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Lipid Characterization of a High-Stearic Sunflower Mutant Displaying a Seed Stearic Acid Gradient

VALLE FERNANDEZ-MOYA, ENRIQUE MARTÍNEZ-FORCE, AND RAFAEL GARCÉS*

Instituto de la Grasa (CSIC), Avenida Padre García Tejero 4, E-41012 Seville, Spain

In the seeds of the high-stearic sunflower mutant CAS-14 a gradient of increasing stearic acid exists from the embryo to the terminal extreme of the cotyledon. This gradient modifies the fatty acid composition of the total lipids, triglycerides, and phospholipids, which can best be appreciated in the triglycerides that pass from 16% stearic acid content in the embryo to 37.1% in the other extreme. This increase in the triglycerides occurs principally at the cost of the oleic acid content. The stearic content at position *sn*-2 of triglycerides is low, rising from 1.3% in the embryo to 3.4%, whereas at positions *sn*-1+3 the stearic content is high and augments from 25.2% in the embryo to 41.0% at the other extreme. The molecular species of triglycerides are also modified; the disaturated triglycerides increase from 15.5 to 51.7%. Furthermore, for the first time in sunflower seeds, it is demonstrated that trisaturated triglycerides are present, arising probably due to a modification in the acyltransferase system that synthesizes the triglycerides.

KEYWORDS: ***Helianthus*; lipid; mutant; oil; seed; stearic; sunflower; triacylglycerol**

INTRODUCTION

The oleaginous seeds, such as those of the sunflower, are composed of various tissues. One of these is the embryo where the cells that form the future plant are found and where the tissue has reserves rich in lipids and proteins. In oleaginous plants such as the sunflower, lipids accumulate above all in the form of triglycerides (TAG), in special organelles denominated oil bodies. Each species and variety of oleaginous plant has a characteristic composition of fatty acids. This composition is controlled by the genotype of the embryo and by some environmental conditions such as temperature. This has made it possible to use the half-seed or the half-cotyledon technique once germination has commenced (*1*), assuming it to be representative of the total. In this way, the composition of the fatty acids of one fragment can be analyzed, reserving the rest of the seed that contains the embryo to select heritable traits in the phenotype. Using this method, various mutant sunflower lines have been selected with a high content of palmitic or stearic acid on a normal or high-oleic background (*2*, *3*), as well as a low-linoleic mutant and another high-palmitic flax line (*4*). Furthermore, this technique has made it possible to study the oleic/linoleic relationship in different genotypes of safflower (*5*).

In the mutant and normal sunflower lines, no important variations have been found in the composition of fatty acids between the different parts of the seed (*1*, *6*). Thus, the compositions of oil fatty acids from the half seed and the whole seed are practically the same. However, more recently a temperature-dependent high-stearic mutant line of CAS-14 was isolated, which reached levels of 37% stearic acid in optimal growth conditions (*7*). Important differences were encountered in the fatty acid composition in portions of the seeds of this mutant with respect to the distance from the embryo. Indeed, an increasing gradient of stearic acid existed from the embryo up to the distal part of the cotyledons, suggesting that this nonhomogeneous distribution of the fatty acids in the mutant seed might be regulated by a morphogen (*8*). The idea of a gradient is intimately associated with the concept of positional information. In developmental biology, the emission of a signal, a morphogen, from one part of the embryo can determine the localization, differentiation, and destiny of many neighboring cells (*9*). In plants, various known examples of gradients produced by morphogenes exist. In the stem of the poplar hybrid (*Populus tremula* L. × *Populus tremuloides* Michx.), the indole-3-acetic acid is distributed in a concentration gradient through the region of the cambial tissues. Indole-3-acetic acid plays a role in regulating not only the velocity of physiological processes such as cell division but also the duration of developmental processes such as the extension of the fiber of the xylem. Thus, indole-3-acetic acid acts as a morphogen, imparting positional information during the development of the xylem (*10*). In our case, it appears that a gradient of a given signal is influencing the desaturation of stearic acid along the length of the sunflower seed.

The differential accumulation of stearoyl-ACP in the plastid or of stearoyl-CoA in the cytoplasm, reflected in the gradient of stearic in the seed, can promote other types of effects along the seed of this mutant. In this study, we set out to determine how this gradient of stearic acid might influence the different types of lipids that can be found from the embryo up to the distal part of the cotyledon in the sunflower.

* Author to whom correspondence should be addressed (telephone 34-954611550; fax 34-954616790; e-mail rgarces@cica.es).

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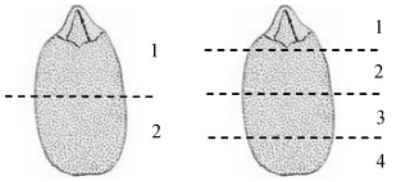

Figure 1. Two- and four-sliced seed fragments for lipid analysis.

MATERIALS AND METHODS

Plant Material and Growth Conditions. Sunflower (*Helianthus annuus* L.) seeds from the mutant CAS-14 line that have a high stearic acid content (*7*) were used in this study. This mutant plant was always maintained at high temperatures during seed formation to achieve good expression of the mutant phenotype. The control line used was CAS-10, a standard isogenic line of the mutant CAS-14. The plants were cultivated in growth chambers at 25/15 °C (day/night), with a 16-h photoperiod and 300 μEinstein m^{-2} s^{-1} light intensity, unless otherwise indicated. Plants were transferred, 3−5 days before flowering, to a very high temperature growth chamber 39/24 °C (day/night). Seeds were harvested at 35 days after flowering and processed immediately. The seeds were sliced into two or four parts as described in **Figure 1**. Three replicates of each experiment were performed, and a different capitulum was analyzed independently in each replicate.

Lipid Extraction and Separation. The lipids were extracted from two or four seed fragments (**Figure 1**); experiments with two fragments were carried out to avoid unnecessary and repetitive data. The seed fragments were ground in a screw-cap glass tube (10 × 13 mm) with a pestle and sand. The total lipids were extracted (*11*) and fractionated into triacylglycerols, diacylglycerols, and phospholipids on TLC silica gel plates (0.25 mm thickness) that were developed with hexane/ethyl ether/formic acid (75:25:1 by vol). Lipid fractions were scraped off the plates and eluted from silica with chloroform/methanol (1:2; by vol).

Lipid Analysis. Fatty acid methyl esters were obtained from oil samples by heating the samples to 80 °C for 1 h in a 3 mL solution of methanol/toluene/H_2SO_4 (88:10:2 by vol). After cooling, 1 mL of heptane was added and mixed (*12*). The fatty acid methyl esters were recovered from the upper phase, then separated and quantified using a Hewlett-Packard 5890A gas chromatograph (Palo Alto, CA) with a Supelco SP-2380 capillary column of fused silica (30 m length, 0.32 mm i.d., 0.20 μm film thickness; Bellefonte, PA). Hydrogen was used as the carrier gas, and the linear gas rate was 28 cm/s. The injector and detector temperatures were 200 °C, the oven temperature was 170 °C, and the split ratio was 1:50. The fatty acids were identified by comparison with known standards.

Lipase Hydrolysis. For TAG analysis, TAGs were eluted from silica−Celite (80:20) columns with hexane and ethyl ether (95:55, v/v) after the silica−Celite mix had been previously activated at 110 °C for 30 min. For TAG fatty acid positional analysis, 10 mg of purified TAGs was hydrolyzed with 2 mg of pancreatic lipase 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 TAGs 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, and the reaction products were separated by TLC (see above). Free fatty acids and *sn*-2-monoacylglycerol bands representing the *sn*-1,3 and *sn*-2 positions of TAGs, respectively, were scrapped off the plate and transmethylated (see above). The procedure was checked by comparing the fatty acid composition of the original TAGs 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 (*13*), using a macro worksheet for Microsoft Excel 97 developed by ourselves (available at http://www.cica.es/aliens/glsoig/).

TAG Analysis by GLC. TAG species were separated 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 with a hydrogen flame ionization detector (FID). TAGs were identified and the data corrected for the relative response of the FID (*14*). After a 5 min hold, the oven temperature was increased by ramping from 340 to 355 °C at 1 °C/min, detector and injector temperatures were maintained at 400 °C, and the split ratio was 1:100. Hydrogen was used as carrier gas at a linear rate of 31 cm/s.

The distribution of saturated fatty acids in the *sn*-1 and *sn*-3 external positions of TAGs was calculated as the coefficient of asymmetry α (*15*). This coefficient was determined as the α of the TAG of the type SatUnsSat/SatUnsUns (αSUU/SUS) as recommended for vegetable oils with low saturated fatty acid content in the *sn*-2 position. An α value of 0.5 indicates that saturated fatty acids are distributed equally between the *sn*-1 and *sn*-3 TAG stereochemical positions.

Statistical Analysis. The chi-square test for unpaired observations was used to determine significant differences in fatty acid and TAG compositions between seed fragments. All tests of significance were made at the 0.05 level of probability.

Table 1. Fatty Acid Composition of Total Lipids (TL) and Triacylglycerols (TAG) from Control CAS-10 and Mutant CAS-14 Seeds Divided into Two or Four Fragments As Shown in **Figure 1**[a]

			fatty acid composition (mol %)					
line	lipid	fragment	16:0	18:0	18:1	18:2	20:0	22:0
CAS-10	TL	1 a	5.5	6.9	53.4	32.4	0.5	1.2
		2 a	5.9	7.3	51.7	33.2	0.5	1.5
	TAG	1 b	5.9	6.9	53.3	32.6	0.4	0.8
		2 b	5.8	8.2	49.4	34.4	1.0	1.2
CAS-14	TL	1 c	7.9	14.6	16.4	58.9	0.8	1.4
		2 d	8.4	23.7	13.4	51.6	1.3	1.5
		3 e	9.5	29.4	8.9	48.7	1.7	1.8
		4 f	9.9	35.1	5.0	46.3	2.1	1.6
	TAG	1 g	8.1	16.0	13.2	60.8	0.8	1.1
		2 h	8.5	25.0	9.4	54.7	1.3	1.2
		3 i	9.5	31.0	6.0	50.6	1.6	1.4
		4 j	10.9	37.1	3.6	45.6	1.9	1.0

[a] SD < 10% of mean value. Rows followed by the same letter are not significantly different at the 0.05 probability level based on chi-square tests.

RESULTS AND DISCUSSION

Fatty Acid Composition of the Total Lipids and Triglycerides. The composition of the fatty acids in the total lipids (TL) and in the TAGs was initially determined, in the case of the mutant, from the four seed fragments, whereas the control seeds were analyzed in two fragments. In both cases, fragment 1 corresponded to that with the embryo. Once the lipids were extracted, the TAGs were purified, and the results obtained are shown in **Table 1**. In both lines, it could be seen that the composition of fatty acids in the TAGs was very similar for each of the fragments with respect to the TL, as might be expected given that the TAG are the major constituents of the seed oil (*1*). Moreover, when the fragments of the control CAS-10 line were compared, there was very little variation between the fatty acids found in the two fragments, although the stearic content was slightly higher than what might be expected from a normal sunflower. It must be taken into account that CAS-10 is a standard isogenic line of CAS-14 and, as such, it might have some of the genes related to the mutant characteristics as has been seen in other high-stearic mutants (*16*). In the CAS-14 line a very different composition can be appreciated, whereby the fatty acids that display the greatest changes are those of stearic and oleic. The main variation can be seen in the TAGs, where the percentage of stearic acid increases from 16.0% in fragment 1 to 37.1% in the fragment 4, whereas in the TL the increase is from 14.6% in fragment 1 to 35.1% in the fragment 4. This increase in stearic acid is produced at a cost of oleic acid, which is reduced from 13.2% in the TAGs of fragment 1

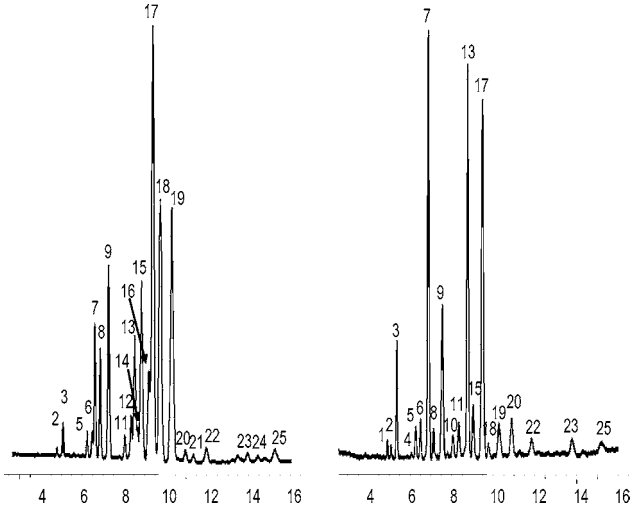

Figure 2. GLC chromatogram of purified triacylglycerols (TAG) from fragments 1 and 4 of CAS-14 mutant seeds. The retention time in the abscissa is expressed in minutes. Numbers were assigned to each TAG species as in **Table 3**.

to 3.6% in fragment 4 and in the TL where this fatty acid falls from 16.4 to 5.0%. Furthermore, we also observed an increase in the palmitic acid content of fragment 4, above all in the TAGs, where it went from 8.1% in fragment 1 to 10.9% in fragment 4, as reflected in the TL by an increase from 7.9 to 9.9%. These results are in accordance with those expected if we take into account those obtained previously with the mutant CAS-14 line when the gradient of fatty acids was discovered along the length of the seed (*7*).

Analysis of the Fatty Acid Distribution in the Positions of the Triglycerides. With respect to the analysis of the *sn*-2 and *sn*-1,3 positions of the TAGs, there is no 18:0 detected in the control line CAS-10 at the *sn*-2 position, although the content of this fatty acid in the *sn*-1,3 positions is higher, as would be expected for the saturated fatty acids in the oils from a sunflower seed. In the TAGs of the mutant, although important variations in the 18:0 content were detected at the *sn*-2 position, in both fragments the 18:0 content was low. Nevertheless, it was more than double that found in the control, augmenting from 1.3% in fragment 1 to 3.4% in fragment 2. In fragment 1 this line had a content similar to that of the other recombinant lines with a very high stearic content; in fragment 2 this value was the highest found to date in the sunflower (*17*, *18*). However, it must be kept in mind that this gradient does not exist in other high-stearic sunflower mutants. At the *sn*-1,3 positions, the palmitic and stearic contents are higher, with important increases being observed from fragment 1 to 2, the content in the fragment 1 being 25.2%. This was similar to the situation in the medium-stearic lines (*6*, *18*); indeed, the content in fragment 2 was as high as in the lines obtained by recombination to obtain such characteristics (*17*, *18*). Nevertheless, once again it should be remembered that no gradient was observed in these seeds. In all cases, an important reduction of oleic acid was observed between fragments 1 and 2, and there was a complete absence of long-chain fatty acids at *sn*-2 in all of the fragments.

Analysis of the TAG Species in Fragments along the Length of the Seed. The TAG species have been determined only in two fragments of the seeds from the control line CAS-10 and in four fragments of the mutant CAS-14. From the chromatograms of fragments 1 and 4 of the mutant CAS-14, the differences between both lines can be readily appreciated (**Figure 2**); the oil from each of the fragments from the mutant

Table 2. Analysis of the Fatty Acid Distribution in the *sn*-2 and *sn*-(1+3) Positions of Triacylglycerols (TAG) from Control CAS-10 and Mutant CAS-14 Seeds Sliced into Two Fragments As Shown in **Figure 1**[a]

			fatty acid composition (mol %)					
line		fragment	16:0	18:0	18:1	18:2	20:0	22:0
CAS-10	*sn*-2	1 a	0.6	0.6	62.1	36.6		
		2 a	0.6	0.3	59.8	39.2		
	sn-(1+3)	1 b	8.1	10.6	52.7	26.0	0.7	1.8
		2 b	8.0	10.2	52.8	26.3	0.7	1.9
CAS-14	*sn*-2	1 c	1.2	1.3	50.6	47.0		
		2 d	3.1	3.4	36.2	57.3		
	sn-(1+3)	1 e	9.0	25.2	35.9	26.5	1.4	2.0
		2 f	11.3	41.0	19.0	23.5	2.3	2.9

[a] SD < 10% of mean value. Rows followed by the same letter are not significantly different at the 0.05 probability level based on chi-square tests.

Table 3. Triacylglycerol (TAG) Molecular Species in Oils from Control CAS-10 (Two Fragments) and CAS-14 (Four Fragments) Sunflower Seeds[a]

		TAG (mol %)					
		CAS-10 fragment		CAS-14 fragment			
IN[b]	TAG	1 a	2 a	1 b	2 c	3 d	4 e
1	PSP	–[c]	–	–	–	0.2	0.3
2	POP	0.5	0.4	0.2	0.2	0.2	0.5
3	PLP	–	0.3	1.2	1.4	1.8	2.6
4	PSS	–	–	0.2	0.3	0.6	1.1
5	POS	1.2	1.1	0.8	0.9	1.0	1.4
6	POO	5.7	5.4	0.6	0.3	0.1	–
7	PLS	0.7	0.7	5.4	8.6	11.6	15.5
8	POL	6.1	6.2	3.8	2.4	1.6	0.8
9	PLL	1.6	1.7	9.2	7.9	6.8	6.2
10	SSS	–	–	–	0.3	0.7	0.9
11	SOS	0.8	0.7	0.6	1.1	1.4	1.7
12	SOO	7.4	6.7	1.1	0.9	0.5	–
13	SLS	0.5	0.4	6.0	11.8	18.0	26.0
14	OOO	17.9	17.3	0.5	–	–	–
15	SOL	7.9	7.8	6.9	6.6	5.3	3.6
16	OOL	28.3	28.5	2.8	1.4	0.7	–
17	SLL	2.1	2.2	23.3	28.5	29.3	29.8
18	OLL	14.8	15.6	12.5	7.5	4.1	0.8
19	LLL	2.5	2.9	20.8	13.8	8.8	3.2
20	SLA	–	–	0.9	1.6	2.3	3.3
21	OLA	0.5	0.5	0.6	0.4	0.3	0.3
22	LLA	–	–	1.0	1.3	1.4	0.9
23	SLB	–	–	0.4	1.2	1.4	0.6
24	OLB	1.3	1.3	0.4	0.5	0.5	–
25	LLB	–	–	0.6	1.0	1.3	0.5

[a] Columns followed by the same letter are not significantly different at the 0.05 probability level based on chi-square tests. [b] IN, identification number. [c] –, <0.2%.

line has a distinct TAG profile, these variations being due to the differences in the fatty acids that are found in the fragments. The majority of the TAG species are perfectly identified on the basis of the results obtained with other high-stearic sunflower lines (*19*) and the prior analysis of the TAGs from normal and high-oleic sunflower oils (*14*). The identification number assigned depended on the order of the peaks that were found in these lines and corresponded to that shown in **Table 3**. Some of the peaks in the chromatogram, assigned as 1, 4, and 10, corresponded to TAGs in the CAS-14 mutant that have not previously been detected in sunflower. To identify the new TAG species, the molecular species were reconstructed in accordance with the 2-random 1,3-random theory (*13*) and on the basis of the lipolysis data presented in **Table 2**. In contrast to other

mutant lines with a high saturated fatty acid content that have been studied previously, the CAS-14 line displayed higher levels of these fatty acids at position *sn*-2 of the TAG, both palmitic and stearic, the sum of these two being 6.5%, whereas in other mutants with a similar stearic acid content it was between 2.5 and 3.6% (*6*, *18*).

The reconstruction predicts the appearance of three new TAG species in the CAS-14 oil, each of these with the saturated fatty acids PSP, PSS, and SSS. The retention times in the chromatogram of these new TAG species, calculated by the numbers of carbons and double bonds in the fatty acids of the TAG molecule (*14*), coincide with those of the unidentified peaks in the chromatogram (1, PSP; 4, PSS; and 10, SSS). This indicates that for the first time in these oils, trisaturated TAGs appear. Given that the stearic acid content of this oil is similar to that which is found in the other high-stearic sunflower mutants and because no trisaturated TAGs have been identified in these, it seems to be most likely that in the CAS-14 mutant, the TAG fatty acid distribution is different. Once all of the TAGs with a content >0.2% have been identified, the relative abundance of each can be calculated by analyzing the chromatogram, taking into account the relative response of the flame ionization detector to each of these TAG species (*14*). As a result, significant differences can be perfectly distinguished between the chromatograms as will be seen below.

The percentages of each of the TAG species from the two fragments of the control the CAS-10 seeds and the four fragments of the CAS-14 mutant line are shown in **Table 3**. The CAS-14 mutant line contains a greater proportion of TAG species with one or two saturated fatty acids when compared to the control, CAS-10, as occurred in all of the highly saturated mutant lines analyzed previously (*19*). The most abundant TAG species in this line are distinct in each of the fragments; whereas in fragment 1 they are SLL, LLL, and OLL, in fragment 4 they are SLL, SLS, and PLS. The TAG species in the oil of each of the four fragments in which the seeds from the CAS-14 line are divided reflect the gradient that was found in the fatty acid composition. The increase in stearic acid and the decrease in unsaturated fatty acids from the embryo to the extreme of the cotyledon of the seed corresponded with an appearance and increment of trisaturated TAGs (PSP, PSS, and SSS), an increase in the disaturated species of fatty acids (POP, PLP, POS, PLS, SOS, SLS, and SLA) and the decrease in those containing oleic and linoleic (POO, POL, PLL, SOO, OOO, SOL, OOL, OLL, and LLL) in the distal part of the seed. This is the first time that trisaturated TAGs have been found in sunflower seeds, and they are found at levels similar to those of some tropical fats, such as the aceituno and cacao, but inferior to the fats of illipe, shea, or palms (*20*). Other peculiarities are the increase of SLL due to the large increase of stearic acid and the small reductions or increases in the minority TAGs with long fatty acids.

To confirm the results of the TAGs, the composition of fatty acids was calculated and from these, the total content of saturated TAGs and the value of the asymmetry coefficient of the saturated fatty acids αSUU/SUS were derived. Furthermore, the triglyceride species were grouped together on the basis of the fatty acid saturation from those that harbored three saturated fatty acids to those in which three unsaturated fatty acids were present and where the tendencies could be more clearly observed (see **Table 4**). In the CAS-10 line, no important variations were observed in the groups of triglycerides and no trisaturated fatty acids appeared in any of the seed fragments. Furthermore, no important modifications in the composition of the fatty acids generated from the TAGs were observed. The opposite occurred in CAS-14, where it could seen from the composition of fatty acids generated from the TAGs that the content of stearic augmented and, above all, the oleic content diminished. Indeed, the total saturated fatty acids increased from 26.4 to 50.8% from fragment 1 to 4, this saturated content being the highest yet found in sunflower, greater than the highest value found to date of 45.3% in CAS-29 (*18*). There is a great difference in the groups of triglycerides in the distinct fragments of the CAS-14 line, as was more clearly seen when the TAGs were grouped together. On the one hand, the appearance of trisaturated can be seen, and they increase from the embryo until the final part of the cotyledon. It is also important to highlight the elevated disaturated fatty acid content observed in triglycerides, above all, in the most distal fragment where the trisaturated fatty acids were highest. The monounsaturated TAGs rise and fall from one extreme to the other and, finally, the triunsaturated acids fall rapidly from the embryo to the distal extreme. The asymmetry coefficient αSUU/SUS of all the fragments is 0.36−0.37, indicating certain asymmetry in the saturated content between positions *sn*-1 and *sn*-3. This asymmetry coincides with earlier data demonstrating a similar phenomenon in highly saturated sunflower mutants (*15*, *21*). However, what is most important is that this does not vary along the length of the seed in the mutant, suggesting that the value of this coefficient is not related either with the gradient or with the stearic acid content of the mutant.

Table 4. Fatty Acid Composition Calculated from the Composition of Triacylglycerol (TAG) Molecular Species and TAG Subclasses Grouped by Degree of Unsaturation in Control CAS-10 and CAS-14 Mutant Seeds

	sunflower seed fragment					
	CAS-10		CAS-14			
	1 a	2 a	1 b	2 c	3 d	4 e
fatty acid (mol %)						
16:0	5.4	5.4	7.6	7.9	8.7	10.6
18:0	7.2	6.8	17.5	25.2	31.2	38.4
18:1	55.6	54.4	12.1	8.3	5.7	3.0
18:2	29.5	30.5	61.3	56.6	51.9	46.3
20:0	0.2	0.2	0.8	1.1	1.3	1.5
22:0	0.4	0.4	0.5	0.9	1.1	0.4
Sat	13.2	12.8	26.4	35.1	42.3	50.8
TAG classes[b] (mol %)						
SSS			0.2	0.6	1.3	2.0
SUS	3.7	3.6	15.5	26.8	37.7	51.7
SUU	32.2	31.3	47.5	49.8	47.1	42.2
UUU	62.4	62.9	36.6	22.7	13.6	4.0
α(SUS/SUU)	0.33	0.33	0.37	0.36	0.37	0.37

[a] Columns followed by the same letter are not significantly different at the 0.05 probability level based on chi-square tests. [b] S, saturated fatty acid; U, unsaturated fatty acid.

With respect to the appearance of trisaturated species, this is the most peculiar modification. Given that they do not appear in other high-stearic mutant sunflower seeds, the most coherent explication is that the lysophosphatidyl acyltransferase is in some way affected in the CAS-14 line. This enzyme is responsible for the introduction of fatty acids at position *sn*-2 of lysophosphatidic acid. In sunflower and other seeds, this enzyme displays a strong preference for unsaturated fatty acids, 18:1 and 18:2, and totally excludes the saturated ones (*6*). This would imply that in this line the distribution of the fatty acids in the TAG occurs in a manner different from that previously studied.

Composition of Fatty Acids in Phospholipids. The fatty acid composition of the phospholipids in the oils from the seed fragments of these two lines is shown in **Table 5**. In general,

Table 5. Analysis of the Fatty Acid Composition of the Phospholipids from Control CAS-10 and Mutant CAS-14 Seeds Divided into Two Fragments As Shown in **Figure 1**[a]

		fatty acid composition (mol %)					
	fragment	16:0	18:0	18:1	18:2	20:0	22:0
CAS-10	1 a	8.8	6.2	36.4	47.1	0.2	1.3
	2 a	10.1	6.6	35.8	45.7	0.3	1.6
CAS-14	1 b	10.5	13.8	21.4	52.7	0.5	1.1
	2 c	10.6	21.5	17.8	48.4	0.7	1.1

[a] SD < 10% of mean value. Rows followed by the same letter are not significantly different at the 0.05 probability level based on chi-square tests.

we could see that in both fragments, the phospholipid fatty acids were enriched in palmitic acid with respect to the total lipid content (**Table 1**), which is normal both in the standard and in the high-stearic mutant sunflower seed (*6*). The stearic acid content is lower in phospholipids than in the total lipids, which can be explained if we take into account that these lipids have only two esterified fatty acids at positions *sn*-1 and *sn*-2 and that at the second position the saturated fatty acids are not usually esterified. The stearic content is the same in the two fragments of the CAS-10 control line and, as in other types of lipids in CAS-14, the stearic content increases toward the distal extreme of the seed, passing from 13.8 to 21.5% from fragment 1 to 2. The oleic acid and linoleic acid contents are also reduced. The fatty acid composition of phospholipids is very important to maintain the correct functioning of the biological membranes during the formation of the seed and in its subsequent germination (*22*). For this motive, the composition of the phospholipids always tends toward certain determined fatty acids. Indeed, mechanisms to retrieve fatty acids that are not usually found in membrane phospholipids exist, as can be seen in the castor bean (*23*).

In conclusion, we can say that the stearic acid gradient and those of other fatty acids possibly associated with this are reflected both in the neutral lipids such as TAGs and in the polar lipids in the mutant CAS-14 line. In the seeds of this line, the fragment corresponding to the embryo contains less stearic acid. The most readily appreciated differences can be found in the stearic acid of the TAGs. An important characteristic of this mutant is the presence of trisaturated TAGs, which augment proportionally to the stearic acid content. To date, this is the only sunflower line that has detectable quantities of this type of TAG.

ABBREVIATIONS USED

A, araquidic acid; B, behenic acid; L, linoleic acid; O, oleic acid; P, palmitic acid; S, stearic acid; Sat, saturated fatty acid; TAG, triaglyceride; TL, total lipids; U, unsaturated fatty acid.

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