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Triacylglycerol Structure and Composition of Hydrogenated Soybean Oil Margarine and Shortening Basestocks

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The composition and structures of triacylglycerols (TAG) in a commercially prepared hydrogenated soybean oil margarine basestock [iodine value (IV) 65, 39.7% trans fatty acids] were determined by high-performance liquid chromatography (HPLC) in tandem with atmospheric pressure chemical ionization (APCI) mass spectrometry (MS). The basestock was separated by preparative HPLC into four fractions. Fractions 1 and 4, constituting ~8% of the total, were shown to consist of LOO, PLO, and LLS and OSS and PSS, respectively (where L = linoleic, O = oleic, S = stearic, and P = palmitic). APCI will not distinguish between O, oleic cis C18:1, and E, elaidic trans C18:1. Thus, O and E may be used interchangeably in discussion of TAG isomer structures. Fraction 2 consisted of OOO and POO. Fraction 3 consisted of OOO, POO, OOS, and POS. About 80% of the total triglycerides consisted of OOO, POO, and OOS. The trans fatty acid content of the fractions was determined, and the results showed that 92% of the total trans content was found in fractions 2 and 3. A shortening basestock (IV 81.7, 31.8% trans fatty acids) was partially characterized.

KEYWORDS: Triacylglycerols; trans fatty acids; gas−liquid chromatography (GLC); high-performance liquid chromatography (HPLC); atmospheric chemical ionization mass spectrometry (APCI-MS)

INTRODUCTION

Previous papers from our laboratory have described the characterization of complex natural and interesterified mixtures of triglycerides by atmospheric pressure chemical ionization (APCI) mass spectrometry (MS) in tandem with high-performance liquid chromatography (HPLC) (*1*−*5*). Relatively little has been published on the structures of triglycerides obtained from hydrogenation. Accurate knowledge of the trans fatty acid content of food oils has assumed new importance since the announcement by the U.S. Food and Drug Administration that, by January 1, 2006, trans fatty acids must be listed on food labels. We report here applications of HPLC/APCI-MS to the analysis of the compositions and structures of triglycerides in commercially processed hydrogenated soybean oil intended for shortening and margarine/spread formulation.

EXPERIMENTAL PROCEDURES

The unhydrogenated and hydrogenated soybean oils were supplied by C & T Refinery, Charlotte, NC. The unhydrogenated oil had the following composition: C16:0, 10.5%; C18:0, 3.4%; C18:1, 24.0%; C18:2, 54.0%; C18:3, 7.4%. It contained <1% trans fatty acids. The hydrogenated margarine basestock showed the following composition by gas−liquid chromatography (GLC) of methyl esters as described previously (*6*): C16:0, 11.3%; C18:0, 13.6%; C18:1, 75.2%; C18:2, 0.0%; C18:3, 0.0%, with a calculated iodine value (IV) of 64.7. The trans monoene content was 39.7%. The shortening basestock showed the following composition: C16:0, 11.2%; C18:0, 5.1%; C18:1, 72.6%; C18:2, 11.0%; C18:3, 0.0%, with a calculated IV of 81.5. The trans monoene content was 31.8%. The soybean oils containing various levels of trans fatty acids were commercial margarine oils (*4*).

Preparative HPLC. A Waters model 40 HPLC system was used. The column was a Dynamax 60A, C18, 30 × 2.25 cm, 60 μM particle size, operated with isocratic flow at 4.0 mL/min. The mobile phase consisted of a 1:1 mixture of methylene chloride and acetonitrile. Triglycerides (500 mg/mL) were dissolved in the mobile phase, and 50 μL (25 mg) was injected onto the column. Pooled fractions from multiple runs were used for further analysis by HPLC/APCI-MS. The margarine basestock yielded four fractions by preparative HPLC: fraction 1, 4.8%; fraction 2, 63.2%; fraction 3, 29.4%; fraction 4, 2.6%. The trans isomer contents of fractions 1−4 were 2.6, 27.7, 9.0, and 0.5%, respectively; the IV of fractions 1−4 were 7.5, 45.2, 14.0, and 0.6, respectively, for a total of 67.3. When the shortening basestock (IV 81.5) was subjected to preparative HPLC, it also yielded four fractions: fraction 1, 4.1%; fraction 2, 27.0%; fraction 3, 57.4%; fraction 4, 11.5%. The trans isomer contents of these fractions were 0.8, 6.1, 18.6, and 2.8%, respectively, for a total trans content of 28.3%; the IV were 6.8, 26.0, 43.9, and 3.1, respectively, for a total of 79.8.

HPLC. For RP-HPLC/APCI-MS, a Thermo Separation Products (Shaumburg, IL) LDC 4100 MS quaternary pump system with a membrane degasser was used. Two Inertsil ODS-80A (GL Sciences, Keystone Scientific, Bellefonte Park, PA), 25 cm × 4.6 mm, 5 μm,

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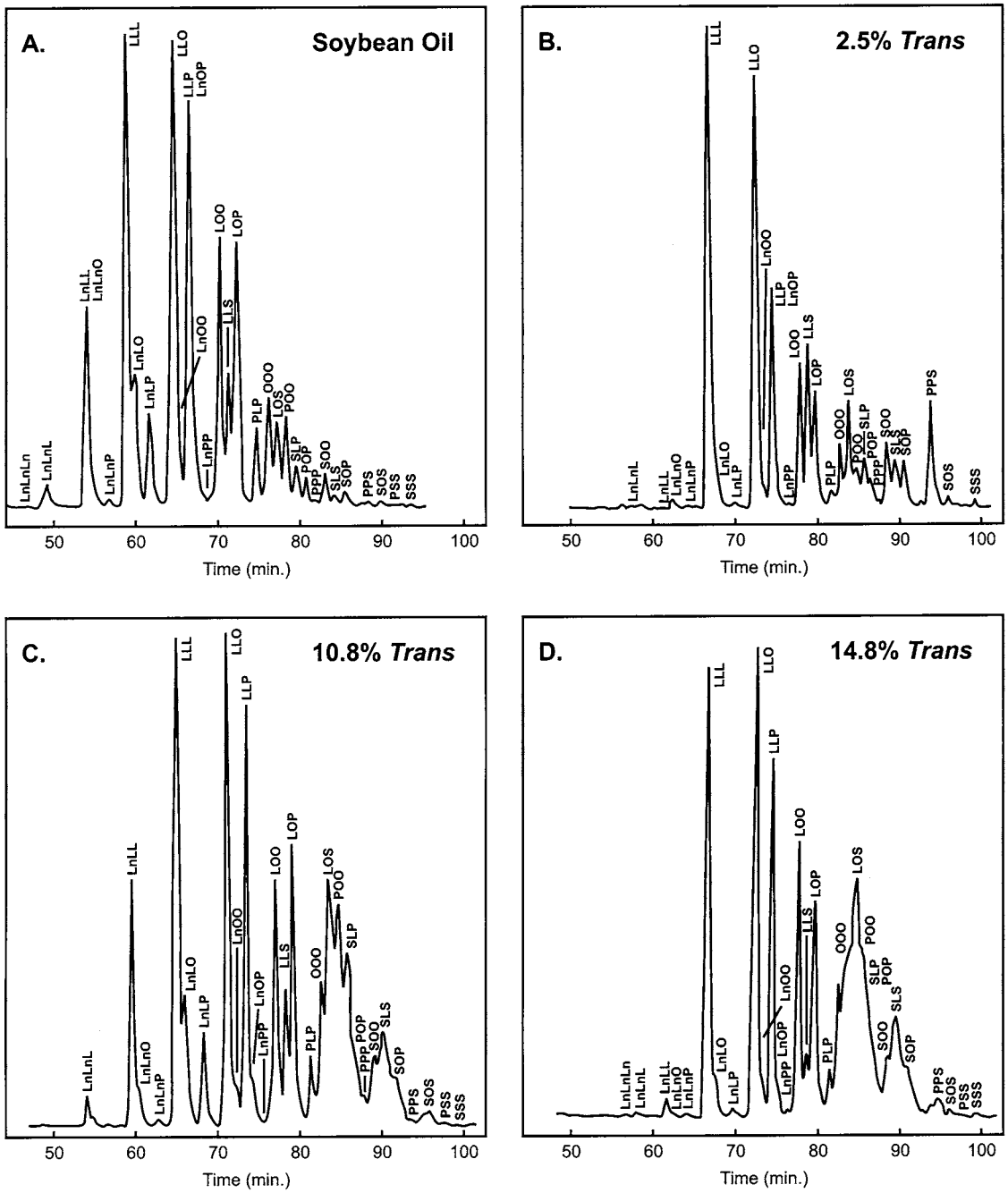

Figure 1. Total ion APCI-HPLC of soybean oils: (**A**) unhydrogenated; (**B**) 2.5% trans; (**C**) 10.8% trans; (**D**) 14.8% trans (for peak identification see ref *6*).

columns were used in series. The gradient elution was as follows: 70% acetonitrile/30% dichloromethane held for 40 min; to 65% acetonitrile/35% dichloromethane at 45 min, held until 55 min; to 60% acetonitrile/40% dichloromethane at 60 min, held until 70 min; to 55% acetonitrile/45% dichloromethane at 80 min. The flow rate was 0.85 mL/min throughout. The column effluent was split so that ~720 μL/min went to an evaporative light scattering detector and ~130 μL/min went to the APCI interface. A shorter gradient than that used for analysis using the RP-HPLC−flame ionization detector had to be used for RP-HPLC/APCI-MS because the MS data acquisition software limited RP-HPLC runs to 99.9 min. For RP-HPLC with flame ionization detector, the pump used was a Thermo Separation Products SP 8800 system. A linear gradient solvent program from 60% acetonitrile/40% dichloromethane to 30% acetonitrile/70% dichloromethane, as described previously, was used (*3*). Ten microliters of a 25 μg/μL sample in dichloromethane was injected. The flame ionization detector was a Tracor model 945 HPLC detector (Finnigan, Inc., Austin, TX). The flame ionization detector operating conditions were as follows: block temperature of 130 °C, 140 mL/min hydrogen detector gas, 250 mL/min hydrogen cleaning flame gas, 175 mL/min oxygen, and 0.4 ft^3/min of air. The data output from the flame ionization detector was integrated by a mainframe computer system. The quantitation of triglycerides was obtained from the integrated areas under chromatogram peaks in ion chromatograms of the $[M + H]^+$ ion and diacylglycerol fragment ion $[DAG]^+$ without response factors. The precision in the integrated area percent compositions ranged from 0.0 to 0.7%.

MS. A Finnigan MAT (now ThermoElectron, San Jose, CA) SSQ 710C single-quadrupole mass spectrometer fitted with an atmospheric pressure chemical ionization source was used to acquire mass spectral data. Conditions have been described previously (*6*).

GC. Fatty acid methyl esters were prepared by the potassium hydroxide catalyzed transmethylation of the triglyceride mixtures. The fatty acid methyl esters were analyzed using calibrated gas chromatography with a flame ionization detector according to this procedure. The sample solution, 5 μL (5 mg/mL sample in hexane), was analyzed by direct injection capillary gas chromatography. The capillary column was an SP2380 column, 30 m × 0.25 mm i.d. with a 0.2 μm film thickness from Supelco, Inc. (Bellefonte, PA). The gas chromatograph

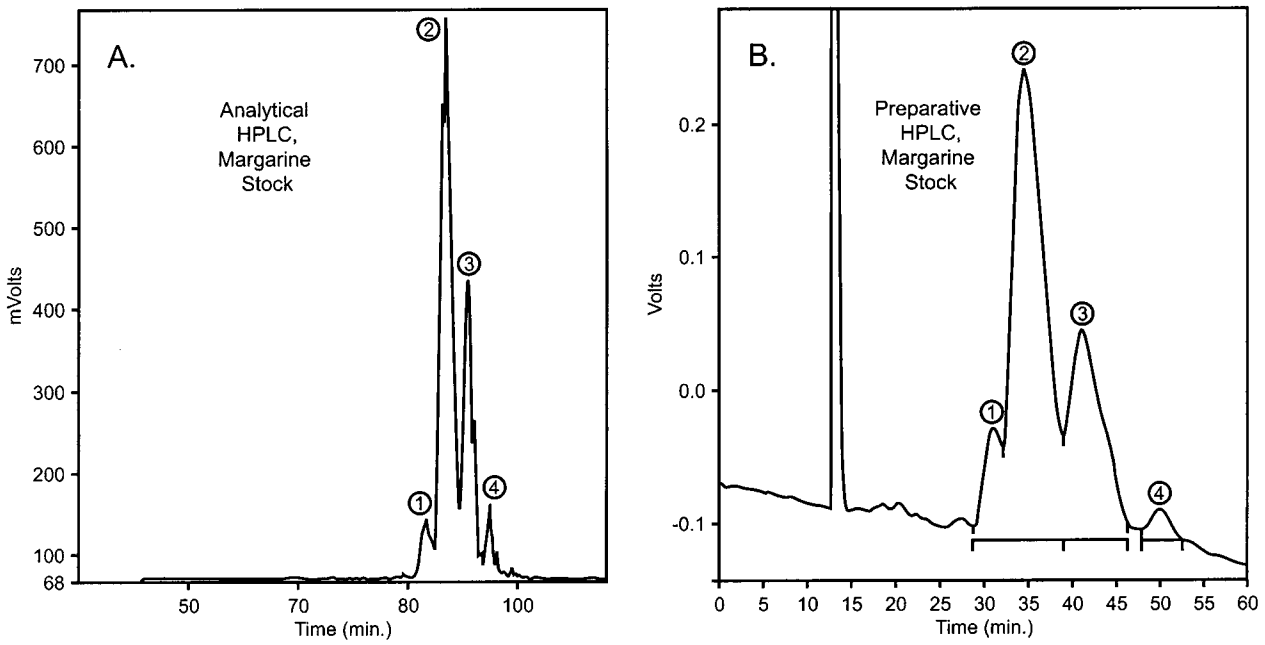

Figure 2. Total ion APCI-HPLC of margarine basestock: (**A**) analytical; (**B**) preparative.

Table 1. Composition of Preparative HPLC Fractions by APCI-LC: Margarine Basestock

	% HPLC		mass balance %[a]		triglycerides % APCI-LC								
fraction	analytical	preparative	trans	IV units	LOO	PLO	LSS	OOO	POO	OOS	POS	OSS	PSS
whole oil			39.7	64.6	4.1	0.8	0.1	42.6	26.3	17.7	7.7	2.2	0.4
1	5.6	4.8	2.6	7.5	81.7	16.2	2.0						
2	61.7	63.2	27.7	45.2				64.3	34.8				
3	28.1	29.4	9.0	14.0				4.3	12.1	58.4	25.2		
4	4.5	2.6	0.5	0.6								83.2	16.8
total	99.9	100.0	39.8	67.3									

[a] From GLC data.

Table 2. Mass Balance Data: Shortening Basestock, IV 81 Soybean Oil

	% HPLC		mass balance %[a]	
fraction	analytical	preparative	trans	IV units
whole oil			31.8	81.5
1	3.9	4.1	0.8	6.8
2	24.8	27.0	6.1	26.0
3	61.2	57.4	18.6	43.9
4	10.2	11.5	2.8	3.1
total	99.90	100.00	28.30	79.80

[a] From GLC data.

was a Star model 3400 equipped with a flame ionization detector from Varian, Inc. (Walnut Creek, CA). The gas chromatography column was operated at a starting temperature of 150 °C. The column was programmed at 150 °C, held for 35 min, then heated at 2 °C/min to 210 °C, then to 220 °C, and held at 220 °C for 5 min. The helium carrier gas had a column head pressure of 15 psi. The injector and detector were maintained at 240 and 280 °C, respectively. The calibration mixture for gas chromatography calibration was fatty acid methyl ester mixture 20-A from Nu-Chek-Prep, Inc. (Elysian, MN).

RESULTS AND DISCUSSION

An APCI-MS total ion current chromatogram (TIC) of natural, unhydrogenated soybean oil triglycerides is shown in **Figure 1** along with data from some margarine oils formulated for soft-tub margarine (*4*). Typically, soft margarines/spreads contain <15% trans acids, whereas stick products typically contain 15−25% trans FA (*4*). As shown in **Figure 1**, the presence of trans fatty acid (FA) isomers affects the resolution achieved by HPLC. Unfortunately, the portions affected correspond to the products of hydrogenation. For example, unhydrogenated soybean oil contains substantial amounts of LLL, LLO, LLP, LOO, and LOP (where L = linoleic, O = oleic, and P = palmitic), which are well separated and readily quantified. However, the presence of trans isomers adversely affects the separation and quantification of products arising from hydrogenation. Most notably, resolution of OOO, LOS, POO, SLP, and POP (where S = stearic) is affected.

Margarine/spread oils are commonly formulated by blending a highly hydrogenated (IV 65) soybean oil basestock with additional liquid oil. The HPLC/APCI-MS TIC of basestock with an IV of 65 is shown in **Figure 2**. Parts **A** and **B** illustrate analytical and preparative separations, respectively.

The composition of the preparative HPLC fractions as determined by HPLC/APCI-MS is shown in **Table 1** along with mass balance data (**Table 2**) for the trans FA content and iodine values. Fraction 1 (~5%) contains primarily LOO, PLO, and LSS and accounts for <3% of the total trans content. Although GLC of the methyl esters of the IV 65 soybean oil showed that linoleate was essentially absent, the preparative HPLC data show traces of linoleate-containing triglycerides.

Fraction 2 (27.7% trans) consists of two triglycerides, OOO and POO. Fraction 3 (0.9% trans) consists of primarily POO, OOS, and POS. Fraction 4 consists of OOS and PSS and is essentially trans free.

The shortening basestock (**Table 2**) was not characterized by HPLC/APCI-MS. However, analytical HPLC/APCI-MS of this basestock shows a chromatogram much like that of the IV 65 soybean oil except that the relative amounts of trans FA are reversed. Most of the trans FA are found in fraction 3, whereas in the IV 65 soybean oil, the major portion of the trans FA are found in fraction 2.

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