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Correlation of reversed-phase high-performance liquid chromatography and gas–liquid chromatography for fatty acid compositions of some vegetable oils

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ABSTRACT

The triacylglycerol compositions of six genetically modified soybean oils, and commercial soybean, corn, safflower and sunflower oils were determined by reversed-phase high-performance liquid chromatography with flame ionization detection. Triacylglycerol molecular species were identified based on theoretical carbon numbers. Fatty acid compositions of the above soybean oils were estimated from concentrations of the triacylglycerol molecular species. Fatty acid composition was calculated by multiplying the concentration of each triacylglycerol by the percent of each fatty acid species in the triacylglycerol molecule. The fatty acid composition calculated from triacylglycerol analysis was compared with the composition determined by gas–liquid chromatography. There was good agreement between the fatty acid composition as calculated from the high-performance liquid chromatographic data and as determined by gas–liquid chromatography. These results support the findings that triacylglycerol molecular species could be identified based on theoretical carbon numbers and that fatty acid composition of soybean oil can be obtained from the data of reversed-phase high-performance liquid chromatography with flame ionization detection. The triacylglycerol composition for soybean oil obtained by this procedure required no response factors for quantitation.

INTRODUCTION

The quantitative measurement of triacylglycerol (TAG) and fatty acid composition of lipids is required in many biological research areas, like food, medicine and agriculture. Studies of genetic control of fatty acid distribution in TAG of vegetable oil depend firmly upon knowledge of TAG molecular species. Likewise, TAG molecular species analysis is necessary for the studies regarding natural and synthetic fats like cocoa butter and its substitutes, and for the nutritional evaluation of edible oil [1–4]. Several reports have been published on the separation and identification of TAG by using high-performance liquid chromatography (HPLC). In the studies by El Hamdy and Perkins [5,6], reversed-phase HPLC (RP-HPLC) was used and demonstrated improved TAG separation. Phillips and coworkers [7,8] adopted flame ionization detection (FID) connected to RP-HPLC for quantitative analysis of TAG. Nurmela and Satama [9] reported that FID showed a more quantitative response than that obtained by ultraviolet (UV) and refractive index (RI) detection. Christie [10] reported that FID had a good linear response to sample size and gave satisfactory results with microgram amounts of sample. Also, FID did not show baseline drift during gradient elution because the volatile solvent was vaporized and removed before the nonvolatile sol-

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utes were carried into the flames of the detector for combustion [10]. Christie [11,12] concluded that the best and ideal detection system available currently for TAG molecular species analysis by HPLC was based on the FID principle which allowed a wide use of solvents and gave quantitative response. We have previously used RP-HPLC–FID to determine the TAG composition for oxidative studies [13] and studies of the interesterification of blends of vegetable oils with hydrogenated soybean oil [14].

The retention times or elution volumes of TAG molecular species are best predicted by theoretical carbon number (TCN) [11,13,14] which considers other elution factors determined experimentally in addition to the numbers of total carbon and carbon–carbon double bond, associated with the previously adopted equivalent carbon number (ECN).

In order to confirm that TAG molecular species can be identified based on TCN, the correlation of RP-HPLC–FID and gas–liquid chromatography (GLC) methods to obtain fatty acid composition of vegetable oils is reported here.

EXPERIMENTAL

Materials

Soybeans were commercial cultivars obtained from Randall L. Nelson, Curator, US Department of Agriculture, Germplasm Collection (University of Illinois, Urbana, IL, USA), and included plant introductions selected on the basis of fatty acid composition. Experimental lines were provided by Walter R. Fehr (Iowa State University, Ames, IA, USA). Soybean, corn, safflower and sunflower oils were obtained from a commercial source. The solid-phase extraction (SPE) columns (6.5 ml volume, loaded 2000 mg silica) used for purification of TAG were purchased from Baxter Health Care (Muskegon, MI, USA). All organic solvents used in this research were HPLC grade. TAG reference mixtures used for identification and quantitation were obtained from NuChek Prep (Elysian, MN, USA), and Sigma (St. Louis, MO, USA).

Methods

Crude oil (1.4–2.6 g) was obtained from genetically modified soybeans by hexane extraction of 15 g of soybean [11]. The beans were ground in a Varco coffee bean grinder (Type 228; Mouli Manufacturing, Belleville, NJ, USA) and soaked in 30 ml hexane at room temperature for 10 min. Oil was extracted by sonication for 5 min with an ultrasonic homogenizer Model 4710 sonicator (Cole Parmer, Chicago, IL, USA) with outlet setting at 7. After sonication, the hexane–bean mixture was cooled in ice to room temperature. This mixture was filtered over 0.6 g Celite filter aid and 1.2 g activated carbon, and the filter cake was rinsed five times with 80 ml hexane. The filtrate was dried with 4 g sodium sulfate and then filtered through folded filter paper (2V). Hexane was removed in 45 min on a rotoevaporator with water bath at 27°C.

The vegetable oils were stripped of non-TAG components by SPE chromatography with a hexane–diethyl ether gradient elution of each oil (1.2 g) mixed with 0.485 g activated carbon [11].

Purity and identification of TAG were determined by thin layer chromatography developed by diethyl ether–hexane (20:80, v/v), visualized by iodine and ultraviolet radiation.

TAGs (0.5 mg/5–10 μl hexane) were resolved by RP-HPLC [8,11,12] equipped with two Zorbax C_{18} columns (25 cm × 0.49 cm I.D., 5 μm particles, DuPont, Wilmington, DE, USA) in series, with linear gradient elution program [0.8 ml/min, acetonitrile–methylene chloride (70:30 to 60:40, v/v) over 120 min], followed by column clean up with 100% methylene chloride. The detection of eluents was done by FID, Tracor Model 945 HPLC detector (Austin, TX, USA) [9], block temperature: 180°C; detector and cleaning flame gas flow-rates: 140 and 160 ml/min hydrogen, respectively, and 300 ml/min oxygen. The noise filter was set high and baseline correction was used. The quantification of TAG molecular species by FID was checked with standard mixtures of trilinolenin, trilinolein, triolein, tristearin and tripalmitin.

Fatty acid methyl esters (FAMEs) of oils were prepared by potassium hydroxide-catalyzed transmethylation [13] and analyzed with a Hewlett-Packard (Avondale, PA, USA) Model 5710 A gas chromatograph equipped with a flame ionization detector. The glass column (180 cm × 0.31 cm I.D.) packed with 10% SP 2330 on 100–120-mesh Chromosorb W, AW, obtained from Supelco (Bellefonte, PA, USA), was operated at 160°C with a helium carrier gas flow-rate of 20 ml/min. The injection port and detector were held at 250°C. Methyl esters

TABLE I

FATTY ACID COMPOSITION OF SOYBEAN OILS

P = Palmitic; S = stearic; O = oleic; L = linoleic; Ln = linolenic acid. Quantitation was confirmed with NuChek. Prep standard 15 A.

Sample	Composition (%)				
	P	S	O	L	Ln
1	9.9	4.6	30.2	51.7	3.6
2	12.2	4.2	16.6	55.3	11.7
3	9.0	3.3	50.5	34.4	2.8
4	12.9	3.7	15.5	55.7	12.2
5	10.9	4.4	28.1	50.2	6.4
6	10.2	3.7	24.5	53.6	8.0
Commercial	9.7	3.1	22.0	56.6	8.6

were identified and their quantitation was calibrated with the NuChek Prep soybean methyl ester standard 15A (methyl palmitate, 6.0%; methyl stearate, 3.0%; methyl oleate, 35.0%; methyl linoleate, 50%; methyl linolenate, 3.0%; methyl arachidate, 3.0%).

RESULTS AND DISCUSSION

The fatty acid compositions of the soybean oils as determined by GLC-FID are presented in Table I.

The TAG molecular species (TAGMSs) were identified by matching calculated and experimental TCN values [5,8,14] to those of known TAGs in reference mixtures. Retention times and TCNs were linearly correlated, as reported previously [14].

TABLE II

TRIACYLGLYCEROL MOLECULAR SPECIES ANALYSIS OF A GENETICALLY MODIFIED SOYBEAN OIL BY RP-HPLC-FID, CALCULATION FOR FATTY ACID COMPOSITION AND THOSE DETERMINED BY GLC-FID

Ln, L, O, S, P are linolenic, linoleic, oleic, stearic and palmitic acids, respectively.

Molecular species	RP-HPLC (%)	Calculated fatty acid concentration (%)[a]				
		P	S	O	L	Ln
LnLnL	0.2	–	–	–	0.07	0.13
LnLL	3.1	–	–	–	2.07	1.03
LnLnO	0.3	–	–	0.10	–	0.20
LLL	16.3	–	–	–	16.30	–
LnLO	3.2	–	–	1.07	1.07	1.07
LnLP	1.1	0.37	–	–	0.37	0.37
LLO	19.6	–	–	6.53	13.06	–
LnOO	1.1	–	–	0.73	–	0.37
LLP	10.3	3.43	–	–	6.86	–
LNOP	0.7	0.23	–	0.23	–	0.23
LOO	12.7	–	–	8.47	4.23	–
LLS	3.7	–	1.23	–	2.47	–
LOP	10.0	3.33	–	3.33	3.33	–
PLP	1.5	1.0	–	–	0.50	–
OOO	4.9	–	–	4.90	–	–
LOS	4.2	–	1.40	1.40	1.40	–
POO	3.5	1.17	–	2.33	–	–
SLP	1.1	0.37	0.37	–	0.37	–
POP	0.4	0.27	–	0.13	–	–
SOO	1.3	–	0.43	0.87	–	–
SLS	0.3	–	0.20	–	0.10	–
SOP	0.5	0.17	0.17	0.17	–	–
	100.0					
Fatty acid composition, HPLC[b]		10.34	3.80	30.26	52.20	3.40
Fatty acid, composition, GLC		9.86	4.61	30.16	51.74	3.63

[a] Concentration calculated from RP-HPLC-FID data.

[b] Quantitation was confirmed with NuChek Prep standard 15 A.

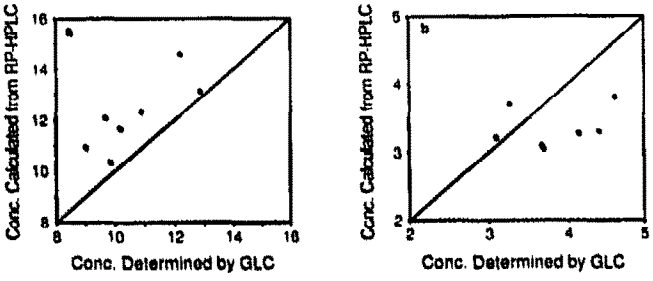

Fig. 1. Plot of palmitic (a) and stearic acid (b) concentrations in soybean oils determined by GLC–FID *versus* values of these acids calculated from RP-HPLC–FID.

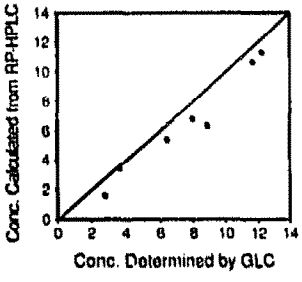

Fig. 3. Plot of linolenic concentrations in soybean oils determined by GLC–FID *versus* values of this acid calculated from RP-HPLC–FID.

TAGMS concentrations were calculated from their peak areas which correspond to the concentrations based on mass without requirement of correlation factors, as reported previously [14]. Fatty acid compositions were calculated from concentrations of TAGMSs by summation of the values calculated by multiplying the concentration of each TAGMS by the percent of the specific fatty acid in that TAGMS. As an example, the calculated fatty acid composition of a genetically modified soybean oil is presented in Table II. Close agreement was observed between calculated fatty acid composition obtained from TAG analysis by RP-HPLC–FID and fatty acid composition determined by GLC–FID (Table II).

Each fatty acid concentration, in oils from different soybean varieties, calculated from RP-HPLC–FID data was compared with the concentration determined by GLC–FID. Correlation coefficients between the concentrations of soybean oils determined using the two methods for five fatty acids (Figs. 1–3) were: palmitic, 0.982, stearic, 0.932, oleic, 0.998, linoleic, 0.999 and linolenic acids 0.986. Palmitic acid concentrations calculated from RP-HPLC–FID were slightly higher than those determined by GLC–FID (Fig. 1a); while linolenic acid concentrations calculated from RP-HPLC–FID tended to be slightly lower than those determined by GLC–FID (Fig. 3).

Fatty acid compositions of corn, safflower and sunflower oils as determined by the two methods were in good agreement and did not show the variation of palmitic acid and linolenic acid concentrations as found with soybean oils (Table III).

CONCLUSIONS

The high correlation obtained between fatty acid composition calculated from RP-HPLC–FID TAG

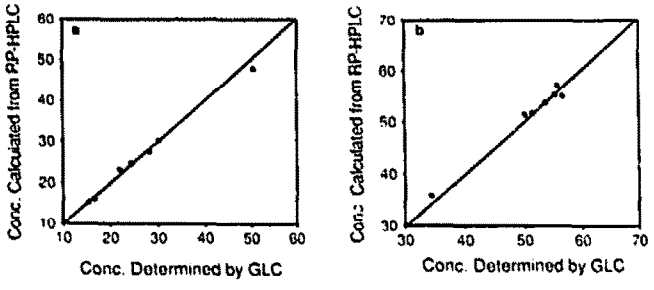

Fig. 2. Plot of oleic (a) and linoleic acid (b) concentrations in soybean oils determined by GLC–FID *versus* values of these acids calculated from RP-HPLC–FID.

TABLE III

COMPARISON OF FATTY ACID COMPOSITIONS CALCULATED FROM RP-HPLC–FID AND THOSE DETERMINED BY GLC–FID

Fatty acid	Composition (%)					
	Corn oil		Safflower oil		Sunflower oil	
	HPLC	GLC	HPLC	GLC	HPLC	GLC
Palmitic	11.0	11.3	6.8	6.8	6.9	7.0
Stearic	1.7	1.7	1.8	2.4	3.8	4.5
Oleic	24.2	26.1	13.9	14.9	17.9	17.8
Linoleic	62.3	59.8	77.4	75.4	71.1	70.2
Linolenic	0.7	1.1	0.7	0.5	0.1	0.5

analysis and the composition determined by GLC–FID FAME analysis indicates that TAG molecular species can be identified based on TCNs. RP-HPLC–FID analysis of soybean oil TAG can be quantitated without response factors. Fatty acid compositions of vegetable oils can be obtained by calculation from the results of RP-HPLC–FID.

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