABLE 2
High Palmitic Acid Soybean Oil Mass Spectral Results Determined by Reversed-Phase HPLC Coupled with Quadrapole Mass Spectrometer *via* Atmospheric Pressure Chemical Ionization[a]

TAG name[b]	Mol. wt	DG1 Mass/Int.%	DG2 Mass/Int.%	DG3 Mass/Int.%	[TAG + 1] + Mass/Int.%	Ret. times	% Total
LnLnL	874	595 (5.02)	597(10.66)		875 (100)	20:05	1.6
LLLn	876	597 (9.85)	599 (7.45)		877 (100)	24:53	6.9
LnLnP	850	573 (9.62)	595(12.39)		851 (100)	27:24	1.7
LLL	878	599(21.32)			879 (100)	31:14	10.7
LnLO	878	597 (8.41)	599(18.67)	601 (6.37)	879 (100)		
PLLn	852	573 (4.28)	575 (7.67)	597(14.69)	853 (100)	34:28	18.7
LLO	880	599(24.67)	601(23.16)		881 (100)	40:43	5.1
LLP	854	575(19.16)	599(28.76)		855 (100)	43:39	24.8
PPLn	828	551(13.94)	573(60.97)		829 (100)	48:33	2.0
OOL	882	601 (100)			883(73.5)	53:28	0.9
POL	856	575(47.75)	601 (100)		857(59.55)	57:41	7.6
PPL	830	551(24.17)	575 (100)		831 (5.17)	1:02:12	13.9
OOP	858	577 (100)	603(50.28)		859 (8.31)	1:20:58	1.8
PSL	858	575 (100)	579(49.74)		859(10.33)	1:19:38	1.6
PPO	832	551(16.77)	577 (100)		833 (5.13)		

[a]See Table 1 and the Experimental Procedures section for definitions and analysis conditions. [b]TAG identified in the chromatogram peaks in Figure 1B.

TX) was operated with block temperature 130°C, detector gas, 140 mL/min hydrogen; cleaning flame, 600 mL/min hydrogen, 300 mL/min oxygen; and compressed air, 0.4 ft^3/min. The FID response was processed by a real-time computer (37). Quantitation was verified by analysis of standard TAG mixture 406 (Nu-Chek-Prep, Inc., Elysian, MN).

GC analysis. The FA composition of the TAG was determined by GC of the methyl esters. A 10-mg sample of oil 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), washed three times, and dried with 5 mL acetonitrile azeotrope. FA methyl ester (FAME) samples were analyzed by direct injection capillary GC using an SP 2380 column (30 m × 0.25 mm i.d. with 0.2 mm film thickness; Supelco, Inc., Bellefonte, PA) in a Varian Gas Chromatograph (Star 3400; Varian, Walnut Creek, CA) equipped with an FID; column temperature gradient, 150°C for 35 min, then 3°C/min to 210°C; helium head pressure, 10 psi; injector temperature, 240°C; detector temperature, 280°C. The GC–FID area percent quantitation was verified by analysis of standard by weight FAME mixture 15 A (Nu-Chek-Prep, Inc.).

RESULTS AND DISCUSSION

TAG were resolved by RP-HPLC and conclusively identified by RP-HPLC–MS–APCI for oils from an unmodified or normal (SBO), Hi-P-SBO, and Hi-S-SBO varieties. RP-HPLC–MS–APCI identification data or mass spectral results for normal SBO are given in Table 1. The TAG are arranged in Table 1 with increasing elution time with respect

TABLE 3
High Stearic Soybean Oil Mass Spectral Results Determined by Reversed-Phase HPLC Coupled with Quadrapole Mass Spectrometer *via* Atmospheric Pressure Chemical Ionization[a]

TAG name[b]	Mol. wt	DG1 Mass/Int.%	DG2 Mass/Int.%	DG3 Mass/Int.%	[TAG + 1] + Mass/Int.%	Ret. times	% Total
LnLnL	874	595 (5.11)	597 (7.21)		875 (100)	19:59	1.0
LLLn	876	597 (9.43)	599 (5.42)		877 (100)	24:44	7.1
LLL	878	599(17.32)			879 (100)	31:00	11.6
LnLO	878	597 (6.33)	599(13.77)	601 (3.99)	879 (100)	32:09	0.4
PLLn	852	573 (4.86)	575 (6.45)	597(16.27)	853 (100)	34:21	2.6
LLO	880	599(19.59)	601(19.64)		881 (100)	40:45	9.7
LLP	854	575 (17.5)	599(28.74)		855 (100)	43:46	10.9
OOL	882	601 (100)	603(34.61)		883(94.61)	54:10	1.1
LLS	882	599(26.27)	603(22.27)		883 (100)	56:19	6.3
SOLn	882	599(24.93)	601(81.69)	605 (5.95)	883 (100)	58:24	2.0
POL	856	575(41.73)	577(26.63)	601 (100)	857(62.42)		
PPL	830	551(23.40)	575 (100)		831 (3.59)	1:02:57	0.9
SOL	884	601 (100)	603(56.47)	605(34.19)	885(74.02)	1:15:45	4.5
PSL	858	575(95.84)	579(42.19)	603 (100)	859 (10.9)	1:20:32	7.6
LNSS	884	607 (8.57)	602(14.31)		885 (100)	1:20:32	
SLS	886	603 (100)	607(25.04)		887 (8.29)		
SOS	888	605 (100)	607(20.79)		889 (4.11)	1:27:35	5.4

[a]See Table 1 and the Experimental Procedures section for definitions and analysis conditions. [b]TAG identified in the chromatogram peaks in Figure 1C.

to the RP-HPLC–ELSD, as presented in Figure 1A. The RP-HPLC column effluent was split between the quadrapole MS and the ELSD. This was required because the MS ionization curve contained considerable instrument noise. However, the ELSD chromatogram in Figure 1 mirrored the MS total ionization curve pattern. This pattern fit allowed conclusive mass spectral identification of the peaks in Figure 1. The baseline in the ELSD chromatogram rose at the end of the run because the drift tube temperature was not at its maximum. The TAG containing mostly saturated long-chain fatty acids were sprayed less efficiently from the ELSD inlet nozzle.

The example mass spectra obtained for trilinoleoyl (same FA), oleoyldilinoleoyl (two of the same FA, one different FA), and linoleoyloleoylpalmitoyl (all different FA) glycerols are presented in Figure 2 (A, B, and C, respectively). For the first two of these TAG, the base peak is the protonated TAG molecular ion (M + 1). Other spectra peaks are the distinctive diacylglycerol fragment masses which are presented in Table 1. The diacylglycerol fragments conclusively identify different TAG with the same molecular weight (16). Also, protonated molecular ions plus PrCN (TAG + 1 + 55) are observed in the mass spectra. Inspection of Table 1 showed that RP-HPLC–MS–APCI produced simple spectra for normal SBO, which conclusively identified the TAG represented by the chromatogram peaks of the RP-HPLC–ELSD in Figure 1. Also, it can be observed in Table 1 and Figure 2 that the protonated molecular ion is the base peak in the mass spectra for TAG LnLnL through LLS. TAG POL through SOO showed one of the diacylglycerol fragments as the mass spectral base peak. (S = stearic acid; P = palmitic acid; O = oleic acid; L = linoleic acid; Ln = linolenic acid.) The shift in the base peak from the protonated molecular ion to the diacylglycerol fragments may suggest the involvement of double bonds in the stabilization of molecular ions.

The quantitative TAG composition data for normal SBO obtained by RP-HPLC–ELSD is also presented in Table 1. However, our work (unpublished data) and that of others (28,38) indicates that ELSD requires response factors for good quantitation. Therefore, the TAG composition data obtained *via* ELSD in Table 1 for normal SBO, in Table 2 for Hi-P-SBO, and in Table 3 for Hi-S-SBO should be considered approximate. However, HPLC–FID has been determined to be linear to TAG weight percent (13,21,38,39). Valid quantitative data for TAG composition for the samples is presented in Table 4. Our RP-HPLC–MS–APCI system was equipped with an ELSD rather than an FID, which would have given quantitative TAG analysis. The ELSD proved suitable for facilitating identification or location of TAG on the mass spectrometer total ionization curve. Response factors for the ELSD have not yet been determined.

Identification data for Hi-P-SBO are given in Table 2. The RP-HPLC–ELSD chromatogram, which resembled the MS total ionization curve, is presented in Figure 1B. As accomplished previously for normal SBO (Table 1), the protonated molecular ion and diacylglycerol fragment data in Table 2 conclusively identified the Hi-P-SBO TAG. Similar to normal SBO TAG, the spectra obtained for the later eluting Hi-P-SBO TAG no longer had the protonated molecular ion as the base peak, but one of the diacylglycerol fragments be-

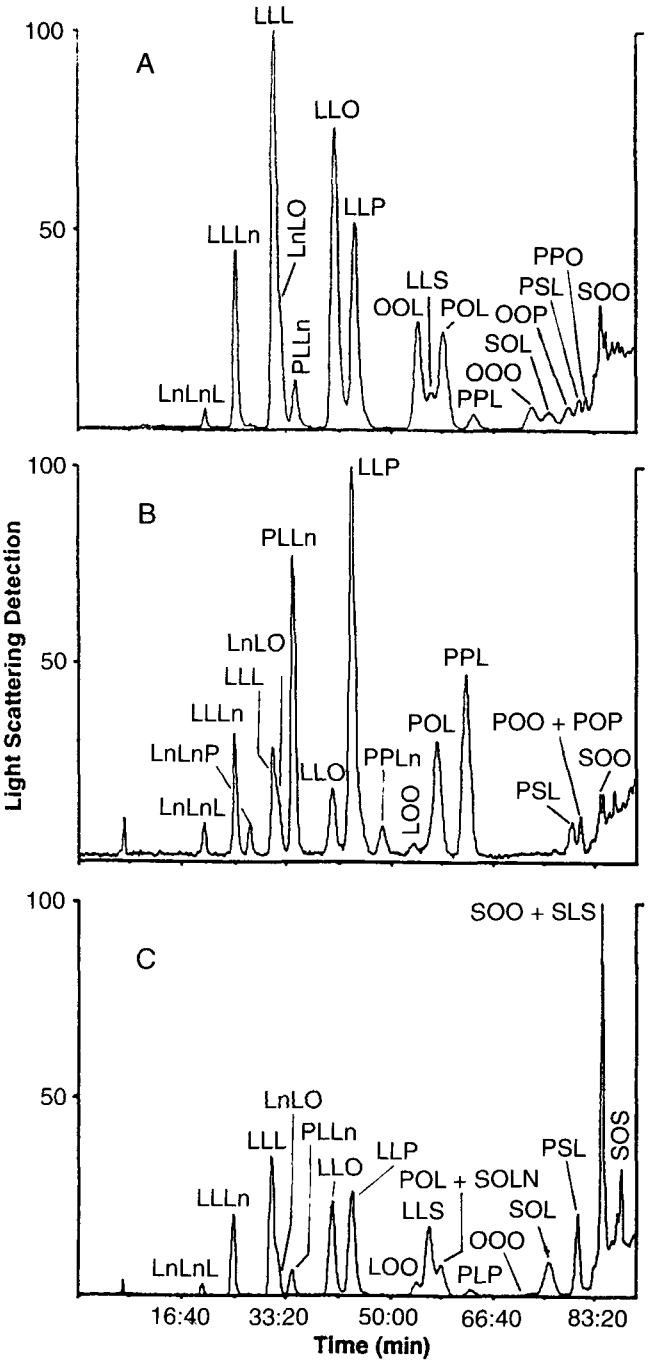

FIG. 1. A. Chromatogram of normal soybean oil triacylglycerols by reversed-phase high-performance liquid chromatography–evaporative light-scattering detector. Effluent also introduced into a quadrapole mass spectrometer *via* atmospheric pressure chemical ionization. Chromatography and mass spectrometer conditions are given in the Experimental Procedures section. Triacylglycerol fatty acids: S, stearic; P, palmitic; O, oleic; L, linoleic; Ln, linolenic. B. Chromatogram of high-palmitic soybean oil triacylglycerols. Mass spectrometric identifications of the chromatogram peaks are given in Table 2. C. Chromatogram of high-stearic soybean oil triacylglycerols. Mass spectrometric identifications of the chromatogram peaks are given in Table 3.

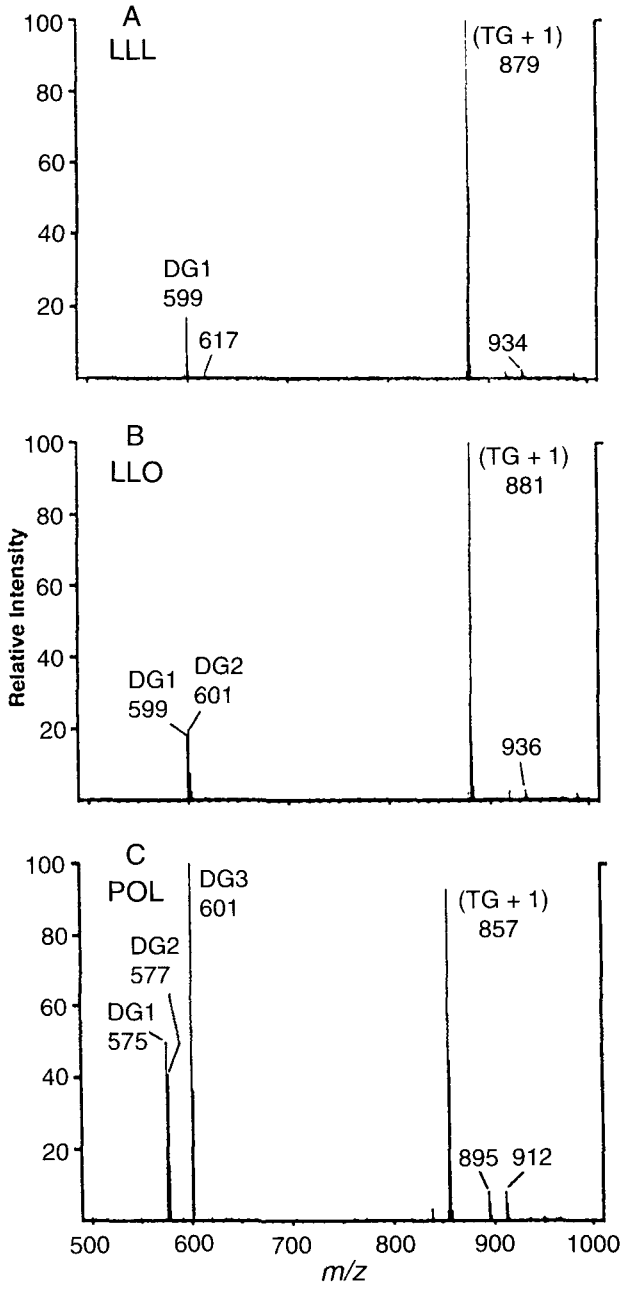

FIG. 2. A. Mass spectrum of trilinoleoylglycerol obtained by reversed-phase high-performance liquid chromatography coupled with a quadrapole mass spectrometer *via* atmospheric pressure chemical ionization. Analysis conditions are given in the Experimental Procedures section. TG + 1 is the protonated trilinoleoylglycerol molecular ion. DG1 is the characteristic diacylglycerol fragment for trilinoleoylglycerol. B. Mass spectrum of oleoyldilinoleoylglycerol. See Experimental Procedures section for analysis conditions. TG + 1 is the protonated molecular ion. DG1 and DG2 are the two required characteristic diacylglycerol fragments for the triacylglycerol. C. Mass spectrum of palmitoyloleoyllinoleoylglycerol. See Experimental Procedures section for analysis conditions. TG + 1 is the protonated molecular ion. DG1, DG2, and DG3 are the three required diacylglycerol fragments for the triacylglycerol. Abbreviations as in Figure 1.

TABLE 4
Triacylglycerol Analyses *via* Reversed-Phase HPLC with Flame-Ionization Detection of Normal, Palmitic, and Stearic Acid Containing Soybean Varieties[a]

	Soybean variety		
TAG[b]	"Normal" SBO[c]	High stearic	High palmitic
LnLnLn	0.2[d]	0.1[d]	0.4[d]
LnLnL	1.1	1.2	2.0
LLLn	6.0	4.8	1.5
LnLnO	1.4	1.3[d]	4.8[d]
LnLnP	0.5	0.4[d]	2.3
LLL	17.3	9.0	6.8
LnLO	5.1	4.9	2.3
PLLn	3.1	3.8	13.6
LLO	17.2	9.2	4.5
LnOO	1.3[d]	1.1[d]	0.0
LLP	12.1	10.6	19.4
LnOP	1.4[d]	1.6[d]	2.6[d]
PPLn	0.3[d]	0.3[d]	2.5
OOL	8.4	3.0	1.2
LLS	3.0	9.7	0.0
POL	8.3	7.3	12.4
		(POL and SOLn)	
PPL	1.5	2.2	16.0
OOO	2.9	1.4[d]	0.6[d]
SOL	2.6	8.3	0.5[d]
OOP	2.2	1.0[d]	0.9
PSL (LnSS)[e]	1.0 (SLP)[e]	5.6[e]	2.6 (PSL)[e]
		(LnSS and PSL)	
PPO	0.3	0.6[d]	1.4
PPP	0.1[d]	0.4[d]	0.1
OOS	0.2	1.2	0.1
SLS	0.9[d]	5.8	0.2[d]
SOP	0.4[d]	1.2[d]	0.4[d]
PPS	0.2[d]	0.7[d]	0.4[d]
SOS	0.2[d]	1.5	0.1[d]
PSS	0.1[d]	0.3[d]	0.2[d]
SSS	0.1[d]	0.1[d]	0.1[d]
Unidentified	0.6	1.4	0.2

[a]Determined by reversed-phase HPLC with flame-ionization detection; analysis conditions in the Experimental Procedures section and in Figure 3. Abbreviations as in Table 1.
[b]TAG confirmed by reversed-phase HPLC–quadrapole mass spectrometer analysis (Tables 1–3).
[c]SBO soybean oil.
[d]TAG confirmed by HPLC retention with respect to standard TAG (Ref. 21).
[e]TAG PSL and LnSS have the same HPLC retention times. These TAG were confirmed in the respective soybean varieties by HPLC quadrapole mass spectrometry analysis (Tables 1–3).

came the base peak. In the Hi-P-SBO chromatogram, palmitic containing TAG (Fig. 1B, Table 2), such as LnLnP, PLLn, LLP, POL, and PLP, were more abundant than in the normal SBO chromatogram (Fig. 1A, Table 1). These TAG are more oxidatively stable than TAG such as LnLO, LLO, and LnLL, which are more abundant in normal SBO (7,8).

Identification data for Hi-S-SBO are given in Table 3. The RP-HPLC–ELSD chromatogram, which resembled the MS total ionization curve, is presented in Figure 1C. The protonated molecular ion and diacylglycerol fragment data conclusively identified the Hi-S-SBO TAG, and, again, one of the diacylglycerol fragments became the base peak. In the

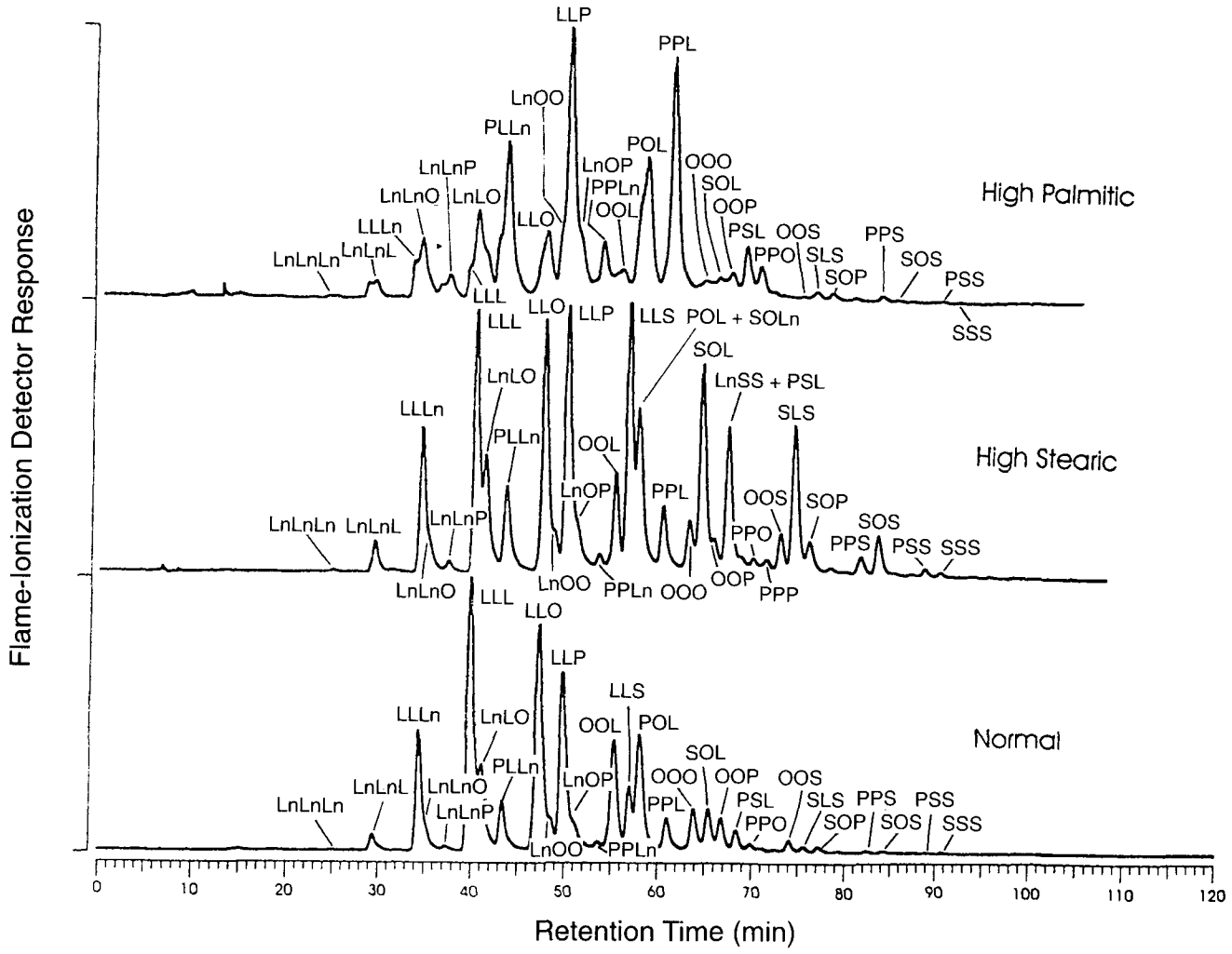

FIG. 3. Reversed-phase high-performance liquid chromatography with flame-ionization detector chromatogram of normal, high stearic, and high palmitic soybean oil triacylglycerols. Analysis details are given in the Experimental Procedures section. Quantitative composition for the soybean oils and fatty acid identifications are given in Table 4. Abbreviations as in Figure 1.

Hi-S-SBO, TAG containing stearic acid (Fig. 1C, Table 3) were more abundant, such as LLS, SOLn, SOL, OOS, and PSL, as compared to normal SBO (Fig. 1A, Table 1). These TAG are more oxidatively stable than TAG such as LLL, LLO, and LOO, which are more abundant in normal SBO (7,8).

As previously mentioned, the Hi-P-SBO and Hi-S-SBO TAG were conclusively identified by the RP-HPLC–MS–APCI procedure. However, the ELSD used with the MS system, as well as MS total ionization current chromatograms, gave only an approximate quantitative TAG analysis (Tables 1–3). RP-HPLC–FID has previously been determined to give quantitative TAG composition without response factors (13,21,38). The RP-HPLC–FID chromatograms are given in Figure 3, and the peak profiles correlate with the RP-HPLC–ELSD chromatograms (Fig. 1), permitting peak identifications in the RP-HPLC–FID chromatogram. The RP-HPLC–FID chromatograms show finer resolution than the RP-HPLC–ELSD chromatograms. Also, for later eluting TAG such as SOO, SLS, PPS, SOS, and PSS, the ELSD chromatograms showed poor resolution. These TAG were identified in the RP-HPLC–FID chromatograms from a plot of TAG theoretical carbon number with HPLC retention time with respect to standard TAG (21).

The SBO, Hi-P-SBO, and Hi-S-SBO quantitative TAG composition data are presented in Table 4. FA composition calculated from the TAG composition is in good agreement with the GC analysis of the FAME (Table 5). These results confirm the accuracy of the quantitative composition analyses for TAG of SBO, Hi-P-SBO, and Hi-S-SBO obtained by RP-HPLC–FID (39).

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TABLE 5
Fatty Acid Composition of Normal, High Palmitic, and High Stearic Soybean Oils (calculated[a] vs. experimental composition[b])

		Fatty acids (methyl esters) area percent				
Soybean variety	Method	16:0	18:0	18:1	18:2	18:3
Normal	Calculated[a]	11.1	3.5	24.0	53.6	7.8
	Observed[b]	10.0	4.1	25.2	53.4	7.3
High stearic	Calculated[a]	11.2	15.9	17.5	45.9	9.5
	Observed[b]	8.9	17.2	16.7	47.2	10.0
High palmitic	Calculated[a]	27.4	2.2	12.6	43.6	14.2
	Observed[b]	25.8	3.5	13.6	43.1	13.8

[a]Calculated from HPLC triacylglycerol composition data in Table 4. See the Experimental Procedures section for analysis details.
[b]Obtained by gas chromatography of the respective oils after transmethylation. See the Experimental Procedures section for analysis details. See Table 1 for abbreviation.

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