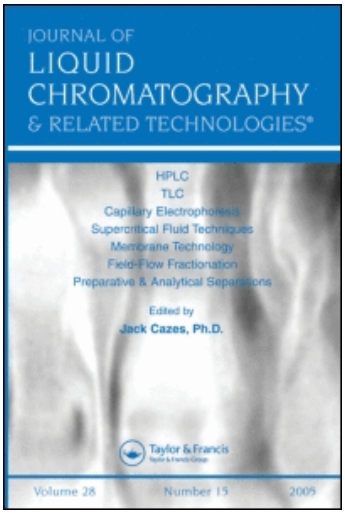

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Analyses of Vegetable Oil Triacylglycerols by Silver Ion High Performance Liquid Chromatography with Flame Ionization Detection

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ANALYSES OF VEGETABLE OIL TRIACYLGLYCEROLS BY SILVER ION HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH FLAME IONIZATION DETECTION

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ABSTRACT

Silver ion high performance liquid chromatography with a commercially available column with a simple isocratic mobile phase of acetonitrile in hexane and flame ionization detection was employed to separate and quantitate triacylglycerol species of vegetable oils. Coconut, palm, cottonseed, olive, safflower, sunflower, corn, pumpkinseed, linseed, soybean, and canola oils were analyzed, as well as randomized corn and soybean oils, and the blends and interesterified products of corn and soybean oil with cottonseed oil stearine. Fractionated triacylglycerol species were identified by gas chromatography of their methyl esters. Triacylglycerol composition was obtained by reversed phase and silver ion high performance liquid

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chromatography of the same oil. Oil fatty acid composition was determined by gas chromatography of the transmethylated oil and correlated with that calculated from the triacylglycerol composition by silver ion chromatography of the same oil. The triacylglycerol separation was mostly based on the total unsaturation of the fatty acids attached to the glycerol moiety. However, partial separation of triacylglycerols with the same unsaturation content but different fatty acids indicates that other separation mechanisms, in addition to fatty acid complexation with silver ions, such as adsorption and partition, are involved. The flame ionization response (area percent) was determined to be proportional to weight percent for oil triacylglycerol composition. In addition to analyses of vegetable oils, the efficacy of the silver ion high performance liquid chromatography method with flame ionization detection for analyses of margarine base stocks produced from corn and soybean oils was demonstrated.

INTRODUCTION

Triacylglycerol (TAG) composition of seed oils has been determined by high temperature gas chromatography (GC) and reversed phase high performance liquid chromatography (RP-HPLC) (1-3). High temperatures of GC may adversely effect analysis of TAG with unsaturated fatty acids (FA) through thermal alteration. RP-HPLC avoids thermal alteration but the TAGs are resolved with respect to both FA unsaturation and chain length, which may give HPLC chromatograms that are difficult to interpret. Silver ion high performance liquid chromatography (Ag-HPLC) resolves TAG primarily by FA unsaturation, resulting in simpler chromatograms for TAG identification (2,4). Previously reported Ag-HPLC systems for TAG and FA separations use complex mobile phases (4,5). A simple isocratic mobile phase, acetonitrile in hexane, and a commercially available Ag-HPLC column were recently used to resolve

cis and trans FA methyl ester isomers (6) and TAG of *Crepis alpina oil* (7).

Several types of HPLC detectors have been used for TAG analysis. Light scattering and ultraviolet detectors have been used for semi-quantitative determination of TAG composition (5), but these lack linear response and require response factors. We have previously used the transport flame ionization detector (FID) with RP-HPLC analysis of vegetable oils (8-11), vegetable oil blends and interesterified products for margarine basestocks (12) and for the analysis of *Crepis alpina* and *Vernonia galamensis* oils (13). The FID detector was determined to give a linear response without the need for response factors for quantitative TAG analysis. Ag-HPLC with an FID detector was previously used for the quantitative determination of TAG of cocoa butter, palm oil, and soybean oil (14).

We used the Ag-HPLC FID system with the isocratic acetonitrile/hexane mobile phase for the analysis of TAG of *Crepis alpina* oil, an oil with an unusual fatty acid which contains an alkyne bond (7). Here we report the broad applicability of the technique in the analysis of fats and oils which have common fatty acids with alkene bonds.

EXPERIMENTAL

Material

A set of complex and varied oils and products were selected to illustrate the applicability of the Ag-HPLC-FID technique for TAG analysis. Vegetable oils (olive, soybean, sunflower, corn, cottonseed, pumpkinseed, peanut, safflower, canola, coconut, linseed, and palm) were obtained from either local

market or industrial sources as finished oils. Soybean and corn oil each was blended (1:1, wt/wt) with cottonseed stearine and then interesterified using 0.5% sodium methoxide catalyst. Also, soybean and corn oil were each randomized in the presence of 0.5% sodium methoxide catalyst.

Methods

Pure TAGS were prepared from the oils and products by solid phase extraction chromatography described previously (9).

Fatty acid location in the TAG was determined by the enzymatic lipolysis and capillary gas chromatography as reported previously (15) and was also used to verify vegetable oil randomization.

To verify the results of Ag-HPLC-FID, TAGS were resolved and quantitated by RP-HPLC-FID (8, 9). Quantitation was verified against weighed TAG standards (7).

For TAG analysis by Ag-HPLC-FID, a Chromosphere Lipids column (4.6 mm I.D. x 250 mm, 5 micron particle size) (Chrompack International, Middleburg, The Netherlands) was used. The TAG (10 μl; 50 mg solute per 2 ml hexane) were injected in triplicate. All TAG were eluted in 120 min by an isocratic mobile phase of 0.5% acetonitrile in hexane (v:v) at a flow rate of 1.0 ml/min. HPLC flame ionization detector (Tracor Model 945, Trimetrics, Houston, TX), response was monitored by a real time computer and calibrated against weighed TAG samples. Fractions were collected via a splitter between the column and detector as previously described (7).

TAGs were characterized and identified by gas chromatography flame ionization detection of methyl esters prepared by transmethylation of each Ag-HPLC

fraction. TAG identifications were confirmed by comparison of TAG composition obtained by Ag-HPLC with that obtained by RP-HPLC. Further, TAG identification was supported by comparison of the TAG fatty acid composition calculated from Ag-HPLC fractions with the fatty acid composition determined experimentally for the starting TAG mixture (10).

Transmethylation was performed on the TAG by heating the sample in 0.5 N KOH in methanol at 50°C for 30 min. The reaction was stopped with acetic acid and the mixture was extracted with 5 ml petroleum ether:diethylether (1:1 v/v), water washed to neutral Ph and dried with 5 ml acetone azeotrope under helium.

Fatty acid methyl esters (FAME) samples were analyzed by direct injection capillary-GC utilizing an SP2380 column (30 m, 0.25 mm ID and 0.2 micron film thickness (Supelco, Inc., Bellefonte, PA) in a Varian 3400 Gas Chromatograph (Walnut Creek, CA) equipped with a flame ionization detector. The column was operated isothermally at 150°C for 35 min and then programmed to 210°C at 3°C/min with helium head pressure 10 psi. The injector and detector temperature were 240°C and 280°C respectively. GC chromatogram peak integration was by computer. The FAME were identified by matching their retention times with respect to authentic standards.

RESULTS AND DISCUSSION

Quantitative analyses of vegetable oil TAG are presented in Tables 1-3. The methyl ester quantitation by GC-FID was used to characterize the TAG species with respect to amount of saturated (S), monenoic (M), dienoic (D), and trienoic (T) fatty acids per TAG components represented by the Ag-HPLC chromatogram

TABLE 1

Vegetable Seed Oil Triacylglycerol Composition by Silver Ion and Reversed-Phase High Performance Liquid Chromatography with Flame Ionization Detection[a]

Resolution			Quantitation (Area Percent)[b]									
			Coconut		Palm		Cottonseed		Olive		Peanut	
Triacylglycerol saturated, monenoic, dienoic, trienoic fatty acid species[c]	Number of Double Bonds	Triacylglycerol Molecular Species Reversed Phase[d,e]	Reversed Phase	Silver Ion	Reversed Phase	Silver Ion	Reversed Phase	Silver Ion	Reversed Phase	Silver Ion	Reversed Phase	Silver Ion
SSS	0	PPP,PPS,PSS,SSS	89.3	86.9	7.7	7.2	0.3	0.1			3.9	3.1
SSM	1	POP,SOP,SOS	0.1	0.2	41.8	38.7	2.0	2.6	3.2	3.2	1.7	1.4
SSD	2	PLP,SLP,SLS	0.3	0.3	6.8	7.8	12.5	11.5	2.2	2.1	4.0	5.7
SMM	2	POO,SOO	0.6	0.4	27.6	28.5	3.9	3.6	26.4	27.5	9.2	9.3
SMD	3	LOP,LOS	0.5	1.5	8.3	9.0	13.3	12.3	11.0	10.9	17.6	16.5
MMM	3	OOO	0.4	1.0	2.0	4.4	0.7	1.5	33.1	34.1	15.5	14.0
SDD	4	LLS,LLP	2.3	2.3	1.5	1.9	30.0	30.1	5.1	2.3	5.4	6.9
MMD	4	LOO	1.4	1.6	1.3	1.9	2.6	4.3	14.4	14.4	21.6	20.3
DDM,TMM	5	LLO,LNOO	2.1	2.3	0.3	0.6	11.4	13.4	3.4	4.4	14.1	14.5
DDD	6	LLL	3.0	3.4			21.3	19.8	1.0	1.0	2.7	4.5
TDD	7	LNLL					0.1	0.1			0.1	0.8
UNIDENTIFIED			0.0	0.1	2.7	0.0	0.0	0.7	0.1	0.1	4.2	2.8

[a]HPLC and Flame Ionization Conditions given in Experimental Section.
[b]Area percent standard deviation ± 01. to 0.2%.
[c]S,M,D,T = saturated (palmitic and stearic acids except for coconut, which also contains caprylic, capric, lauric and myristic acids (19)); monoenoic (oleic); dienoic (linoleic) and trienoic (linolenic) acids, respectively, attached to the triacylglycerol glycerol moiety.
[d]S,P,O,L and Ln = stearic, palmitic, oleic, linoleic and linolenic acids, respectively, attached to the triacylglycerol moiety.
[e]Coconut oil also contains saturated triacylglycerols with caprylic, capric, lauric and myristic acids (19).

TABLE 2
Vegetable Seed Oil Triacylglycerol Composition by Silver Ion and Reversed-Phase High Performance Liquid Chromatography with Flame Ionization Detection[a]

Resolution			Quantitation (Area percent)[b]							
			Safflower		Sunflower		Corn		Pumpkinseed	
Triacylglycerol saturated, monenoic, dienoic, trienoic, fatty acid species[c]	Number of Double Bonds	Triacylglycerol Molecular Species Reversed Phase[d]	Reversed Phase	Silver Ion	Reversed Phase	Silver Ion	Reversed Phase	Silver Ion	Reversed Phase	Silver Ion
SSS	0	PPP,PPS,PSS,SSS	0.8	0.1	0.3	0.4	0.2	0.4	0.3	0.1
SSM	1	POP,SPO,SOS	0.6	0.6	0.7	1.1	0.5	1.3	2.2	1.2
SSD	2	PLP,SLP,SLS	1.4	1.6	1.7	1.4	2.3	2.9	8.3	8.1
SMM	2	POO,SOO	0.9	1.4	1.8	1.4	3.0	3.8	3.2	3.8
SMD	3	LOP,LOS	5.3	6.3	8.1	7.9	11.9	10.8	13.9	14.7
MMM	3	OOO	0.8	0.7	1.5	1.4	3.0	3.8	4.2	4.5
SDD	4	LLS,LLP	17.6	18.7	20.4	20.8	18.7	17.8	20.6	20.7
MMD	4	LOO	3.5	4.5	6.0	6.0	10.0	11.8	9.6	10.5
DDM	5	LLO	19.6	19.8	23.4	23.3	23.3	23.7	17.7	18.9
DDD	6	LLL	47.4	46.1	35.8	35.8	26.3	22.8	18.9	16.7
TDD	7	LNLL	0.2	0.2	0.3	0.3	0.7	0.9	0.3	0.4
UNIDENTIFIED			1.9	0.0	0.0	0.2	0.1	0.0	0.8	0.4

See notes in Table 1

TABLE 3
Vegetable Seed Oil Triacylglycerol Composition by Silver Ion and Reversed-Phase High Performance Liquid Chromatography with Flame Ionization Detection[a]

Resolution			Quantitation (Area Percent)[c]					
			Linseed		Soybean		Canola	
Triacylglycerol saturated, monenoic, dienoic, trienoic, fatty acid species	Number of Double Bonds	Triacylglycerol molecular species reversed phase[b]	Reversed Phase	Silver Ion	Reversed Phase	Silver Ion	Reversed Phase	Silver Ion
SSS	0	PPP, PPS, PSS, SSS	0.1	0.1	0.5	0.4	0.5	0.1
SSM	1	POP, SOP, SOS	0.8	0.8	0.7	1.4	0.9	0.7
SSD	2	PLP, SLP, SLS	1.0	0.3	3.7	3.9	0.7	1.3
SMM	2	POO, SOO	2.2	2.3	3.8	4.2	5.7	6.5
SMD	3	LOP, LOS	4.3	3.3	12.9	11.6	8.0	7.0
MMM	3	OOO	2.1	2.1	2.9	3.4	21.1	21.9
SDD	4	LLS, LLP	2.1	4.8	16.9	15.5	3.9	5.1
MMD	4	LOO	1.9	5.2	11.1	9.4	22.4	21.7
DDM, TMM, SMT	4-5	LLO, LNOO, LNOP	12.1	14.2	17.7	21.2	20.1	21.2
DDD, TDS, TDM	5-6	LLL, LNLO, LNLP	14.3	17.9	21.8	20.5	11.7	9.8
TDD	7	LNLL	16.8	12.4	6.8	6.7	3.5	3.1
TTM	7	LNLNO	6.5	5.7			0.1	0.0
TTD	8	LNLNL	12.7	11.7	1.0	0.9	0.6	0.8
TTT	9	LNLNLN	22.2	19.2				
UNIDENTIFIED			0.3	0.0	0.2	0.9	0.8	1.1

See notes in Table 1

peaks. Some linolenic acid containing TAG eluted with linoleic TAG during Ag-HPLC of linolenic acid containing vegetable oils. (Christie, using a different Ag-HPLC column and a more complex mobile phase system, also observed that TAG containing T and D were unresolved with linolenic acid rich oils like linseed [16].) The GC-FID procedure was particularly useful for analysis of fractions which contained both dienoic and trienoic acid containing TAG. GC analysis was previously demonstrated by Christie for identification of TAG components represented by Ag-HPLC chromatogram peaks (16).

The retention volumes of the vegetable oil TAG increased as the number of double bonds increased from zero to nine (by the total number of fatty acid double bonds in the triacylglycerol). TAG containing the same number of double bonds (for example, SSD vs. SMM and SDD vs. MMD) showed partial resolution, perhaps due to secondary partition or adsorption mechanisms. However, computer integration of the TAG chromatogram peaks allowed estimation of the amount of each TAG. Other TAG with the same number of double bonds, like the pair MDD and TMM and the pair DDD and TDM showed no resolution and estimation of these components was based on GC-FID analysis of the TAG fraction after transmethylation.

The accuracy of the TAG quantitation by Ag-HPLC-FID with 0.5% acetonitrile/hexane system relative to TAG standards known by weight was previously reported (7). We found area percent compared well to weight percent without having to apply response factors for tristearoyl (SSS), trioleoyl (MMM), trilinoleoyl (DDD), triarachidonoyl (tetraenoic fatty acids), and tricrepenynoyl (monenoic alkynoic fatty acids) glycerols. Also, FID linear response had been demonstrated for another Ag-HPLC column with a

different mobile phase, for tripalmitoyl and trilauroylglycerols (14). Unlike TAG analyses obtained by Ag-HPLC systems with light scattering and ultraviolet detectors which required response factors for quantitation (5, 16), the TAG quantitation reported for the vegetable oil TAG resolved by Ag-HPLC-FID in Tables 1-3 gave good quantitation without response factors of the oil composition.

Previously, good TAG quantitation without response factors was demonstrated for the HPLC FID detector in combination with reversed phase HPLC (RP-HPLC FID) analyses of vegetable oils (8, 17). TAG quantitation by RP-HPLC-FID, which resolves TAG based on both fatty acid carbon number and unsaturation is also presented in Tables 1-3. Quantitative results for many of the TAG resolved by Ag-HPLC-FID were in agreement with those for RP-HPLC-FID analysis of the same oil. Thus, identification of the TAG resolved by Ag-HPLC is further supported by RP-HPLC analyses.

Fatty acid composition calculated from the quantitation of resolved TAG (10,16) by Ag-HPLC-FID and the experimental GC analysis of the methyl esters of the same oil are presented in Table 4. These results further confirm the TAG identity and quantitation determined by Ag-HPLC FID analyses.

In a previous study of margarine basestock formulations, we used the RP-HPLC-FID method for TAG analyses of interesterified blends of fully hydrogenated soybean oil with nine commonly used vegetable oils (12). TAG composition data obtained by Ag-HPLC-FID and by RP-HPLC-FID of corn oil and the basestocks produced by randomization of corn oil, the corn oil blend with cottonseed stearine and its interesterified product are presented in Table 5.

TABLE 4
Fatty Acid Composition of Selected Vegetable Oils as Calculated from Triacylglycerol Composition and as determined by Gas Chromatography of the Respective Oils after Transmethylation[a]

Vegetable Oil	Method	Area Percent Fatty Acid Composition			
		Saturated	Oleic	Linoleic	Linolenic
Coconut	HPLC	88.7	3.4	7.9	0.0
	GC	88.3	2.9	8.8	0.0
Palm	HPLC	49.3	40.8	9.8	0.0
	GC	48.9	41.1	9.9	0.0
Cottonseed	HPLC	25.7	15.9	58.2	0.2
	GC	25.1	16.1	58.6	0.2
Olive	HPLC	16.5	70.0	13.3	0.3
	GC	16.8	69.9	12.3	1.0
Peanut	HPLC	16.7	49.2	33.3	0.8
	GC	19.8	48.0	31.9	0.3
Safflower	HPLC	9.2	14.9	75.1	0.8
	GC	9.3	14.8	75.2	0.2
Corn	HPLC	13.9	26.1	59.6	0.4
	GC	13.8	26.4	59.0	0.8
Pumpkinseed	HPLC	21.0	24.6	54.1	0.3
	GC	18.7	25.7	54.8	0.8
Linseed	HPLC	9.4	17.3	31.2	42.1
	GC	9.6	19.3	29.9	41.2
Soybean	HPLC	14.3	24.8	53.3	7.6
	GC	14.9	25.1	52.8	7.2
Canola	HPLC	8.7	57.3	27.4	6.6
	GC	8.5	56.3	26.2	9.0

[a]Conditions given in Experimental Section.

Composition data for soybean oil, randomized soybean oil, soybean oil blend with cottonseed stearine and its interesterified product are given in Table 6. Ag-HPLC chromatograms of the corn oil products are given in Fig. 1 and for the soybean oil products in Fig. 2.

There is good agreement between the composition data obtained by RP-HPLC FID or by Ag-HPLC FID (Tables 5 and 6). These results show Ag-HPLC FID is a suitable method for analyses of the TAG composition changes

TABLE 5
Triacylglycerol Composition of Corn Oil, Randomized Corn Oil, Corn Oil and Cottonseed Stearine (CSOST) Blend and Interesterified Product, by Silver Ion and Reversed-Phase HPLC with Flame Ionization Detection[a]

Resolution		Area Percent[b]							
				Randomized		Blend		Interesterified	
		Corn Oil		Corn Oil		Corn Oil + CSOST		Corn Oil + CSOST	
Triacylglycerol saturated, monenoic, dienoic, trienoic fatty acid species silver ion[c]	Triacylglycerol molecular species reversed phase[d]	Reversed Phase	Silver Ion	Reversed Phase	Silver Ion	Reversed Phase	Silver Ion	Reversed Phase	Silver Ion
SSS	PPP,PPS,PSS,SSS	0.2	0.4	0.2	0.1	19.8	20.5	2.3	3.0
SSM	POP,SOP,SOS	0.5	1.3	0.5	0.7	0.5	1.2	5.6	5.0
SSD	PLP,SLP,SLS	2.3	2.9	2.8	1.9	2.3	2.5	10.9	12.8
SMM	POO,SOO	3.0	3.8	2.5	4.5	2.7	3.3	4.1	4.0
SMD	LOP,LOS	11.9	10.8	13.0	9.8	10.2	8.8	20.7	18.9
MMM	OOO	3.0	3.8	2.3	5.1	2.3	3.2	0.7	0.7
SDD	LLS,LLP	18.7	17.8	17.9	14.2	15.4	13.2	21.6	22.4
MMD	LOO	10.0	11.8	10.1	14.2	8.0	9.9	4.7	4.9
DDM	LLO	23.3	23.7	24.1	24.8	17.1	17.9	14.5	15.4
DDD	LLL	26.3	22.8	24.4	22.7	20.3	17.7	12.0	12.5
TDD	LNLL	0.7	0.9	0.8	0.9	0.6	0.9	0.6	0.4
UNIDENTIFIED		0.1	0.0	1.4	1.1	0.8	0.9	2.3	0.0

See notes in Table 1

TABLE 6
Triacylglycerol Composition of Soybean Oil, Randomized Soybean Oil, Soybean Oil and Cottonseed Stearine (CSOST) Blend and Interesterified Product, by Silver Ion and Reversed-Phase HPLC with Flame Ionization Detection[a]

Resolution		Area Percent[b]							
				Randomized		Blend		Interesterified	
		Soybean Oil		Soybean Oil		Soybean Oil + CSOST		Soybean Oil + CSOST	
Triacylglycerol saturated, monenoic, dienoic, trienoic, fatty acid species silver ion[c]	Triacylglycerol molecular species reversed phase[d]	Reversed Phase	Silver Ion	Reversed Phase	Silver Ion	Reversed Phase	Silver Ion	Reversed Phase	Silver Ion
SSS	PPP,PPS,PSS,SSS	0.5	0.4	0.6	1.6	16.1	16.6	2.3	3.1
SSM	POP,SOP,SOS	0.7	1.4	1.4	2.2	1.0	1.5	5.8	2.5
SSD	PLP,SLP,SLS	3.7	3.6	3.4	1.1	3.5	2.8	1.3	2.2
SMM	POO,SOO	3.8	4.2	6.0	5.9	3.4	3.9	13.4	16.0
SMD	LOP,LOS	12.9	11.6	14.5	8.8	11.6	9.1	20.5	18.6
MMM	OOO	2.9	3.4	1.4	6.1	2.4	3.0	0.5	0.4
SDD	LLS,LLP	16.9	15.5	13.4	9.0	13.2	13.3	19.3	22.5
MMD	LOO	11.1	9.4	8.4	14.8	6.5	7.8	3.9	3.6
MDD,SMT,MMT	LLO,LNOP,LNOO	17.7	21.2	22.9	23.9	14.9	17.3	12.5	14.1
DDD,TDS,TDM	LLL,LNLP,LNLO	21.8	20.5	18.8	19.6	20.6	16.9	13.3	10.7
TDD	LNLL	6.8	6.7	6.2	5.4	5.7	5.2	3.5	3.2
TTD	LNLNL	1.0	0.9	0.9	0.8	0.8	1.2	0.0	0.4
UNIDENTIFIED		0.2	1.2	2.1	0.8	0.3	1.3	3.7	2.7

See notes in Table 1

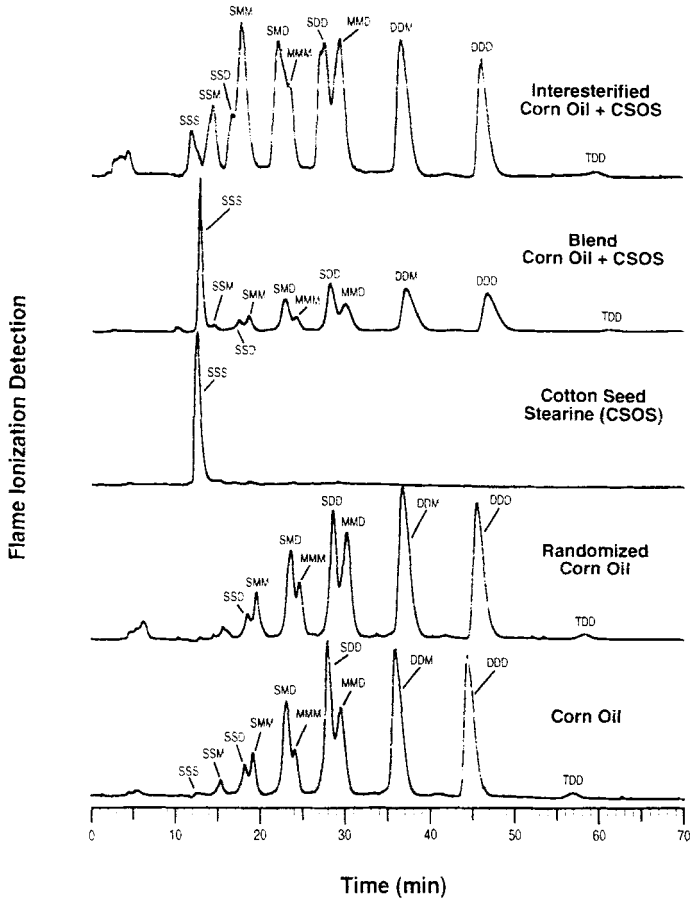

FIGURE 1. Triacylglycerol analyses by Ag-HPLC-FID of corn oil and randomized corn oil, blend of corn oil and cottonseed oil stearine and its interesterified product. See notes in Table 1.

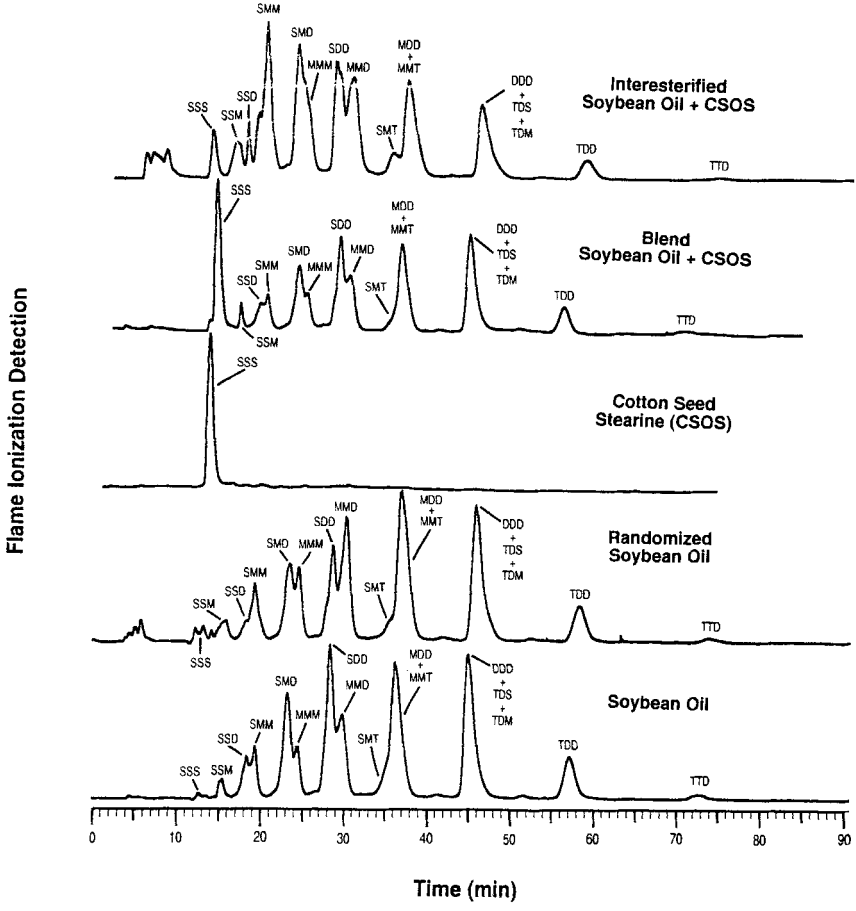

FIGURE 2. Triacylglycerol analyses by Ag-HPLC-FID of soybean oil and randomized soybean oil, blend of soybean oil and cottonseed oil stearine and its interesterified product. See notes in Table 1.

obtained by randomization, blending and interesterification of fats. For example, in Table 5, TAG composition of randomized corn oil relative to that of the starting oil showed slight decrease in SMD compared to MMM and slight increase in MMD compared to SDD. The blend of corn oil and cottonseed oil stearine showed the high concentration of SSS which was greatly reduced with increase in other TAG after interesterification. Similar results were obtained from the soybean oil study. TAG composition of randomized soybean oil relative to that of the starting oil showed a decrease in SMD compared to MMM and an increase in MMD compared to SDD. The blend of soybean oil showed a high initial concentration of SSS, which was greatly reduced after interesterification.

Ag-HPLC FID, with isocratic mobile phase of acetonitrile/hexane, is thus a suitable method for vegetable oil TAG analysis, yielding chromatograms which are easy to interpret and to quantitate. The chromatograms for TAG eluted by Ag-HPLC compared to RP-HPLC are less complex due to TAG elution with respect to fatty acid double bond number as opposed to both unsaturation and carbon chain length for TAG eluted by RP-HPLC (8,9,12,18).

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