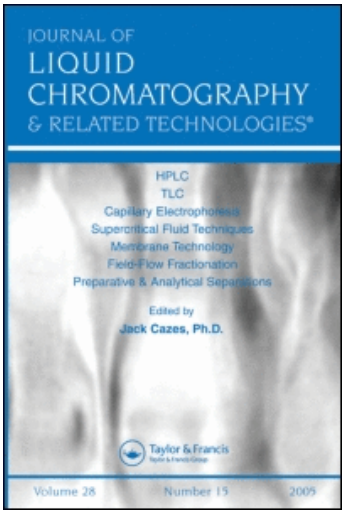

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QUANTITATIVE COMPOSITION OF HIGH PALMITIC AND STEARIC ACID SOYBEAN OIL TRIACYLGLYCEROLS BY REVERSED PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY: UTILIZATION OF EVAPORATIVE LIGHT SCATTERING AND FLAME IONIZATION DETECTORS

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ABSTRACT

Triacylglycerol species (TAG) of oils from five genetically modified soybean varieties were resolved by gradient reversed phase high performance liquid chromatography. The eluting TAG were detected and quantitated without the requirement for TAG response factors or calibration curves for the detector response by an improved evaporative light scattering detector and by a flame ionization detector. The accuracy of the improved light scattering detector for TAG analysis was checked by comparing the absolute error and average absolute error between experimental fatty acid composition obtained by calibrated gas chromatography of the transmethylated soybean oil and the calculated fatty acid composition obtained from the TAG composition of the soybean oil.

Low absolute and average absolute errors were obtained for the quantitative TAG analysis by the improved light scattering detector. Thus, this light scattering detector is suitable without the need for detector calibration for routine quantitative analysis of vegetable oil TAG mixtures. The evaporative light scattering detector showed absolute and average absolute errors compared to TAG quantitative analysis obtained by the flame ionization detector.

INTRODUCTION

Improvement of the functional properties of vegetable oils (VGO) such as soybean oil (SBO) for food uses (i.e., salad dressings, frying oils, margarine base stocks) has recently been obtained by alteration of fatty acid (FA) composition.[1-6] What was really changed was the triacylglycerol (TAG) composition (i.e., kinds and quantities of individual TAG) and TAG structure (i.e., kinds and quantities of individual FA located at the TAG glycerol moiety carbons) of the altered VGO or VGO product. Changes in TAG composition and structure effected the VGO functional properties such as melting point range, solid fat index, and crystal structure, which in turn affected food properties from texture to taste. Also, the VGO oxidative stability was in part dependent on TAG composition and structure.[7-11] The oxidative stability affected the storage and cooking life times of the VGO and VGO containing food products. Thus, knowledge of the kinds and quantities of individual TAG in VGO is important in food chemistry.

Previously, identification of TAG resolved by reversed phase high performance liquid chromatography (RP-HPLC) of VGO was conducted by collection of HPLC fractions for subsequent gas chromatography 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.[12-14] Recently, RP-HPLC coupled with atmospheric pressure chemical ionization mass spectrometry (APCI-MS) has been utilized to conclusively identify eluting TAG during RP-HPLC of VGO.[15] The MS of the TAG through the utilization of the APCI-MS method gave spectra, which contained a simplified number of very distinctive fragment ions that included diacylglycerol, protonated molecular and molecular related ions that conclusively identified individual TAG in VGO-TAG mixtures. Thus, this new methodology permitted the facile determination of the kinds of TAG in VGO mixtures.

The weakness in quantitative TAG analysis has been the method of detection, since TAG do not have strongly chromatophoric groups and the gradient solvent system needed for RP-HPLC TAG resolution present

absorbance problems for the commonly used HPLC ultraviolet absorbance detector. Christie, and others have written extensive reviews on possible HPLC detectors for quantitative TAG analysis.[16-21] The consensus of these authors is that while not perfect, the commercial HPLC flame ionization (FID) and evaporative light scattering (ELSD) detectors are the preferred detectors for quantitative TAG analysis.

Previously, we have made extensive use of a commercial FID for quantitative HPLC analysis of individual TAG in VGO mixtures.[14] This detector allowed TAG quantitation without the need for detector response factors. Thus, TAG quantitation was obtained as area percent, which for the FID is related to weight percent, obtained by computer integration of the RP-HPLC TAG chromatogram peak areas. Accuracy of the TAG composition could then be checked by comparison of the FA calculated from the experimental TAG composition against experimental FA composition obtained from GC analysis of the transmethylated VGO mixture.

Unfortunately, the FID detector is no longer commercially available. We have also utilized the APCI-MS as a quantitative detector.[15,16] This detector was determined to give quantitative results for TAG through facile calculation of individual response factors of individual TAG based on the raw MS response and the FA composition previously obtained by GC of the VGO mixture. However, from an economic view, the APCI-MS is a very expensive HPLC, detector for routine TAG analysis.

Christie and others have utilized the evaporative light scattering detector (ELSD) for quantitative TAG analysis.[17-23] However, earlier versions of the ELSD required an extensive calibration to produce response factors for accurate TAG quantitation.[17-23] New versions of the ELSD have improved linear detector response.[18,19]

The improvement is believed due to more uniform droplet size, which is responsible for the light scattering, without respect to solute type. Thus, the new versions of the HPLC ELSD may produce TAG quantitation without the need for response factors.

In this report, the new version of the ELSD is investigated as a quantitative HPLC detector for TAG analysis of oils from genetically modified soybean varieties. The ELSD results are compared with the previously used HPLC FID for quantitative TAG analysis. Soybean oil was used as a model TAG mixture, since it contains TAG found in other VGO such as canola, olive, corn, cottonseed, and palm. It is demonstrated here that the ELSD produced quantitative results without the need for response factors for the RP-HPLC analysis of TAG.

EXPERIMENTAL[†]

Materials

Five oil samples, 69, 75, 78, 81, and 85 were extracted, refined, bleached, and deodorized from seeds of five genetically modified soybean varieties. These oils were chromatographed to remove non TAG components, which might interfere with the chromatography, before HPLC by a silica solid phase procedure previously discussed.[9] HPLC mobile solvents acetonitrile (ACN) and dichloromethane (DCM) were HPLC grade and were purchased EM Science (Gibbstown, NJ) and Fisher Scientific (Fair Lawn NJ) respectively and used without further purification.

High Performance Liquid Chromatography

RP-HPLC was performed with a Thermo Separation Products (Schaumburg, IL.) (Model SP 8800) ternary solvent system with two RP-HPLC columns with bonded silyl (CT8) ODS, Inertsil ODS-80A, GL Sciences, Keystone Scientific (Bellefonte Park, PA), 25 cm 4.6 mm, 5 μm in series.

The gradient elution was as follows: 80% acetonitrile (ACN), 20% dichloromethane (DCM) to 20% ACN, 80% DCM after 120 min. The flow rate was 0.6 mL/min throughout. Sample size (25 micrograms) injected was 10 μL of 25 mg solute/ mL DCM.

Evaporative Light Scattering Detector

The ELSD was a Sedex Model 55, Sedone (Altontville, France). The drift tube was set at 32 C. The gas flow was set at a pressure of 1.6 bars. The photomultiplier gain was times 5. High purity N_2 was used as the nebulizer gas.

Flame Ionization Detector

The FID was a Tracor Model 945 HPLC detector, Finnigan, Inc., (Austin, TX). The FID operating conditions were: 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 cubic ft/min air. The detector electronic noise filter was set on high.

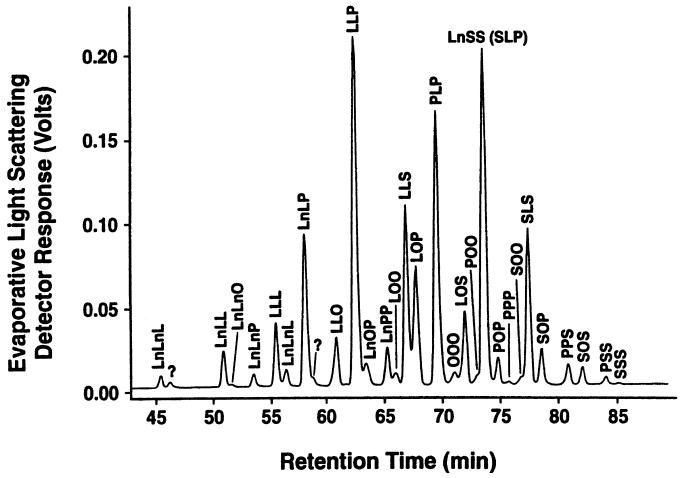

Figure 1. Triacylglycerol (TAG) analysis by gradient reversed phase HPLC coupled with an evaporative light scattering detector for soybean oil sample 78, a high palmitic and stearic acid oil, from a genetically modified soybean variety. TAG identifications were made by reversed phase HPLC atmospheric pressure chemical ionization mass spectrometry.[15] Ln, L, O, P, S are fatty acids of the TAG species detected. HPLC conditions are in the Experimental section in the text.

Triacylglycerol Identification

The Triacylglycerol HPLC chromatogram peaks were identified based on earlier analyses of SBO via reversed phase HPLC coupled with an atmospheric pressure chemical ionization mass spectrometer.[15,16]

Data Processing

The data output from ELSD was processed or integrated by a Star Chromatography Workstation with version 4.0 software, Varian Associates, Inc. (Walnut Creek, CA).

Gas Chromatography

Fatty acid methyl esters (FAME) were prepared by the potassium hydroxide catalyzed transmethylation of the TAG mixtures and the FAME were analyzed using calibrated gas chromatography (GC) according to a previous procedure.[9]

RESULTS AND DISCUSSION

Genetically modified soybeans with high palmitic and stearic acids were processed into refined, bleached, and deodorized oils. The qualitative and quantitative TAG composition of each oil was determined by reversed phase HPLC coupled with the ELSD and FID.

A RP-HPLC-ELSD chromatogram of a high palmitic and high stearic SBO from a genetically modified soybean variety (sample 78) is given in Figure 1. Identification of the TAG was based on the elution pattern previously published for RP-HPLC APCI-MS of high stearic and high palmitic SBO.[16] Twenty-eight TAG are identified in the HPLC chromatogram for the high saturated FA sample 78. In addition to the TAG given for the high saturated FA SBO sample 78, normal SBO contained TAG such as LnOO and SLP.[16] The elution times for these two TAG are indicated in Figure 1. The TAG identified or indicated for SBO 78 in Figure 1 are found in VGO. Of course, zero or trace Ln containing oils such as corn and cottonseed oils contain little Ln TAG such as LnLnL, LnOO and LnLO. The major TAG in SBO 78 are LnLP, LLP, LLS, LOP, PLP, LnSS, and SLS (Figure 1), which reflect the high palmitic, stearic, and linolenic acid content of this oil. Normal SBO contains major TAG such as LLLn, LLL, and LLO.[16] In the normal SBO TAG such as LnLP, LLS, PLP, LnSS, and SLS occur in minor amounts.

Increasing TAG with stearic and palmitic acids in SBO through genetic modification of soybean varieties assist the development of improved base stocks for food products like margarines.[4] Except for SBO 69, which has low content of saturated fatty acids, the other SBO have high content of saturated fatty acids and thus may be useful for base stocks for margarine**.** Thus, from the discussion above it is observed the importance of knowledge of accurate qualitative and quantitative TAG composition of products pertinent to food chemistry.

The TAG composition data determined by RP-HPLC-ELSD and FID for all the oils from the five soybean varieties are listed in Tables 1 and 2, respectively. The ELSD and FID detectors showed good agreement with each other for each TAG species in the oils of the five soybean varieties listed. These oils were utilized for this detector study, since there was considerable variation in TAG species amounts across the oils. For example, the following TAG had these percent composition ranges based on ELSD response (Table 1): LnLL (1.2-3.2**),** LLL (3.7-30.0)**,** LnLP (0.4-9.4), LLO (3.6-26.9), LLP (6.4-21.4)**,** LOO (1.3-13.9), LLS (2.0-13.0), PLP (0.8-14.8), OOO (0.8-4.6), LOS (1.3-11.8), SLS (0.2-12.3), SOS (0.0-3.4), and SSS (0.0-0.1). Thus, we analyzed via. RP-HPLC-ELSD and FID, TAG species which contained stearic, palmitic, oleic, linoleic, and linolenic acids to observe how well the detectors agreed with each other over a wide TAG species concentration range.

Also, these SBO TAG mixtures were utilized to show, as discussed below and as previously known for the FID,[21] that the ELSD response could be used without response factors for quantitative analysis of a large variety of VGO TAG species over a large concentration range.

A check on the identification accuracy of the TAG presented in Figure 1 and in Tables 1 and 2 is the agreement between experimental fatty acid composition obtained by calibrated gas chromatography of the methyl esters from the transmethylated SBO and the fatty acid composition calculated from the experimental TAG composition of the SBO obtained by RP-HPLC coupled with a linear mass detector such as the ELSD and FID. These data are listed in Tables 3 (ELSD detector) and in Table 4 (FID detector) for all the oils from the five soybean varieties. Also listed are the absolute difference (absolute error) between accurate fatty acid composition obtained by calibrated GC of the transmethylated oil and uncorrected fatty acid composition obtained by ELSD and FID per fatty acid per SBO.

Also, an average absolute error across all the absolute errors for the five fatty acids listed per SBO is given for all the SBO listed. For ELSD, SBO 69,75,78, and 81 the AAE are less than 2. SBO 85 has an AAE of 2. The AE are small values per fatty acid are usually less than 2 percent for large fatty acid composition numbers. For FID, SBO 69,75,78, and 81 the AAE are less than 2. SBO 85 has an AAE of 1.9.

As for the ELSD, the AE are small values per fatty acid are usually less than 2 percent for the large fatty acid composition numbers. There is little difference among the AE and AAE values between the ELSD and FID detectors. Thus it can not be stated that ELSD yields more accurate TAG concentration results than the FID or visa versa.

For the APCI-MS detector, we reported the magnitude of AAE for SBO and SBO products ranged from 0.2 to 0.5%. For the APCI-MS, the AE were usually less than 1% per fatty acid. However, the APCI-MS allowed the use of mass chromatography to resolve for quantitation HPLC co-eluting TAG pairs such as SLP/LnSS and partially resolved TAG pairs such as LLO/LnOO and LLP/LnOP. Thus, the mass chromatography assisted production of smaller AE and AAE for the fatty acid data calculated from the TAG composition obtained by APCI-MS.

Mass chromatography is not possible with the ELSD and FID detectors. Therefore, partially resolved and coeluting TAG quantitation accuracy is reduced for these detectors. For the ELSD and FID detectors this problem would be expected to increase the AE and AAE values for the calculated fatty acid vs. the experimental fatty acid composition.

Table 1

Triacylglycerol (TAG) Composition by Reversed Phase HPLC Coupled with Evaporative Light Scattering Detector (ELSD) for Oils from Genetically Modified Varieties[a]

TAG/SBO[b]	69	75	78[c]	81	85
LnLnLn	0.0	0.0	0.0	0.0	0.0
LnLn	0.0	0.0	0.1	0.3	0.3
LnLL	2.0	1.2	1.4	3.2	2.6
LnLnO	0.0	0.1	0.1	0.2	0.1
LnLnP	0.0	0.0	0.4	0.6	0.1
LLL	30.0	11.5	3.7	9.6	6.5
LnLO	1.7	1.5	0.8	2.0	1.9
LnLP	0.4	1.7	6.9	9.4	2.2
LLO	26.9	14.4	3.6	8.7	7.1
LNOO	0.4	0.5	0.1	0.3	0.3
LLP	6.4	20.7	17.5	21.4	11.6
LnOP	0.1	1.0	1.4	2.1	11.6
LnPP	0.1	0.0	1.5	2.0	0.1
LOO	13.9	7.3	1.3	3.1	2.5
LLS	3.6	2.6	7.7	2.0	13.0
LOP	3.7	16.3	7.4	12.2	6.4
PLP	0.8	8.6	13.8	14.8	2.0
OOO	4.6	2.1	1.0	0.8	1.1
LOS	2.6	2.3	3.9	1.3	11.8
POO	0.9	3.2	0.6	1.0	0.5
SLP(LnSS)[d]	0.8(SLP)	2.2(SLP)	16.0(LSS)	3.0(LnSS)	8.8(LnSS)
POP	0.2	1.7	1.2	1.5	0.3
PPP	0.0	0.0	0.1	0.0	0.2
SOO	0.7	0.5	0.3	0.2	2.1
SLS	0.3	0.2	6.5	0.3	12.3
SOP	0.1	0.4	1.3	0.2	1.4
PPP	0.0	0.0	0.6	0.0	0.6
SOS	0.0	0.0	0.6	0.0	3.4
PSS	0.0	0.0	0.2	0.0	0.1
SSS	0.0	0.0	0.0	0.0	0.1

[a] Determined by Reversed Phase- HPLC with evaporative light scattering detector; analysis conditions in the Experimental section. [b]TAG confirmed by Reversed Phase atmospheric pressure chemical ionization mass spec. analysis.[16] See Fig. 1 for triacylglycerol abbreviation. SBO=soybean sample no. [c]SBO #78 HPLC chromatogram in fig.1. [d]TAG SLP and LnSS have the same HPLC retention times.

Table 2

Triacylglycerol (TAG) Composition Determined by Reversed Phase HPLC Coupled with Flame Ionization Detector (FID) for Soybean Oils from Genetically Modified Varieties[a]

TAG/SBO#[b]	69	75	78[c]	81	85
LnLnLn	0.0	0.0	0.0	0.0	0.0
LnLn	0.0	0.0	0.5	0.9	0.3
LnLL	2.7	1.3	1.6	3.6	2.8
LnLnO	0.0	0.0	0.1	0.4	0.1
LnLnP	0.0	0.0	0.5	1.2	0.1
LLL	29.2	10.3	2.7	6.9	6.2
LnLO	2.2	1.9	0.8	3.0	1.8
LnLP	0.0	2.2	7.5	10.6	2.3
LLO	26.4	12.8	2.4	6.9	7.0
LNOO	1.2	0.7	0.0	0.5	0.2
LLP	5.7	18.7	18.0	21.0	11.6
LnOP	0.3	1.6	0.5	3.9	0.4
LnPP	0.0	0.0	0.6	2.7	0.0
LOO	13.5	7.3	1.7	1.3	2.7
LLS	3.8	2.8	8.3	4.3	13.1
LOP	3.7	15.8	6.2	9.9	5.8
PLP	0.5	9.7	14.5	15.1	2.1
OOO	5.0	2.8	0.5	0.0	1.2
LOS	3.0	2.7	3.7	0.6	12.0
POO	1.3	3.6	1.0	0.9	0.6
SLP(LnSS)[d]	0.3 (SLP)	2.7(SLP)	16.0(LnSS)	3.0(LNSS)	8.8(LnSS)
POP	0.3	2.2	1.5	1.9	0.3
PPP	0.0	0.0	0.1	0.0	0.2
SOO	0.9	0.9	0.3	0.1	2.2
SLS	0.0	0.0	7.0	0.5	12.1
SOP	0.0	0.0	1.6	0.5	1.6
PPS	0.0	0.0	1.0	0.3	0.8
SOS	0.0	0.0	0.7	0.0	3.4
PSS	0.0	0.0	0.3	0.0	0.2
SSS	0.0	0.0	0.1	0.0	0.1

[a] Determined by Reversed Phase- HPLC with flame ionization detector; analysis conditions in the Experimental section. [b]TAG confirmed by Reversed Phase atmospheric pressure chemical ionization mass spec. analysis.[16] See Fig. 1 for triacylglycerol abbreviation. SBO=soybean sample no. [c]SBO #78 HPLC chromatogram in Fig.1. [d]TAG SLP and LnSS have the same HPLC retention times.

Table 3

Fatty Acid Composition of Oils from Genetically Modified Soybean Varieties[a]

Sample/Fatty Acid	16:0	18:0	18:1	18:2	18:3	
69 A	3.9	3.3	28.5	61.8	2.5	
B	4.8	2.8	26.9	64.0	1.6	
AE[d]	0.9	0.5	1.6	2.2	0.9	
AAE[e]						1.2
75 A	21.4	3.3	23.6	49.0	2.7	
B	22.0	2.8	22.3	50.8	2.0	
AE	0.6	0.5	1.3	1.8	0.7	
AAE						1.0
78 A	23.6	19.0	9.3	38.0	10.0	
B	23.4	20.2	9.3	37.4	9.8	
AE	0.2	1.2	0.0	0.6	0.2	
AAE						0.4
81 A	28.2	3.9	13.9	43.8	10.2	
B	27.8	3.4	13.2	47.7	8.0	
AE	0.4	0.5	0.7	3.9	2.2	
AAE						1.5
85 A	8.5	26.5	18.0	38.9	8.2	
B	9.9	26.2	15.7	42.5	5.8	
AE	1.4	0.3	2.3	3.6	2.4	
AAE						2.0

[a] A: Fatty acid composition determined experimentally by transmethylation of the oil followed by quantitative gas chromatography of the resultant methyl esters.[b] B: Fatty acid composition calculated from the experimental triacylglycerol composition determined by evaporative light scattering detector listed in Table 1.[c] [b] Obtained by gas chromatography of the respective oils after methylation. See the Exp. section for analysis details. See Fig 1 for fatty acid abbreviation. [c] Calculated from HPLC triacylglycerol composition data Table 1. See the Exp. section for analysis details. [d] AE is the absolute error of the difference between the calculated and experimental fatty acid composition per fatty acid. [e] AAE is the average absolute error, which is the sum of the AE divided by 5 per SBO.

Table 4

Fatty Acid Composition of Oils from Genetically Modified Soybean Varieties[a]

Sample Fatty Acid	16.0	18:0	18:1	18:2	18:3	
69 A	3.9	3.3	28.5	61.8	2.5	
B	4.3	2.7	28.2	62.7	2.1	
AE[d]	0.4	0.6	0.3	0.9	0.4	
AAE[e]						0.5
75 A	21.4	3.3	23.6	49.0	2.7	
B	22.8	3.0	23.5	48.1	2.6	
AE	1.4	0.3	0.1	0.9	0.1	
AAE						0.6
78 A	23.6	19.0	9.3	38.0	10.0	
B	23.6	21.2	8.4	36.9	9.8	
AE	0.0	2.2	0.9	1.1	0.2	
AAE						0.9
81 A	28.2	3.9	13.9	43.8	10.2	
B	29.4	4.3	10.9	44.8	10.8	
AE	1.2	0.4	3.0	1.0	0.6	
AAE						1.2
85 A	8.5	26.5	18.0	38.9	8.2	
B	9.9	26.3	15.8	42.3	5.8	
AE	1.4	0.2	2.2	3.4	2.4	
AAE						1.9

[a] A: Fatty acid composition determined experimentally by transmethylation of the oil followed by quantitative gas chromatography of the resultant methyl esters.[b] B: Fatty acid composition calculated from the experimental triacylglycerol composition determined by evaporative light scattering detector listed in Table 1C. [b] Obtained by gas chromatography of the respective oils after methylation. See the Exp. section for analysis details. See Fig 1 for fatty acid abbreviation. [c] Calculated from HPLC triacylglycerol composition data Table 1. See the Exp. section for analysis details. [d] AE is the absolute error of the difference between the calculated and experimental fatty acid composition per fatty acid. [e] AAE is the average absolute error, which is the sum of the AE divided by 5 per SBO.

We observed that the ELSD gave a linear response for SBO TAG sample weights over the range of 10 to 50 micrograms per HPLC sample injection. The analysis precision for the ELSD was good with a standard deviation for triplicate analysis per SBO TAG mixture of 0.0 to 0.3% per TAG species. These data support the use in place of the HPLC FID, which is no longer commercially available, the improved ELSD such as the Sedex model 55 ELSD with the need for detector response factors or calibration curves for routine survey of the quantitative TAG composition of VO.

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[†] Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may be suitable.

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