Identification of Triacylglycerol Species from High-Saturated Sunflower (*Helianthus annuus*) Mutants

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The triacylglycerol (TAG) composition of oils from new high-saturated sunflower lines has been studied by means of GLC. The TAG profiles have been compared with the TAG reconstruction made after lipase hydrolysis (according to the 2-random 1,3-random theory). New TAG species with asclepic (*cis*,Δ11-octadecenoic acid, isomer of oleic acid), araquidic, or behenic acids have been synthesized and identified in oils from mutant lines. The TAG molecular species that contain asclepic acid instead of oleic acid have a longer retention time. Because each mutant oil has a specific TAG GLC pattern, this method could be used for a more precise validation of oil type than current fatty acid methyl ester analysis. The comparison of the results obtained by GLC with the reconstruction after pancreatic lipase hydrolysis shows, in general, a good agreement between both methods. However, results shown in this paper show that this is not always the case. TAG species containing two molecules of linoleic acid show a higher presence of palmitic or stearic acid than could be expected from a random distribution. The abundance of SLL increased in proportion to the stearic acid content of the oil, and the amount of TAG species with three unsaturated fatty acids (LLL or OLL) was therefore reduced.

Keywords: *Helianthus annuus; germination; saturated fatty acids; seed; sunflower; triacylglycerol*

INTRODUCTION

Triacylglycerols (TAG) are the main components of vegetable oils, accounting for almost 100% of the weight. Each oil has a characteristic pattern of TAG, and the physical and chemical properties of a particular oil are determined mainly by the abundance of different TAG molecular species. The formation of TAG in oilseeds shows some acyl selectivity. In vitro characterization of *sn*-glycerol 3-phosphate acylation in safflower seeds has shown that the acylation of position *sn*-2 has a strong preference for unsaturated fatty acids, saturated fatty acids being completely excluded from this position (Griffiths et al., 1985). Whereas the acylation of position *sn*-1 in safflower has selectivity for saturated fatty acids, position *sn*-3 appears not to have special selectivity (Ichihara and Noda, 1982). The possibility that the presence of two specific acyl moieties in positions 1 and 2 could influence the acyl moiety selected for position 3 has been considered previously (Santinelli and Christie, 1992). Stereospecific analysis of crude sunflower oil revealed that palmitic and stearic acids showed preferences for the *sn*-3 position over the *sn*-1 position, this being more evident in oils with increased amounts of saturated fatty acids (Reske et al., 1997). Thus, the "2-random 1,3-random" theory (Vander Wal, 1960) is not always applicable.

In recent years several new vegetable oils with modified fatty acid compositions have been obtained. Oils with increased amounts of monoenoic fatty acids, such as oleic acid (80−90%), have been obtained from sunflower (Soldatov, 1976) and soybean (Kinney, 1996). Similarly, oils with increased saturated fatty acid contents have also been obtained: 25−30% palmitic acid in sunflower oil (Ivanov et al., 1988; Osorio et al., 1995) and 25−30% stearic acid in sunflower (Osorio et al., 1995) and rapeseed oils (Knutzon et al., 1992). We have shown that the high-palmitic sunflower mutant described in Osorio et al. (1995) has detectable amounts of unusual fatty acids such as palmitoleic (*cis*,Δ9-hexadecenoic acid) and asclepic (*cis*,Δ11-octadecenoic acid) acids, as well as a fatty acid not previously reported in cultivated oilseeds, palmitolinoleic (*cis*,Δ9,12-hexadecadienoic acid) acid (Martínez-Force et al., 1999).

As a consequence of the modification of the fatty acid composition in high-palmitic or high-stearic mutants, the TAG molecular species pattern is also modified. Not only does the relative abundance of the TAG molecules change, but also new species of TAG should be found in these oils. The TAG molecular species found in these oils are due to the available fatty acids and the specificity of the TAG biosynthetic enzymes. This new pattern of TAG can be used as a fingerprint of the oil.

In this work, we identified the TAG molecular species of oils obtained from different sunflower mutant lines with increased amounts of palmitic or stearic acids in linoleic or high-oleic backgrounds. Purified TAG were analyzed by GLC and compared to the results obtained when TAG molecular species were calculated after pancreatic lipase analysis according to the hypothesis of the 2-random 1,3-random fatty acid distribution (Vander Wal, 1960). Some new TAG molecular species and the preference by certain molecules for stearic acid were also identified.

MATERIALS AND METHODS

Plant Material. Sunflower (*Helianthus annuus* L.) seeds from mutant lines with high stearic acid content (CAS-3 and CAS-4) or high palmitic acid content (CAS-5 and CAS-12) were

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used in this work (Osorio et al., 1995; Fernández-Martínez et al., 1997). Line CAS-12 exhibits a high-oleic phenotype having <5% linoleic acid. The control seeds were from public lines RHA-274, with normal fatty acid content, and BSD-2-423, with high oleic acid content.

TAG Purification. Twelve mature seeds were shelled and then ground in a screw-cap glass tube (10 × 13 mm) with a pestle and sand. Total lipids were extracted (Hara and Radin, 1978) and TAG purified in two ways: (i) Thin-layer chromatography (TLC) was carried out on silica gel plates, thickness = 0.25 mm, developed with hexane/ethyl ether/formic acid (75:25:1, by volume). To detect TAG position, TLC plates were partially covered with a glass plate and exposed to iodine vapors; unexposed TAG fractions were scrapped off the plates and eluted from silica with hexane/ethyl ether (95:5 by volume). (ii) Samples were run through activated (110 °C, 30 min) silica−Celite (80:20) columns, and TAG were eluted with hexane/ethyl ether (95:5 by volume).

Oil samples from two plants were used for each TAG analysis. In each case, samples of purified TAG were analyzed both by GLC and by lipase hydrolysis. Data presented are the average of two determinations, the standard deviation (SD) being <10% of the mean value.

Lipase Hydrolysis. For TAG fatty acid positional analysis, 10 mg of purified TAG was hydrolyzed with 2 mg of pancreatic lipase (Mancha and Vázquez, 1970) in 1 mL of 1 M Tris-HCl buffer (pH 8), with 0.1 mL of $CaCl_2$ (22%) and 0.25 mL of deoxycholate (0.1%). After ~60% of TAG had been hydrolyzed (1−2 min), the reaction was stopped by adding 0.5 mL of 6 N HCl. The lipids were extracted three times with 1.5 mL aliquots of ethyl ether. The reaction products were separated by TLC (see above). Free fatty acids and *sn*-2-monoacylglycerol bands representing, respectively, the positions *sn*-1,3 and *sn*-2 of TAG, were scrapped off the plate and transmethylated (see below). The procedure was checked by comparing the fatty acid composition of the original TAG and those remaining after the partial hydrolysis. TAG molecular species were calculated according to the hypothesis of the 2-random 1,3-random fatty acid distribution (Vander Wal, 1960) using a macro worksheet for Microsoft Excel 97 developed by us (available at http://www.cica.es/aliens/glsoig/).

TAG Analysis by GLC. TAG species were separated and quantified using a Hewlett-Packard 6890 gas chromatograph (Palo Alto, CA) with a Quadrex aluminum-clad bonded methyl 65% phenyl silicone 15M, 400-65HT-15-0.1F column (New Haven, CT) and quantified by a hydrogen flame ionization detector (FID). TAG were identified and data corrected for the relative response of the FID according to the method of Carelli and Cert (1993). After a 5 min hold, the oven temperature was ramped from 340 to 355 °C at 1 °C/min, detector and injector temperatures were 400 °C, and the split ratio was 1:100. Hydrogen was used as carrier gas, the linear rate being 31 cm/s.

Lipid Analysis. Fatty acid methyl esters were obtained from isolated lipids (Garcés and Mancha, 1993) by heating the samples at 80 °C for 1 h in 3 mL of methanol/toluene/H_2SO_4 (88:10:2, by volume). After cooling, 1 mL of heptane was added and the samples were mixed. The fatty acid methyl esters were recovered from the upper phase and then separated and quantified using a Hewlett-Packard 5890A gas chromatograph (Palo Alto, CA) with a Supelco SP-2380 capillary column (30 m length; 0.32 mm i.d.; 0.20 μm film thickness) of fused silica (Bellefonte, PA). Hydrogen was used as carrier gas, the linear gas rate being 28 cm/s. Detector and injector temperatures were 200 °C, oven temperature was 170 °C, and the split ratio was 1:50. Fatty acids were identified by comparison with known standards (Sigma).

TAG Synthesis. TAG were synthesized according to the method of Rios et al. (1992) with modifications. 1,2-Dipalmitoyl-3-oleoylglycerol (PPO), 1,2-dipalmitoyl-3-asclepoylglycerol (PPAs), triolein (OOO), 1,2-dioleoyl-3-asclepoylglycerol (OOAs), and 1,3-dioleoyl-2-asclepoylglycerol (OAsO) were synthesized from 14 mg of diacylglycerides (1,2-dipalmitin, 1,2- or 1,3-diolein) and 10 mg of fatty acid chlorides (oleoyl chloride or asclepoyl chloride). 1,2-Dioleoyl-3-araquidoylglycerol (OOA), 1,2-dioleoyl-3-behenoylglycerol (OOB), 1,2-dilinoleoyl-3-araquidoylglycerol (LLA), and 1,2-dilinoleoyl-3-behenoylglycerol (LLB) were synthesized from 14 mg of diacylglycerides (1,2-diolein or 1,2-dilinolein) and 10 mg of fatty acid chlorides (araquidoyl chloride or behenoyl chloride). Oleoyllinoleoylbehenoylglycerol (OLB) was synthesized from 14 mg of 1-monooleoin and 10 mg each of linoleoyl and behenoyl chloride. The reactions were carried out at 30 °C in the presence of pyridine/dichloromethane (7:25 by volume) for 2 h. All chemicals were purchased from Sigma.

Table 1. Fatty Acid Composition of TAG (Mole Percent) of Control and Mutant Sunflower Lines

	TAG fatty acid composition[a]								
	P	Po	Pl	S	O	As	L	A	B
RHA-274	7.4	−[b]	−	5.8	37.3	−	48.6	−	0.9
CAS-3	7.3	−	−	27.8	16.5	−	45.0	1.6	1.5
CAS-4	6.1	−	−	13.9	38.7	−	38.9	0.9	1.4
CAS-5	31.0	4.9	1.3	2.9	5.1	5.9	47.4	0.6	0.8
BSD-2-423	3.1	−	−	5.2	86.2	−	3.3	0.7	1.5
CAS-12	30.4	6.5	−	2.4	52.4	4.4	2.5	−	1.5

[a] A, araquidic acid; As, asclepic acid; B, behenic acid; L, linoleic acid; O, oleic acid; P, palmitic acid; Po, palmitoleic acid; Pl, palmitolinoleic acid; S, stearic acid. [b] − = <0.5%.

RESULTS AND DISCUSSION

Fatty Acid Composition of TAG. The fatty acid compositions of TAG in seeds from the genetically modified sunflower lines used in this study are given in Table 1. The TAG fatty acid compositions are similar to those found in their respective oils (Alvarez-Ortega et al., 1997). These oils have an increased content of saturated fatty acids. Lines CAS-3 and CAS-4 have more stearic acid, around 5 and 3 times, respectively, than normal sunflower lines; CAS-5 and CAS-12 have around 4 times more palmitic acid than the normal type. Additionally, line CAS-12 has a high-oleic background and <5% linoleic acid. Some unusual fatty acids appear in the high-palmitic lines, such as palmitoleic acid (16:1 Δ9), palmitolinoleic acid (16:2 Δ9,12), and asclepic acid (18:1 Δ11) (Martínez-Force et al., 1999). Palmitoleic and asclepic acids are found in normal sunflower and in most of the vegetable oils in a low concentration, 0.5−3% of total fatty acids. However, palmitolinoleic acid is not found in any normal vegetable oil (Gunstone et al., 1994). In previous work carried out with other high-palmitic acid sunflower mutants, palmitolinoleic and asclepic acids have not been reported in those seed oils (Ivanov et al., 1988; Reske et al., 1997). The synthesis of these fatty acids in our mutants is a side effect of the higher palmitic acid concentration inside the plastid (Martínez-Force et al., 1999). Thus, it is likely that palmitolinoleic and asclepic acids, although not reported, are also present in the other high-palmitic sunflower mutants.

TAG Analysis by GLC. As expected, the alterations in the fatty acid compositions of the mutant sunflower oils were reflected in their TAG GLC profiles (Figures 1 and 2). The separation of TAG species by GLC avoids the problem of the solubility of saturated TAG in some HPLC solvents. Additionally, this method exhibits great sensitivity and a good distribution of the chromatographic peaks. Each mutant line has a specific TAG profile completely different from that of normal or high-oleic control sunflower lines. The main variation in the TAG profile is due to the specific fatty acids showing altered abundance in each mutant (Tables 2−4). Most of the TAG species were identified on the basis of

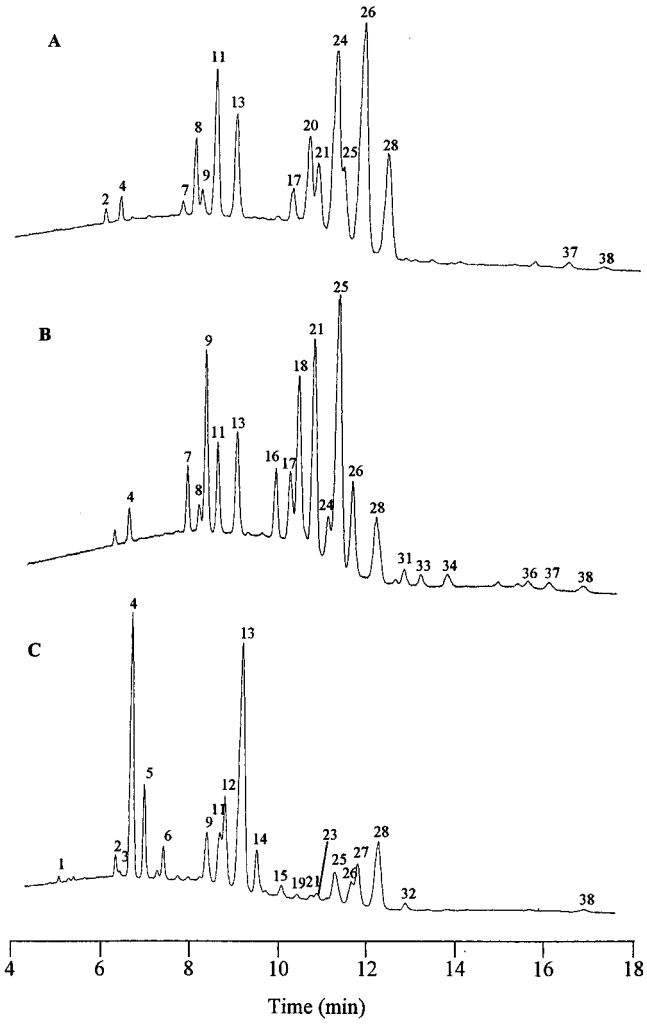

Figure 1. GLC chromatograms of the purified TAG from control RHA-274 (A) and mutant lines CAS-3 (B) and CAS-5 (C). Numbers were assigned to each TAG species as indicated in the tables.

previous analysis of normal and high-oleic sunflower oils (Carelli and Cert, 1993), but other peaks corresponded to previously unidentified TAG (1, 3, 5, 6, 10, 12, 14, 15, 18, 19, 22, 23, 27, 29, 31−34, and 36). Some peaks (3, 10, 12, 22, 23, and 27) appear after TAG with at least one oleic acid molecule, such as POP (2), POO (8), POL (11), OOO (20), SOL (21), OOL (24), and OLL (26). Other unidentified TAG must contain, according to their retention times, long-chain fatty acids in their molecules (29, 31−34, and 36).

Reconstruction of TAG Species after Lipase Hydrolysis. To identify these new TAG species, pancreatic lipase digestions of TAG from mutant lines were carried out and TAG molecular species reconstructed according to the 2-random 1,3-random theory (Vander Wal, 1960). These analyses predicted the appearance of some new TAG species in these oils, some of them containing the unusual fatty acids found in these mutants, such as PPoP, PPlL, PoLL, and PlLL in the high-palmitic mutants and SOS and SLS in the high-stearic mutants, among others. The expected GLC retention time of these predicted new TAG species, calculated from the number of carbons and double bonds of the TAG fatty acids (Carelli and Cert, 1993), matched those of several unidentified GLC peaks (1, 6, 14−16, and 18). Some of the predicted new TAG species had

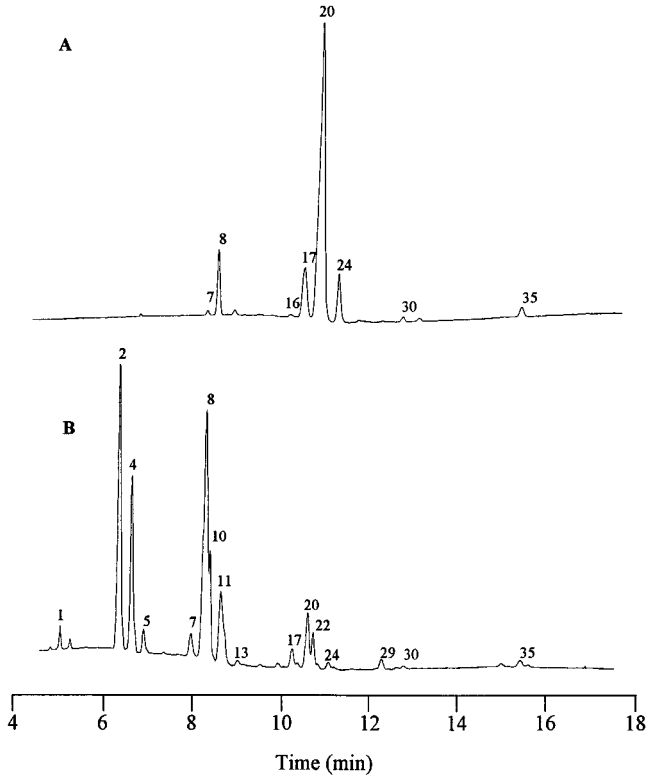

Figure 2. GLC chromatograms of the purified TAG from the high-oleic control line BSD-2-423 (A) and the high-palmitic−high-oleic mutant line CAS-12 (B). Numbers were assigned to each TAG species as indicated in the tables.

Table 2. TAG Molecular Species Composition of Normal (RHA-274), High-Stearic (CAS-3), and Medium-Stearic (CAS-4) Sunflower Seed Oils (Percent)[a]

		RHA-274		CAS-3		CAS-4	
IN[b]		lipase	GLC	lipase	GLC	lipase	GLC
2	POP	0.5	0.3	–[c]	–	–	–
4	PLP	0.7	0.7	0.8	0.8	–	–
7	POS	0.8	0.5	2.9	2.1	2.0	1.5
8	POO	3.4	3.0	1.1	1.0	3.8	3.3
9	PLS	1.1	1.2	5.9	6.8	2.0	2.1
11	POL	8.5	7.4	4.6	3.3	6.8	5.1
13	PLL	5.2	6.1	4.7	4.8	3.0	3.5
16	SOS	–	–	5.1	3.6	2.0	1.9
17	SOO	2.6	2.2	3.9	4.1	7.5	8.6
18	SLS	–	–	10.3	12.0	1.9	1.8
20	OOO	6.3	6.4	0.8	*d*	7.5	9.4
21	SOL	6.4	5.2	16.2	14.6	13.5	12.1
24	OOL	22.3	18.8	4.8	2.7	19.4	15.7
25	SLL	3.9	5.2	16.8	24.6	6.0	9.1
26	OLL	26.5	26.8	10.0	6.5	16.8	15.3
28	LLL	10.4	14.8	7.0	7.6	4.9	8.0
31	SLA	–	–	1.0	1.1	–	–
33	OLA	–	–	0.8	0.8	1.3	0.7
34	LLA	–	–	0.8	1.2	–	–
35	OOB	–	–	–	–	0.7	0.9
36	SLB	–	–	1.0	0.7	–	–
37	OLB	0.9	0.5	0.7	0.8	1.2	1.1
38	LLB	0.5	0.6	0.8	0.9	–	–

[a] Fatty acid position on TAG could not be determined by this method. [b] IN, identification number. [c] – = not detected. [d] OOO was not separated from SLS; the above value represents the sum of both TAG species. (These peaks were not separated and are included in the upper identification number.)

retention times similar to those of known peaks identified from control sunflower oils by GLC (4, 11, 13, and 25). For example, PPoO and PPlO have the same retention time as PLP in the high-palmitic−high-oleic

Table 3. TAG Molecular Species Composition of Normal (RHA-274) and High-Palmitic (CAS-5) Sunflower Seed Oils (Percent)[a]

		RHA-274		CAS-5	
IN[b]		lipase	GLC	lipase	GLC
1	PPoP	–[c]	–	0.6	0.2
2	POP	0.5	0.3	1.3	0.8
3	PAsP	–	–	0.3	0.2
4	PLP	0.7	0.7	15.9	14.5
5	PPoL	–	–	5.1	4.0
6	PPlL	–	–	1.5	1.7
7	POS	0.8	0.5	–	–
8	POO	3.4	3.0	–	–
9	PLS	1.1	1.2	4.2	4.0
11	POL	8.5	7.4	5.2	3.9
12	PAsL	–	–	5.9	6.9
	PoLS	–	–	0.7	*d*
13	PLL	5.2	6.1	25.9	28.9
	PoOL	–	–	0.8	*d*
	PoAsL	–	–	1.0	*d*
14	PoLL	–	–	3.9	3.7
15	PlLL	–	–	1.2	1.1
17	SOO	2.6	2.2	–	–
19	PLA	–	–	0.5	0.2
20	OOO	6.3	6.4	–	–
21	SOL	6.4	5.2	0.7	0.5
23	SAsL	–	–	0.8	0.8
24	OOL	22.3	18.8	–	–
25	SLL	3.9	5.2	3.3	4.9
	OAsL	–	–	0.9	*d*
26	OLL	26.5	26.8	3.4	2.8
27	AsLL	–	–	5.0	5.6
28	LLL	10.4	14.8	10.7	13.6
32	PLB	–	–	0.8	0.9
37	OLB	0.9	0.5	–	–
38	LLB	0.5	0.6	0.7	1.0

[a] Fatty acid position on TAG could not be determined by this method. [b] IN, identification number. [c] – = not detected. [d] These peaks were not separated and are included in the upper identification number.

line (CAS-12). To completely match the predicted TAG lipase profile with the real GLC profile, we need to assume that TAG molecules with one asclepic acid instead of oleic acid must have a longer retention time. This would imply that it is possible to separate TAG species only because they have a different positional isomer of the oleic acid. The rest of the unidentified peaks, as stated above, should contain long-chain fatty acids.

Analysis of Synthesized TAG Species. To test the hypothesis of the influence of asclepic acid in the retention time of corresponding TAG and to identify the TAG species with long-chain fatty acids, we carried out the synthesis of several TAG. Two groups of TAG were synthesized (in this case the fatty acid position in the TAG molecule is as indicated under Materials and Methods): (i) TAG molecules differing in the substitution of one oleic acid by asclepic acid (POP and PAsP; OOO, OOAs, and OAsO) with the latter in different positions to test whether the position of As in the TAG molecule was relevant (OOAs and OAsO); and (ii) TAG molecules with long-chain (araquidic and behenic) fatty acids (OOA and OOB; LLA, LLB, and OLB). The previously unidentified peaks in oils from mutant lines were identified both by comparison with the GLC retention time of the synthesized TAG species (Figure 3) and by mixed injections of samples from mutant lines with the synthesized TAG species.

The results obtained confirmed our hypothesis with respect to the identification of TAG species containing asclepic acid. The comparison of the GLC profiles of POP

Table 4. TAG Molecular Species Composition of High-Oleic (BSD-2-423) and High-Palmitic in High-Oleic (CAS-12) Background Sunflower Seed Oils (Percent)[a]

		BSD-2-423		CAS-12	
IN[b]		lipase	GLC	lipase	GLC
1	PPoP	–[c]	–	0.8	0.9
2	POP	–	–	17.8	19.4
3	PAsP	–	–	0.8	*d*
4	PLP	–	–	1.0	11.5
	PPoO	–	–	9.0	*d*
	PPlO	–	–	0.8	*d*
5	PPoL	–	–	0.3	1.5
	PoPoO	–	–	1.1	*d*
7	POS	0.7	0.4	2.8	2.4
8	POO	7.4	6.4	26.8	30.3
10	POAs	–	–	5.5	5.6
	PoOS	–	–	0.7	*d*
11	POL	–	–	3.3	10.1
	PoOO	–	–	6.5	*d*
	PoOAs	–	–	1.4	*d*
13	PoOL	–	–	0.8	0.8
16	SOS	0.6	0.5	–	–
17	SOO	11.3	11.7	2.0	2.5
20	OOO	69.4	70.9	10.3	6.9
21	SOL	0.7	*d*	–	–
22	OOAs	–	–	3.7	3.9
24	OOL	6.7	6.6	2.1	1.3
29	POB	–	–	1.3	1.3
30	OOA	0.8	0.9	–	–
35	OOB	2.4	2.5	1.0	1.5

[a] Fatty acid position on TAG could not be determined by this method. [b] IN, identification number. [c] – = not detected. [d] These peaks were not separated and are included in the upper identification number.

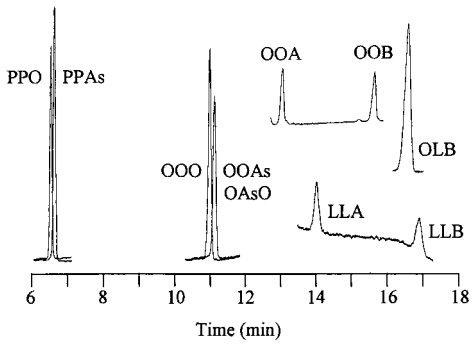

Figure 3. GLC peaks and retention times of the synthesized TAG species.

and PAsP, and also those of OOO, OOAs, and OAsO, show, as predicted, a higher retention time for the TAG containing asclepic instead of oleic acid. However, the fatty acid position *sn*-1,3 or *sn*-2 was not relevant. The retention times for PAsP and OOAs were the same as those of unknown peaks 3 and 22, respectively. Additionally, these retention time data allowed us to identify several other unidentified peaks that appeared inmediately after peaks of TAG containing oleic acid: POAs (10), PoOAs (11), PAsL (12), PoAsL (13), SAsL (23), OAsL (25), and AsLL (27).

The data obtained from synthesized TAG containing at least one molecule of arachidic or behenic acids allowed us to identify new TAG with longer retention times: POB (29), SLA (31), PLB (32), OLA (33), LLA (34), and SLB (36).

Comparative Studies. Once all of the GLC peaks corresponding to TAG molecules with content >0.5% of the total were identified, their relative abundances were calculated from the GLC analysis according to the relative response of the FID (Carelli and Cert, 1993).

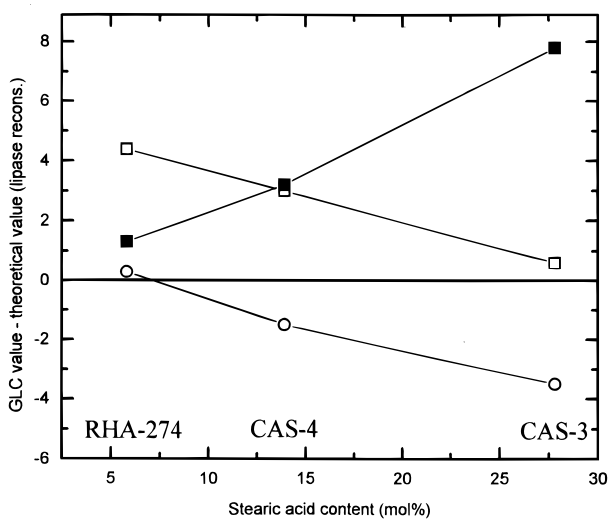

Figure 4. Relationship between the TAG stearic acid content of lines RHA-274, CAS-4, and CAS-3 and the difference between the mean abundances observed by GLC analysis and the theoretical values calculated from the lipase analysis for TAG species SLL (■), OLL (○), and LLL (□).

TAG from mutant lines had specific GLC profiles with increased amounts of TAG species containing one or two saturated fatty acids (Tables 2−4). Whereas in normal sunflower the major TAG found are OLO and OLL, in the high-stearic acid mutant (CAS-3), the major TAG found were SLL, SLO, and SLS. The more abundant TAG in the high-palmitic acid mutant (CAS-5) were PLL and PLP. In the high-palmitic/high-oleic acid mutant (CAS-12), POO and POP were the more abundant TAG.

We have found that the actual GLC profiles of TAG matched closely the theoretical calculations made from the lipolysis data, thus reinforcing the applicability of the theoretical calculations for the identification of the main components of the chromatographic peaks in oils complying with a 2-random 1,3-random distribution (Vander Wal, 1960). In oils with a high proportion of saturated fatty acids, as in the high stearic acid mutant CAS-3, there is some discrepancy. This could be due to the asymmetric distribution of saturated fatty acids between *sn*-1 and *sn*-3 (Reske et al., 1997).

Additionally, there is a discrepancy when we compare our results with those previously obtained by HPLC analysis (Reske et al., 1997). In general, lines with phenotypes similar to those of our lines had the same TAG species. However, in the cases of their high-palmitic line, similar to our CAS-5, and their high-palmitic−high-oleic, similar to our CAS-12, several TAG species, mainly with stearic acid, that we do not found with our method were identified. It is important to note that the stearic acid contents of our CAS-5 and CAS-12 oils and the previously described oils (Reske et al., 1997) were between 2.4 and 2.9%. Thus, to have SLL between 3.5 (in a high-palmitic−high-oleic line) and 5% (in a high-palmitic−high-linoleic line), as it was previously reported (Reske et al., 1997), would require a higher concentration of stearic acid in the high-palmitic−high-oleic oil. Furthermore, some important TAG species such as PPoO in the CAS-12 oil and others containing asclepic acid were not identified by Reske et al. (1997).

There was a significant difference ($P \leq 0.05$) between the observed GLC values and those expected from calculations applying the lipase analysis in the case of some TAG species. Three TAG species (LLL, PLL, and SLL) showed higher values than expected, and five species (POS, POL, SOL, OOL, and OLL) showed lower values by GLC in all lines. We have found, as shown in Figure 4, that in the case of the stearic acid content, the deviations from the expected values for SLL are positively correlated with the total content of stearic acid in the TAG. In contrast, in the case of LLL and OLL the deviations were correlated negatively with the total content of stearic acid in the TAG. This finding suggests a tendency to spread saturated fatty acids within specific unsaturated TAG molecules.

ABBREVIATIONS USED

A, araquidic acid; As, asclepic acid; B, behenic acid; L, linoleic acid; O, oleic acid; P, palmitic acid; Po, palmitoleic acid; Pl, palmitolinoleic acid; S, stearic acid; TAG, triacylglycerols.

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LITERATURE CITED

Álvarez-Ortega, R.; Cantisán, S.; Martínez-Force, E.; Garcés, R. Characterization of polar and nonpolar seed lipid classes from highly saturated fatty acid sunflower mutants. *Lipids* **1997**, *32*, 833−837.

Carelli, A. A.; Cert, A. Comparative study of the determination of triacylglycerol in vegetable oils using chromatographic techniques. *J. Chromatogr.* **1993**, *630*, 213−222.

Fernández-Martínez, J. M.; Mancha, M.; Osorio, J.; Garcés, R. Sunflower mutants containing high levels of palmitic acid in high oleic background. *Euphytica* **1997**, *97*, 113−116.

Garcés, R.; Mancha, M. One-step lipid extraction and fatty acid methyl esters preparation from fresh plant tissues. *Anal. Biochem.* **1993**, *211*, 139−143.

Griffiths, G.; Stobart, A. K.; Stymne, S. The acylation of *sn*-glycerol 3-phosphate and the metabolism of phosphatidate in microsomal preparations from the developing cotyledons of safflower (*Carthamus tinctorius* L.) seed. *Biochem. J.* **1985**, *230*, 379−388.

Gunstone, F. D.; Harwood, J. L.; Padleey, F. B. *The Lipid Handbook*; Chapman and Hall: London, U.K., 1994; p 120.

Hara, A.; Radin, N. Lipid extraction of tissues with a low-toxicity solvent. *Anal. Biochem.* **1978**, *90*, 420−426.

Ichihara, K.; Noda, M. Some properties of diacylglycerol acyltransferase in a particulate fraction from maturing safflower seeds. *Phytochemistry* **1982**, *21*, 1895−1901.

Ivanov, P.; Petakov, D.; Nikolova, V.; Petchev, E. In *Proceedings of the 12th International Sunflower Conference*, Novi Sad; International Sunflower Association: Toowoomba, Australia, 1988; pp 463−465.

Kinney, A. J. Development of genetically engineered oilseeds. In *Physiology, Biochemistry and Molecular Biology of Plant Lipids*; Williams, J. P., Khan, M. U., Lem, N. W., Eds.; Kluwer: Dordrecht, The Netherlands, 1996.

Knutzon, D. S.; Thompson, G. A.; Radke, S. E.; Johnson, W. B.; Knauf, V. C.; Kridl, J. C. Modification of *Brassica* seed oil by antisense expression of a stearoyl-acyl carrier protein desaturase gene. *Plant Biol.* **1992**, *59*, 2624−2628.

Mancha, M.; Vázquez, A. Transformación de los glicéridos durante la maduración de la aceituna. III. Distribución de los ácidos grasos en los triglicéridos. *Grasas Aceites* **1970**, *21*, 127−131.

Martínez-Force, E.; Álvarez-Ortega, R.; Garcés, R. Enzymatic characterization of high palmitic sunflower (*Helianthus annuus* L.) mutants. *Planta* **1999**, *207*, 533−538.

Osorio, J.; Fernández-Martínez, J.; Mancha, M.; Garcés, R. Mutant sunflower with high concentration of saturated fatty acids in their oil. *Crop Sci.* **1995**, *35*, 739−742.

Reske, J.; Siebrecht, J.; Hazebroed, J. Triacylglycerol composition and structure in genetically modified sunflower and soybean oils. *J. Am. Oil Chem. Soc.* **1997**, *74*, 989−998.

Rios, J. J.; Pérez-Camino, M. C.; Márquez-Ruíz, G.; Dobarganes, M. C. Comparison of oxidation of sucrose octaesters and triacylglycerols derived from olive oil. *Food Chem.* **1992**, *44*, 357−362.

Santinelli, F.; Christie, W. W. The triacylglycerol structure of olive oil determined by silver ion high-performance liquid chromatography in combination with stereospecific analysis. *J. Am. Oil Chem. Soc.* **1992**, *69*, 552−556.

Soldatov, K. I. Chemical mutagenesis in sunflower breeding. *Proceedings of the Seventh International Sunflower Association*, Vlaardingen, The Netherlands; International Sunflower Association: Toowoomba, Australia, 1976; pp 352−357.

Vander Wal, R. J. Calculation of the distribution of the saturated and unsaturated acyl groups in fats, from pancreatic lipase hydrolysis data. *J. Am. Oil Chem. Soc.* **1960**, *37*, 18−20.

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