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ANALYSIS OF GENETICALLY MODIFIED CANOLA VARIETIES BY ATMOSPHERIC PRESSURE CHEMICAL IONIZATION MASS SPECTROMETRIC AND FLAME IONIZATION DETECTION

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ABSTRACT

Canola oil triacylglycerols from genetically modified canola lines were conclusively identified by reverse phase HPLC coupled with atmospheric pressure chemical ionization mass spectrometric (APCI-MS) detection. APCI-MS is a soft ionization technique, which gave simple spectra for triacylglycerols. Spectral identification of the triacylglycerols was based on the diacylglycerol fragments and on the protonated molecular ion $[M+H]^+$, except trisaturates which gave no $[M+H]^+$. Triacylglycerols were identified and quantitated in normal, high stearic acid and high lauric acid canola varieties by the RP-HPLC/APCI-MS technique. The LC/APCI-MS identification of canola oil triacylglycerols allowed their quantitation by reverse phase HPLC coupled with a commercial flame ionization detector (FID). There was agreement between fatty acid composition obtained by LC/APCI-MS and LC-FID. However, the triacylglycerol resolution obtained by LC/APCI-MS, was superior to LC-FID in the qualitative identification of triacylglycerols present in amounts even below one percent. The oils of the modified canola varieties, compared to typical canola oil, contained increased content of triacylglycerols known to be more

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oxidatively stable like stearoyloleoyllinoleoyl, distearoyllinoleoyl, stearoyldioleoyl and distearoyloleoyl glycerols in high stearic acid canola oil and dilauroyllinoleoyl, dilauroyloleoyl and lauroyldioleoyl glycerols in high lauric acid canola oil. These oils contained fewer linolenate-containing triacylglycerols known to decrease oxidative stability. The LC/APCI-MS technique gave better resolution of, and quantitation of triacylglycerols in the canola oils, than the LC/FID. However, the LC/FID system gave satisfactory analyses suitable for many research programs, like the development of genetically modified canola varieties with oils of improved oxidative stability.

INTRODUCTION

Recently, some research has been directed toward the improvement, through plant genetic manipulation, of the functional properties of vegetable oils (VGO) for food uses (i.e. frying oils, salad oils, structured fats for margarine basestocks, confectionary products, baking shortenings) by altering the fatty acid (FA) composition and the triacylglycerol (TAG) composition.[1-9] Some of the plants, which are being modified by genetic manipulation, include soybean, canola, sunflower and flax. Other research has been directed toward the correlation of oxidative stability of VGO with TAG composition.[10-12] Understanding VGO oxidative stability is important to develop methods for protection of VGO shelf life and nutritional safety.[13,14] Therefore, it is important for these studies of VGO properties to have a facile method for identification and quantitation of TAG in the complex oil matrix.

Previously, for our VGO oxidative stabiltiy studies of soybean and high oleic acid canola oils from new plant varieties, we used reverse phase high performance liquid chromatography (RP-HPLC) coupled with flame ionization detection (FID) for qualitative and quantitative analysis of the oil TAG.[10,12] The TAG were tentatively identified by correlation of their theoretical carbon number with HPLC retention time.[15] The accuracy of the TAG identification and quantitation were supported by good agreement between FA composition, calculated from TAG composition, obtained by RP-HPLC-FID and experimental FA composition obtained by gas chromatography with FID of the methyl esters of the transmethylated oils.[16]

Recently, our oxidative stability studies have focused on the TAG mixtures obtained from high stearic (16-32 % stearic acid) and lauric (11-32 % lauric acid) canola oils from new genetically modified canola varieties. These oils contained many previously unidentified TAG, some of which coeluted by RP-HPLC and therefore, were not amenable to analysis by our RP-HPLC-FID technique.

Obviously, mass spectrometry, which has the capability of selective ion monitoring, would appear useful for RP-HPLC-MS analysis of co-eluting TAG. However, many MS techniques for TAG require collection of TAG HPLC fractions or, because of the interface between the HPLC column and the MS, have the potential for thermal degradation of the TAG or poor sensitivity for detection of minor TAG.[17] Furthermore, some MS ionization techniques, like electron impact ionization, produce complex spectra for TAG. Electrospray ionization (ESI) mass spectrometric analysis provides a soft ionization method for production of simple spectra with molecular ion information for polar complex molecules.[18] However, for non-polar compounds like TAG, the addition of ionic modifiers to the TAG solvent is required for ESI-MS analysis.[18,19] Moreover, ESI spectra show only molecular ions with no fragmentation into diacylglycerols (DAG), which are required for identification of TAG with identical molecular weights.[19]

We have recently developed satisfactory analytical methodology for qualitative and quantitative analysis of TAG by RP-HPLC, coupled with a quadrupole MS equipped with an atmospheric pressure chemical ionization interface.[17,19,20] The APCI interface, a soft ionization technique, proved suitable for non-polar compounds like the TAG. The resultant simple spectra showed only protonated TAG molecular ion $[M+H]^+$ and the required DAG fragments in sufficient abundance to conclusively identify even minor TAG.

We report here an analytical study, using RP-HPLC/APCI-MS, for qualitative and quantitative analysis of TAG in the complex TAG mixtures from high lauric and high stearic acid containing canola oils (CNO) with widely varying fatty acid composition. The TAG composition and resultant FA composition obtained by RP-HPLC/APCI-MS were compared to the results obtained by LC-FID. The FA compositions calculated from the TAG compositions were compared to the FA composition experimentally determined using GC-FID.

MATERIALS

The seeds from experimental high lauric and high stearic canola varieties were obtained from Calgene Inc. (Davis, CA). Solvents were purchased from Aldrich Chemical Co. (Milwaukee, WI) or EM Science (Gibbstown, NJ). Solvents were HPLC grade or the highest available quality and were used without further purification. The crude oils (4.5-6.0 g) were obtained by extraction of 15 g canola seeds in duplicate using a previously described sonification-hexane extraction procedure.[12] Solid-phase extraction for purification of the crude CNO TAG to avoid interference by non-TAG components during RP-HPLC/APCI-MS, was performed by a reported procedure to give 0.9-1.0 g chromatographed oil per 1.2 g crude CNO.[12]

METHODS

Liquid Chromatography (APCI-MS)

The HPLC pump was an LDC 4100 MS (Thermo Separation Products, Shaumburg, IL) quaternary pump system with membrane degasser. The columns used were an Adsorbosphere C_{18} (Alltech Associates, Deerfield, IL), 25 cm x 4.6 mm, 5 μm (12 % carbon load) in series with an Adsorbosphere UHS C_{18} 25 cm x 4.6 mm, 10 μm (30 % carbon load). The flow rate throughout was 1. mL/min. A gradient solvent program with propionitrile (PrCN), dichloromethane (DCM) and acetonitrile (ACN) was used to separate the canola oil TGs. The gradient used was as follows: initial - PrCN/DCM/ACN (45:20:35, v/v/v); linear from 15 to 20 min. to PrCN/DCM/ACN (45:25:30, v/v/v), held until 35 min.; linear from 35 to 40 min. to PrCN/DCM/ACN (45:30:25, v/v/v), held until 95 min. This combination was used to reduce the undesirably high amount of PrCN adducts formed by this solvent in the absence of other solvents.

The quantitative analysis by RP-HPLC with flame ionization detection, used a linear gradient of ACN/DCM 70:30 to 40:60 v/v over 120 minutes. Columns and conditions were as previously described.[21]

The evaporative light scattering detector (ELSD) was an ELSD MKIII (Varex, Burtonsville, MD). The drift tube was set at 140 °C, the gas flow was 2.0 standard liters per minute. High purity N_2 was used as the nebulizer gas.

Mass Spectrometry

A Finnigan MAT (San Jose, CA) SSQ 710C mass spectrometer fitted with an atmospheric pressure chemical ionization source, was used to acquire mass spectral data. LC column eluent was split so that 600 μL/min was diverted to the APCI inlet, while 400 μL/min went to an evaporative light scattering detector. The APCI vaporizer was operated at 400 °C and the capillary heater was operated at 265 °C. The corona voltage was set at 6.0 μA throughout. High purity nitrogen was used for the sheath and auxillary gases, which were set to 55 psi and 5 mL/min, respectively. Spectra were obtained from 400 amu to 1000 amu or 1100 amu, with a scan time of 1.75 to 2.0 sec. Chromatograms were processed using five-point smoothing for graphical output, but no smoothing was applied during quantitation of extracted ion chromatograms.

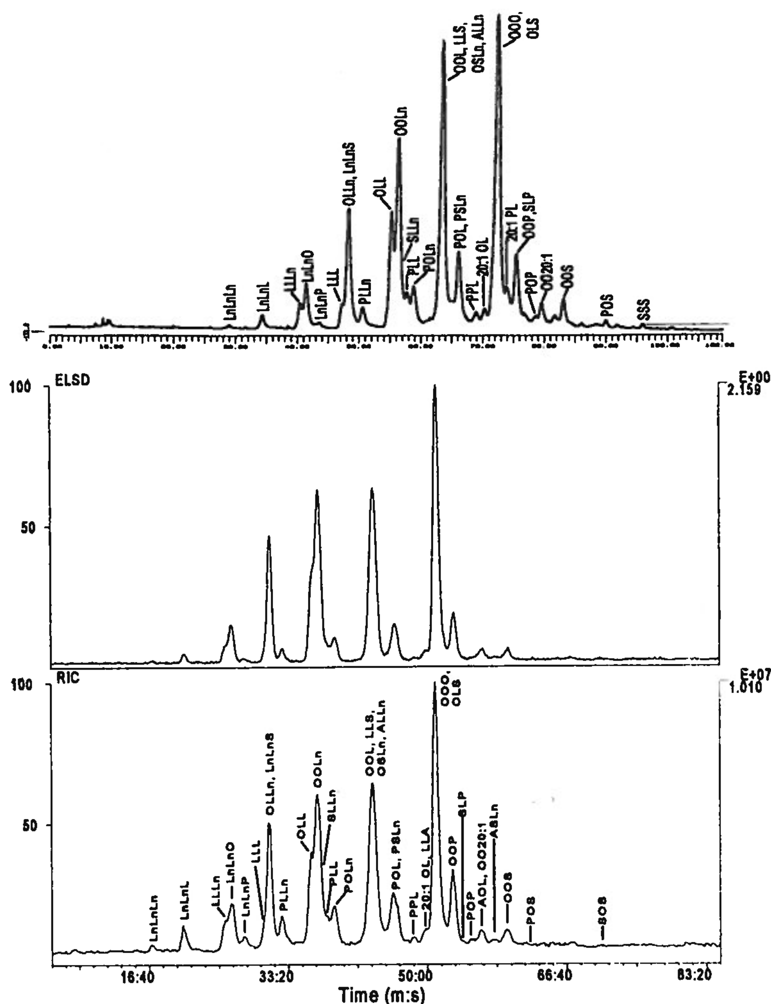

Figure 1. Normal canola oil: reverse phase high performance liquid chromatography/flame ionization detection chromatogram (top), reverse phase high performance liquid chromatography/evaporative light scattering detector chromatogram (middle), reverse phase high performance liquid chromatography/atmospheric pressure chemical ionization mass spectrometric chromatogram (bottom). Liquid chromatographic conditions in text. Abbreviations: Ln=linolenic; L=linoleic; O=oleic; S=stearic; P=palmitic; A=arachidic.

Gas Chromatography

Canola oil TAG were transmethylated according to the method of Glass[22] by reaction with 3 mL of 0.5 N potassium hydroxide in methanol at 50 °C for 15 min. Fatty acid methyl esters (FAMEs) were analyzed using a Varian 3400 (Palo Alto, CA) gas chromatograph equipped with a Supelco (Belefonte Park, PA) SP2380 30 m x 0.25 mm i.d. capillary column. The conditions were: inlet temperature = 240 °C; detector temperature = 280 °C; initial column temperature = 150 °C; initial time = 35 minutes; column temperature increased to 210 °C at 3 °C/min.

RESULTS

Normal Canola Oil

The RP-HPLC/APCI-MS reconstructed ion chromatogram, ELSD chromatogram and RP-HPLC/FID chromatogram are shown in Figure 1. Identification of components was accomplished using the LC/ACPI-MS spectral data. Figure 1 illustrates the chromatographic overlaps which occurred among many of the major TAG components. The LC-FID chromatogram did not have sufficient resolution to allow quantitation of all of the components identified by APCI-MS. The LC/APCI-MS data did allow the resolution of most overlaps by the use of extracted ion chromatograms (EICs), such as those shown in Figure 2. Numerous other TAG species were identified in the canola oil than are labeled in Figure 1, because of space limitations. Many TAG containing gadoleic (20:1) and arachidic (20:0) acids were qualitatively identified but not quantitated.

Areas under peaks associated with diglyceride and protonated triglyceride ions from each triglyceride component were obtained by integration of the extracted ion chromatograms. The integrated areas under these diglyceride and protonated triglyceride peaks were added together to obtain the total integrated areas for each TAG component. A percent composition was calculated from the total area for each TAG, divided by the total area obtained for all TAG components. In the cases where unresolvable DAG fragment overlaps occurred, the areas of the overlapped peaks were apportioned according to their statistically predicted percent composition.

The TAG percent composition obtained from the LC/APCI-MS integrated areas is given in Table 1, column 2. The percent composition obtained from integration of the LC/FID chromatogram is given in Table 1, column 4.

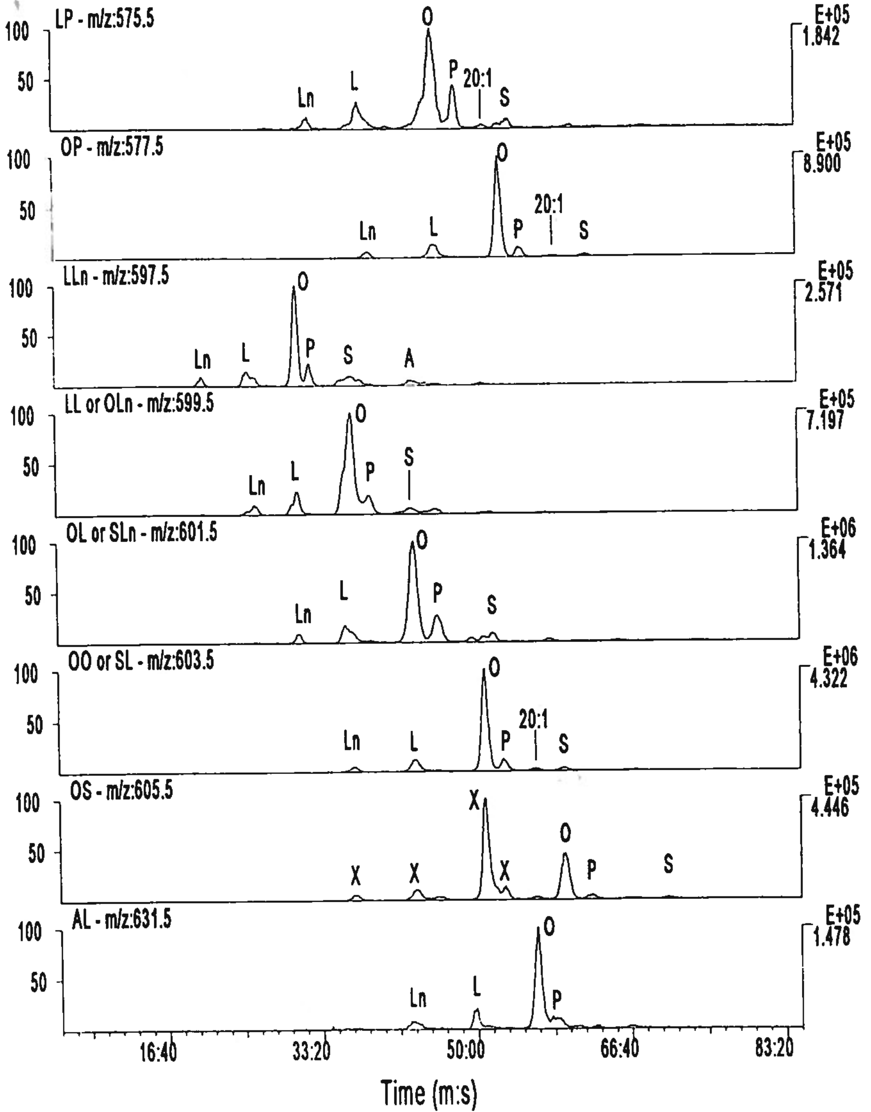

Figure 2. Normal canola oil: extracted ion chromatograms of diacylglycerol fragments. Diacylglycerol identity and mass indicated on the left in each frame. Chromatograms are labeled with the fatty acid which, combined with the diacylglycerol. make up triacylglycerol species. Abbreviations: X=carbon13 isotopic peak; fatty acids as in Figure 1.

Table 1

Normal Canola Oil TAG Composition by LC/MS and LC/FID

TAG	Raw LC/MS	GC-ADJ. %	LC-FID
OOO	22.0	23.7	23.3
OOL	17.1	18.5	19.3
OOLn	12.1	14.1	16.2
LnLO	8.5	9.9	9.0
LLO	7.1	7.9	4.5
LnLnO	3.5	4.3	3.7
OOP	6.7	4.1	4.8
PLO	5.6	3.6	5.2
PLnO	3.5	2.4	3.1
LLLn	1.8	2.1	0.9
OOS	2.0	1.6	1.1
PLnL	1.8	1.3	1.3
LOS	1.4	1.2	1.5
LLP	1.0	0.7	1.4
LLL	0.6	0.6	0.7
LnOS	0.7	0.6	
LnLnP	0.7	0.5	0.4
PoPoLn	0.1	0.3	
LnLS	0.4	0.3	
PLS	0.5	0.2	0.2
PPO	0.5	0.2	
PPL	0.5	0.2	0.2
PoPO	0.1	0.2	
LnLnL	0.1	0.2	0.9
POS	0.4	0.2	0.1
PoLnL	0.1	0.1	
LnLnS	0.1	0.1	
LLS	0.1	0.1	
PLnS	0.2	0.1	
PPLn	0.2	0.1	
SSL	0.1	0.1	
SSO	0.1	0.1	0.1
SSLn	0.1	0.1	
PoPL		0.1	
LnLnLn	0.0		0.2
PPP	0.1		1.0

Table 1 (continued)

Normal Canola Oil TAG Composition by LC/MS and LC/FID

TAG	Raw LC/MS	GC-ADJ. %	LC-FID
PPS			0.3
SSS			0.1
SSP			0.2
Sum	100.0	100.0	100.0

Table 2

Normal Canola Oil Fatty Acid Composition

FA	Raw LC/MS %	Adj. LC/MS %	LC-FID %	GC-FID %
Po	0.1	0.4	0.0	0.3
P	7.8	4.8	7.0	5.1
Ln	12.8	14.0	13.8	14.3
L	19.3	19.6	17.8	19.9
O	57.7	59.5	60.1	58.7
S	2.2	1.6	1.4	1.6
Sum	100.0	100.0	100.0	100.0
AARE %[1]	26.5	3.8	11.1	0.0

[1] Average absolute relative error = sum of the absolute value of relative error, compared to GC-FID, for each FA divided by the number of FA's (n=6).

Fatty acid compositions were calculated from the TAG compositions determined by LC-FID and by LC/APCI-MS. These are given in columns 2 and 4, respectively, of Table 2. The fatty acid composition of the CNO was determined as the fatty acid methyl esters by gas chromatography with flame ionization detection (GC-FID). The fatty acid composition obtained by GC with FID detection, represented a weight percent so, each FA weight percent, was divided by the molecular weight of each FAME to determine a molar percent composition, given in Table 2, column 5. To improve the agreement of the FA composition calculated from LC/APCI-MS data with the FA composition obtained by GC-FID, response factors were calculated for each FA, from the ratio of the FA percent by LC/APCI-MS, to the FA percent by GC-FID. The

response factors obtained for each FA were then multiplied together to produce response factors for each TAG. The FA response factors (normalized to the smallest) which were used to calculate the TAG response factors were: Po = 3.60190; P = 1.00000; Ln = 1.83201; L = 1.70928; O = 1.69596; S = 1.25385. The TAG response factors derived from the GC-FID data, were applied to the uncorrected LC/MS data to produce the TAG percent composition given in Table 1, column 3, referred to as 'GC-Adjusted %'. The FA composition, which resulted from the adjusted LC/MS data, is given in Table 2, column 3. The last row of Table 2 lists the average absolute relative error (AARE) for each result set, compared to GC-FID. The average absolute relative error was obtained by totaling the absolute value of the relative error for each FA and dividing by the number of FA, to give a measure of the average error associated with the FA composition. The AARE of the FA composition, determined by application of GC-FID response factors to the APCI-MS data, was smaller than the error obtained by LC-FID or by uncorrected APCI-MS. Application of the simply-derived response factors to the raw TAG composition, allowed calculation of a corrected TAG composition which had a FA composition which is in excellent agreement with the FA composition determined experimentally.

The results obtained for this study differed somewhat from the TAG and FA compositions reported previously for other canola oils.[12,15]

This was hardly surprising considering the amount of variation in the compositions of the varieties of CNO.

High Lauric Acid Canola Oil

Chromatograms of the separation of TAG in high lauric canola oil, obtained by LC/APCI-MS, ELSD, and FID, are given in Figure 3. The identifications shown in the chromatogram obtained by LC/FID were only possible because of the mass spectral data from the LC/APCI-MS. Standards (chromatographic or quantitative) were not available for the numerous lauric and myristic acid-containing TAG present in this oil sample, so identification by LC-FID alone, based on calculated equivalent carbon number, was not reliable. The extracted ion chromatograms shown in Figure 4, and others not shown, allowed identification of the major and minor TAG components given in Table 3, as well as nearly sixty minor components not listed in Table 3. Many of the minor components (< 0.2%) were included in the percent composition under the label 'Other', while others were identified qualitatively, but not, quantitatively. Identification of a TAG, which was present at a low level, is typified by MMO, present at 0.2%. MMO displayed a distinct oleoyl peak in the EIC, Figure 4, for 'MM' (m/z: 495.4±.5) and a noticeable myristoyl peak in the EIC for 'MO' (m/z:549.5±.5). The peaks in the two EICs occurred at the same time chromatographically, confirming the presence of MMO.

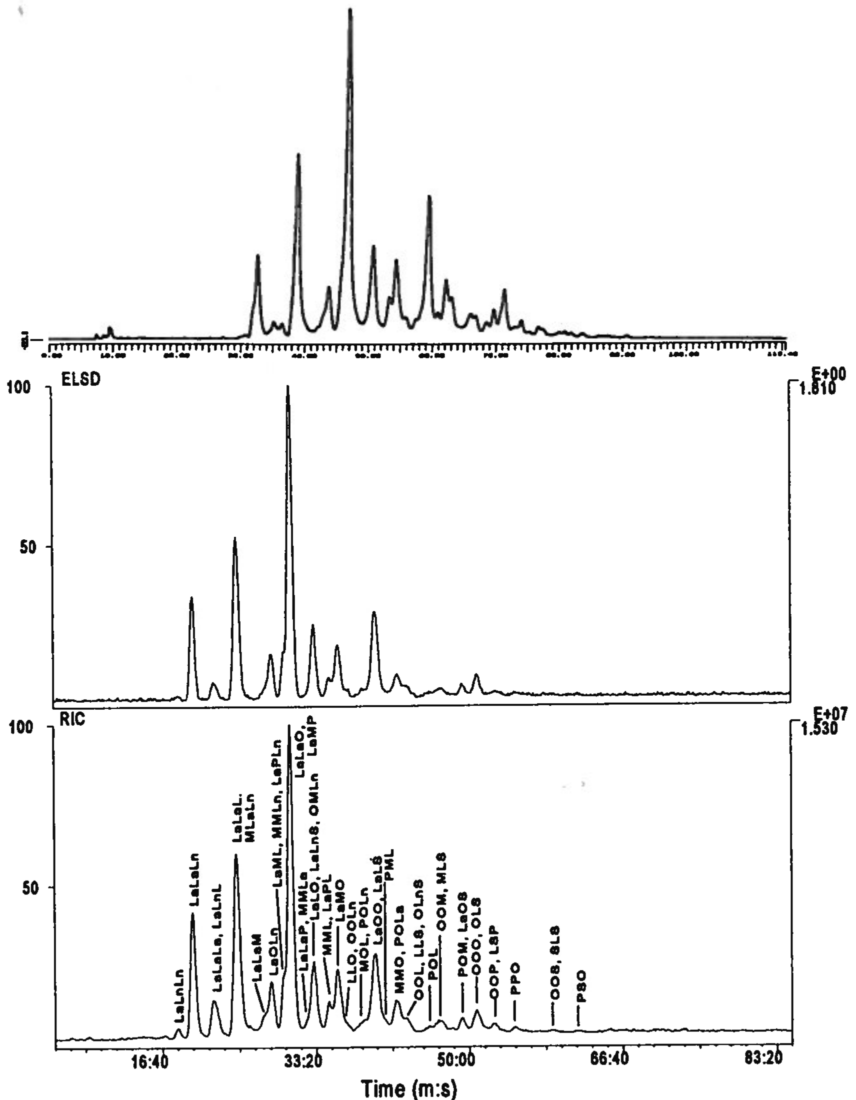

Figure 3. High lauric acid canola oil: reverse phase high performance liquid chromatography/flame ionization detection chromatogram (top), reverse phase high performance liquid chromatography/evaporative light scattering detector chromatogram (middle), reverse phase high performance liquid chromatography/atmospheric pressure chemical ionization mass spectrometric chromatogram (bottom). Liquid chromatographic conditions in text. Abbreviations: La=lauric; M=myristic; other fatty acids as in Figure 1.

Table 3

High Lauric Acid TAG Percent Composition

Triglyceride			Raw	Adj.	LC-FID%
La	La	O	25.4	21.2	27.6
La	O	O	8.4	11.2	11.2
La	La	L	15.2	10.8	14.2
La	La	Ln	8.8	7.5	7.4
La	M	O	5.0	5.1	5.6
La	L	O	3.8	4.3	6.6
La	P	O	2.8	3.3	3.6
La	Ln	O	2.4	3.3	4.0
O	O	O	1.5	3.2	3.3
La	M	L	3.1	2.7	2.0
L	O	O	1.2	2.1	2.1
La	P	L	1.9	1.9	
La	L	L	1.7	1.7	
La	M	Ln	1.6	1.6	
La	La	La	2.6	1.4	
La	Ln	L	1.1	1.3	2.3
P	O	O	0.6	1.2	0.7
M	O	O	0.7	1.2	1.3
La	P	Ln	0.9	1.0	
L	L	O	0.6	1.0	
La	O	S	0.7	0.9	1.4
M	L	O	0.6	0.8	
Ln	O	O	0.4	0.8	
P	L	O	0.5	0.8	
L	O	S	0.4	0.6	
La	Ln	Ln	0.4	0.6	0.1
La	O	20:1	0.7	0.6	
La	L	S	0.6	0.6	
M	Ln	O	0.3	0.5	
Ln	L	O	0.3	0.5	
O	O	S	0.2	0.4	
La	La	M	0.6	0.4	
P	Ln	O	0.2	0.3	
M	P	O	0.2	0.3	
M	L	L	0.2	0.3	
La	La	P	0.3	0.2	
M	M	O	0.2	0.2	

Table 3 (continued)

High Lauric Acid TAG Percent Composition

Triglyceride			Raw	Adj.	LC-FID%
P	Ln	L	0.1	0.2	
La	L	20:1	0.3	0.2	
P	P	O	0.1	0.2	
M	Ln	L	0.1	0.2	
M	P	L	0.1	0.2	
P	L	L	0.1	0.2	
	Other		3.3	3.6	6.6
	Sum		100.0	100.0	100.0

Table 4

High Lauric Acid Canola Oil Fatty Acid Composition

FA	Raw LC/MS %	Adj. LC/MS	LC/FID %	GC-FID %
La	48.6	41.9	48.0	38.7
M	4.7	5.0	3.2	4.6
Po	0.2	0.2	0.0	0.2
P	2.8	3.6	1.7	3.2
Ln	6.1	6.8	4.9	7.9
L	11.8	11.5	9.7	12.8
O	24.1	29.4	31.7	30.8
S	1.0	1.2	0.9	1.1
20:1	0.7	0.6	0.0	0.6
A	0.0	0.0	0.0	0.3
Sum	100.0	100.0	100.0	100.0
AARE%	15.3	10.1	20.6	0.0

A peak, at the same retention time in the EIC corresponding to the protonated triglyceride mass, also added support for this identification. Each TAG was identified based on concurrent appearance of peaks in several EICs at the same retention time. The TAG percent compositions obtained by LC/APCI-MS and LC-FID are shown in Table 3. It was clear, that significantly fewer TAG were identified by LC-FID than by LC/APCI-MS. The FA composition

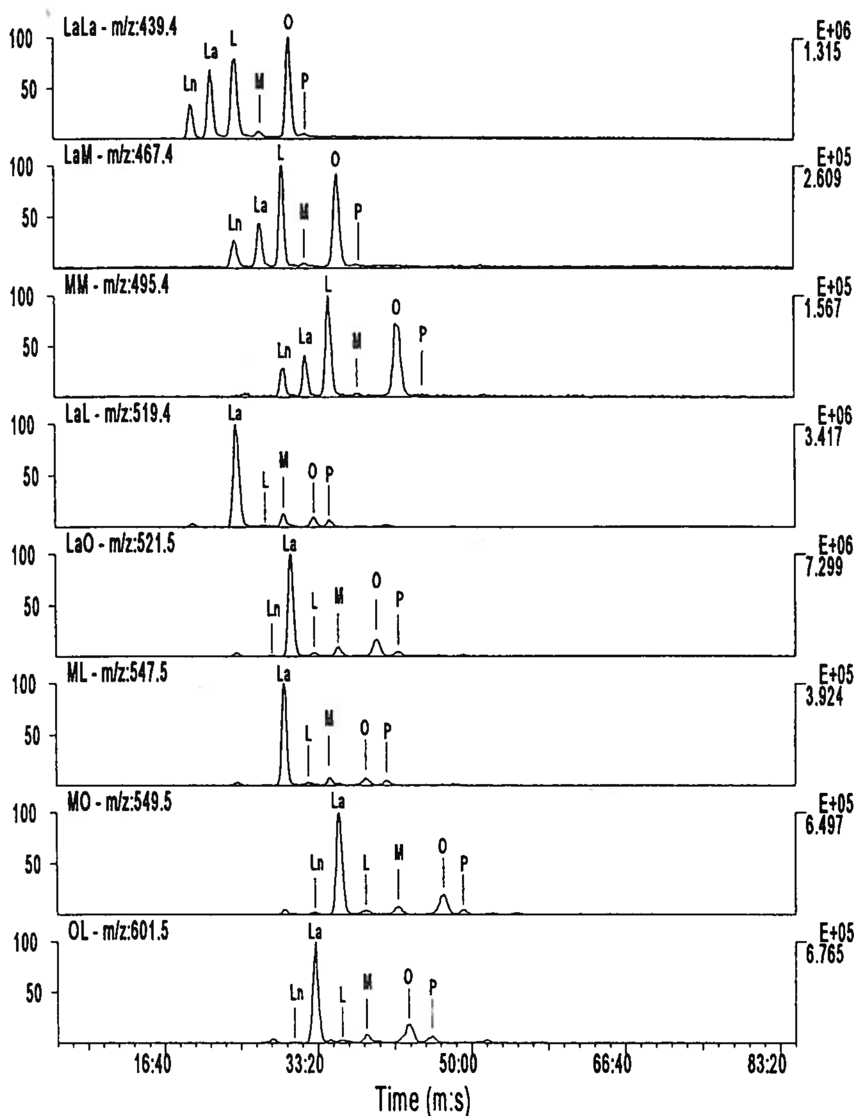

Figure 4. High lauric acid canola oil: extracted ion chromatograms of diacylglycerol fragments. Diacylglycerol identity and mass indicated on the left in each frame. Chromatograms are labeled with the fatty acid which, combined with the diacylglycerol, make up triacylglycerol species. Abbreviations as in Figure 3.

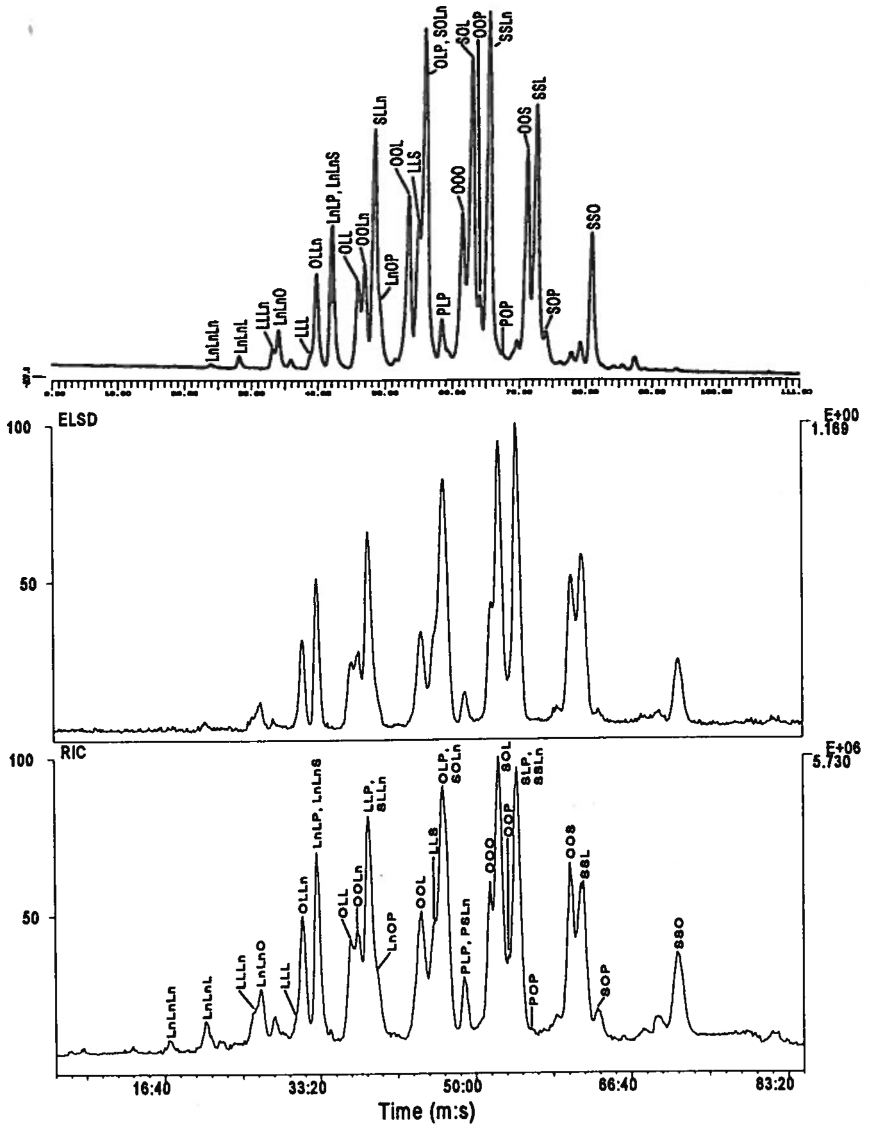

Figure 5. High stearic acid canola oil: reverse phase high performance liquid chromatography/flame ionization detection chromatogram (top), reverse phase high performance liquid chromatography/evaporative light scattering detector chromatogram (middle), reverse phase high performance liquid chromatography/atmospheric pressure chemical ionization mass spectrometric chromatogram (bottom). Liquid chromatographic conditions in text. Abbreviations as in Figure 1.

determined by calibrated GC-FID, and the FA compositions calculated from the TAG compositions, are given in Table 4. As with the normal canola oil, response factors were calculated for each TAG from the FA composition determined by GC-FID. The normalized FA response factors, calculated as the ratio of the GC-FID percent to the LC/APCI-MS percent, were: La = 1.00862; M = 1.24213; Po = 1.15609; P = 1.43088; Ln = 1.64047; L = 1.37635; O = 1.61944; S = 1.40003; 20:1 = 1.00000. The TAG composition obtained by application of the TAG response factors derived form the FA response factors is givn in column 3 of Table 3. The FA composition, which resulted from the GC-adjusted TAG composition, is given in column 3 of Table 4. The FA composition, obtained from the GC-adjusted TAG composition, showed good agreement with the FA composition obtained by GC-FID, and the least AARE compared to the FA composition calculated from the raw LC/APCI-MS, or the LC-FID TAG compositions. The GC-adjusted FA composition had a 10.1 % average relative error, compared to the composition determined by GC-FID. The FA composition calculated from the raw LC/APCI-MS TAG composition, showed better agreement (AARE = 15.3%) with the GC-FID results than did the FA composition calculated from the LC-FID TAG composition (AARE=20.6%).

High Stearic Canola Oil

Chromatograms of high stearic acid-containing canola oil obtained by LC/APCI-MS, ELSD and LC-FID are shown in Figure 5. Extracted ion chromatograms for high stearic acid canola oil are shown in Figure 6. When compared to the normal canola oil chromatograms in Figure 1, the chromatograms of the high stearic acid canola oil distinctly show more large peaks eluting at longer retention times. This was expected for TAG containing mono- and unsaturates, in this chromatographic system. Larger peaks for oleic and stearic acid- containing TAG indicated the larger amounts of these TAG present in this canola variety. The TAG composition determined from the LC/APCI-MS and by LC-FID are given in Table 5. Comparison of this composition to that of normal canola oil in Table 1, showed the increased amounts of oleic and stearic acid-containg TAG achieved in this genetically modified variety. Among the most abundant TAG, Table 5 showed larger amounts of TAG which contain the diacylglycerols OS (oleoylsteroylglycerol), OO (dioleoylglycerol or diolein), and SS (distearoylglycerol or distearin) than normal canola. The fatty acid composition obtained by GC-FID is given in Table 6, column 5. The FA compositions calculated from the LC/APCI-MS and LC-FID data are given in columns 2 and 4 of Table 6, respectively. The LC/APCI-MS TAG composition resulted in a FA composition which had 72.5 % average absolute relative error per FA. Most of this average relative error came from two of the FA, Po and 20:1, present at below 0.5 %. Excluding these two FA from calculation of the average relative error, led to an average absolute

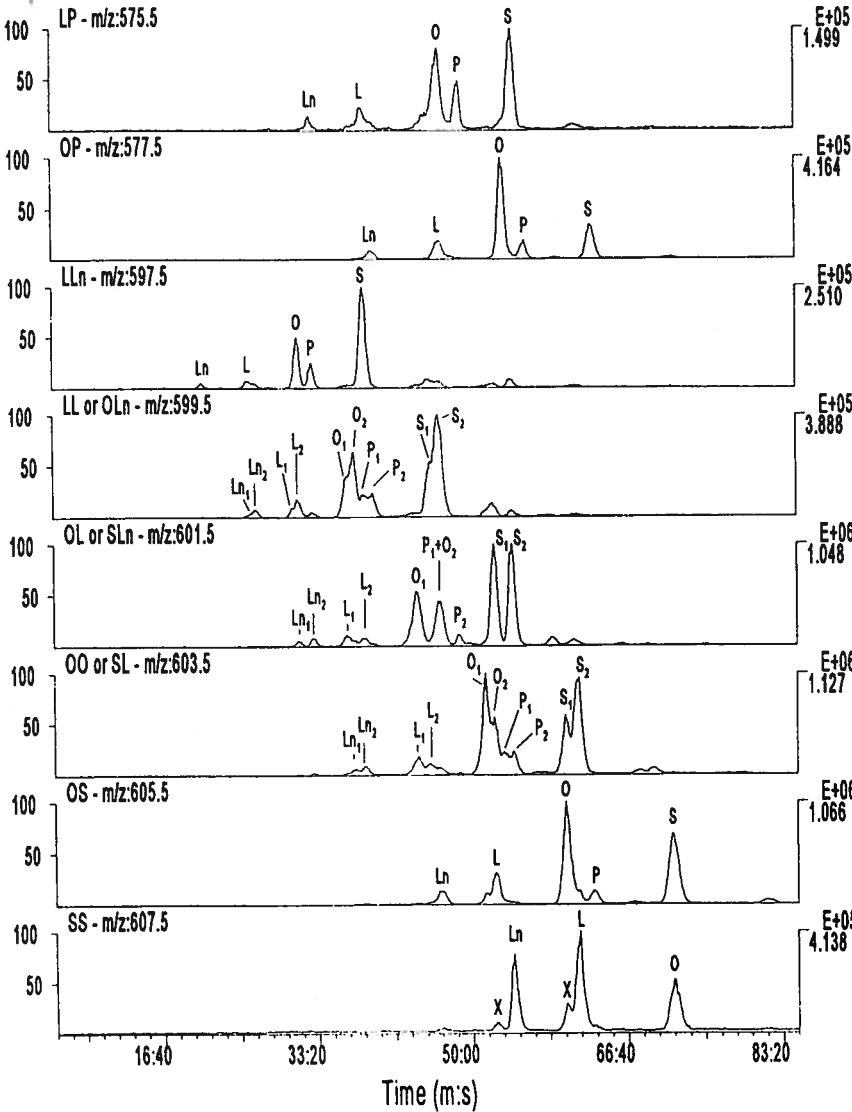

Figure 6. High stearic acid canola oil: extracted ion chromatograms of diacylglycerol fragments. Diacylglycerol identity and mass indicated on the left in each frame. Chromatograms are labeled with the fatty acid which, combined with the diacylglycerol, make up triacylglycerol species. Abbreviations as in Figure 1.

relative error of 11.7 % per FA, much closer to that given by the LC-FID data. The TAG composition determined by LC-FID resulted in a FA composition which had an average relative error of 13.2 % per FA. As with the other canola oils, response factors were calculated for each TAG from the FA composition determined by GC-FID. The normalized FA response factors, calculated as the ratio of the GC-FID percent to the LC/APCI-MS percent, were: Po = 1.00000; P = 1.51979; Ln = 2.10244; L = 1.81170; O = 1.70925; S = 1.94573; 20:1 = 0.34708; A = 1.48662. The TAG composition which resulted from application of TAG response factors derived from the FA response factors, is given in column 3 of Table 5. The TAG composition, thus obtained, resulted in a calculated FA composition which was in excellent agreement with the FA composition obtained by GC-FID. The FA composition from the GC-adjusted TAG composition, had only 3.3 % average relative error per FA. If the error of the FA 20:1, present at below 0.1%, is omitted, then the average relative error for the FA composition is 0.7 % AARE per FA, representing excellent agreement with the FA composition obtained by GC-FID.

DISCUSSION

The chromatograms for normal canola oil were in good agreement with the LC-FID chromatogram published earlier,[12] although we were able to identify more TAG species than was previously possible. The extracted ion chromatograms shown in Figures 2, 4 and 6, were examples of the ability of the APCI-MS technique to qualitatively identify TAG species present even in low quantities. The capability for qualitative identification which LC/APCI-MS allowed, was of utmost importance in analysis of samples containing numerous components for which no commercially available standards were available, such as the high lauric canola oil. Even if chromatographic standards for each TAG were available, LC-FID still was incapable of providing the resolution possible with the LC/APCI-MS system. The ability to differentiate coeluting masses, allowed the LC/APCI-MS system to unambiguously identify many more species than was possible with the LC-FID system alone.

Calculation of the FA composition from the TAG composition gave us the ability to compare results obtained by APCI-MS and LC-FID to results obtained by calibrated GC-FID. We found that the uncorrected LC/APCI-MS composition obtained for a TAG mixture gave a FA composition which had an average relative error which was larger than, or similar to, the error exhibited by the FA composition calculated from the LC-FID results. In the case of all three oil samples studied here, we found that response factors could be calculated in a simple, straightforward manner. These gave TAG compositions for which the calculated FA compositions were in excellent agreement with the experimentally determined FA compositions. The FA compositions calculated

Table 5

High Stearic Acid Canola Oil TAG Composition

Triglyceride			Raw LC/MS	Adj. LC/MS	LC-FID%
L	O	S	14.2	14.0	11.1
Ln	O	S	9.8	11.2	11.6
O	O	S	9.2	8.5	7.8
Ln	S	S	4.8	6.2	12.7
Ln	L	S	5.0	6.1	2.4
O	S	S	4.9	5.1	5.5
L	S	S	4.4	5.0	11.8
Ln	L	O	4.3	4.6	1.3
L	O	O	4.9	4.2	4.1
Ln	O	O	3.6	3.6	6.6
Ln	Ln	S	2.2	3.1	2.3
L	L	S	2.6	2.7	3.1
L	L	O	2.6	2.4	1.4
P	L	S	2.0	1.7	0.0
O	O	O	2.0	1.7	3.9
Ln	Ln	O	1.3	1.6	0.7
P	O	O	2.2	1.6	2.4
P	Ln	O	1.7	1.6	1.4
P	L	O	1.6	1.3	1.1
P	O	S	1.5	1.2	1.4
P	Ln	S	1.2	1.2	0.0
Ln	L	L	0.9	1.0	0.2
Ln	S	A	0.9	0.9	0.0
P	Ln	L	0.9	0.9	0.6
L	S	A	1.0	0.8	0.0
Ln	Ln	L	0.6	0.7	0.2
Ln	O	A	0.8	0.7	0.0
L	O	A	1.0	0.7	0.0
Ln	L	A	0.7	0.6	0.0
O	S	A	0.7	0.5	0.0
P	L	L	0.6	0.5	0.0
P	Ln	Ln	0.5	0.5	0.2
O	O	A	0.7	0.5	0.0
Ln	Ln	A	0.4	0.4	0.0
L	L	L	0.4	0.4	0.6
Ln	Ln	Ln	0.2	0.3	0.1

(continued)

Table 5 (continued)

High Stearic Acid Canola Oil TAG Composition

Triglyceride			Raw LC/MS	Adj. LC/MS	LC-FID%
L	L	A	0.3	0.2	0.0
P	P	L	0.3	0.2	1.0
P	P	O	0.3	0.2	0.2
	Other		3.2	1.4	4.1
	Sum		100.0	100.0	100.0

Table 6

High Stearic Acid Canola Oil Fatty Acid Composition

FA	Raw LC/MS %	GC-Adj. %	LC-FID %	GC-FID %
Po	0.4	0.2	0.0	0.2
P	4.8	4.0	5.8	4.0
Ln	15.5	17.6	15.0	17.8
L	18.9	18.6	15.0	18.7
O	31.0	29.1	29.8	29.0
S	26.4	28.3	34.4	28.1
20:1	0.5	0.1	0.0	0.1
A	2.5	2.0	0.0	2.0
Sum	100.0	100.0	100.0	100.0
AARE%	72.5	3.3	13.2	0.0

after application of GC-FID-derived response factors showed significantly less average relative error than the uncorrected data. The effectiveness of this approach was shown by the FA composition determined for the high lauric canola oil, Table 4. Both the LC/APCI-MS data and the LC-FID data resulted in percentages of lauric acid which were ~10 % too high. This clearly indicated an over-response for lauric acid when using these detectors. This over-response was expected for the LC/APCI-MS system, in which lower molecular weight TAG were propagated through the system with more efficiency. The LC-FID detector, which depended on the carbon content of the TAG, should have given more response for those TAG which contained longer carbon chains, rather than shorter ones. In all cases, the FA composition calculated from the GC-Adjusted

LC/APCI-MS TAG composition agreed with the experimentally determined FA composition better than the FA compositions calculated from the raw LC/APCI-MS or LC-FID.

The TAG and FA results for the oils clearly displayed the changes in composition which were desired for these oils. For instance, the high lauric canola oil clearly contained numerous lauric acid-containing TAG which had a distinct effect on the physical characteristics of the oil. We found that, not only was the high lauric acid variety high in the desired lauric acid, but myristic acid was a major product of this genetic modification, as well. The APCI-MS EICs for the high lauric canola oil indicate that myristic acid behaves very similarly to lauric acid in the formation of TAG from FA. Furthermore, from the EICs we determined that there was a definite tendency for lauric acid and myristic acid to appear together as LaLa, LaM, or MM, even though myristic acid is present in a lower percentage than other FA. The different appearance of the EICs for these three diacylglycerols compared to the EICs for the other DAG revealed a preference for the combination of these FA. The LaLa, LaM, and MM diglycerides form triglycerides primarily with linoleic and oleic acids.

The TAG and FA compositions for high stearic canola oil clearly indicated success in the formation of more stearic acid-containing TAG. The FA percent rose from 1.6 % stearic acid in normal canola oil to 28.1 % in high stearic canola oil. The oleic acid percent was still high, as in normal canola oil, so that the most abundant TAG contained the OS, OO, and SS diacylglycerols. TAG containing these DAG are known to be more oxidatelvely stable than TAG which have a higher unsaturation or polyunsaturation content. The higher stearic content also changed the physical characteristics of the canola oil. The higher content of saturates in this CNO would mean a higher melting point, different mouthfeel, and, as mentioned, greater oxidative stability.

It has been demonstrated that LC/APCI-MS was an excellent tool for unambiguous qualitative identification of numerous TAG species, for which no standards were available and which occurred chromatographically overlapped with other TAG components. It has also been demonstrated that, using the FAME composition determined by GC-FID, TAG response factors were calculated from LC/APCI-MS data which allowed quantitation of TAG components with less average relative error than was obtained by LC-FID results. The relative error in the FA composition obtained by APCI-MS was as low as 0.7 % per FA when minor FA were ignored. It was found, that LC-FID was sufficient to identify the major TAG components of the canola oils when the TAG identities were already known, and that quantitation using LC-FID gave a FA composition which had 11 % to 20 % average relative error compared to the FA composition obtained by calibrated GC-FID. We have shown, that the changes in TAG composition which were induced by genetic modification can be qualitatively and quantitatively analyzed regardless of the

availability of standards. This facile and straightforward method of analysis represents an important technique to complement other analytical and organoleptic methods for seed oil analysis.

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