JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Oils from Improved High Stearic Acid Sunflower Seeds

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

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

Seed oils from new recombinant high-stearic sunflower lines (*Helianthus annuus* L.) have been characterized. These new lines were generated by crossing high stearic acid lines between themselves or by crossing them with standard and high-oleic sunflower lines. Of the novel lines generated, the lines CAS-29 and CAS-30 are on a standard background and contain up to 34.5% of stearic acid. In contrast, CAS-15 and CAS-33 are on a high oleic acid background and contain only 24.9 and 17.4% of stearic acid, respectively. The stearic acid contents of lines CAS-19 and CAS-20 are 10.0 and 21.5%, respectively, and they have only one of the two genes that control the high stearic acid trait. In accordance with their vegetable origin, these lines have a low percentage of stearic acid in the *sn*-2 position of the TAGs, from 0.6 to 2.1%. The amount of disaturated TAGs increases with the stearic acid content, from 1.8% in the standard line to between 5.1% in CAS-20 and 38.5% in CAS-29. There was also a concomitant reduction in triunsaturated TAGs, which were reduced to levels as low as 8.4% in CAS-29, as opposed to the 67.9% that they constitute in the standard line RHA-274. The asymmetrical distribution of the saturated fatty acids between the *sn*-1 and *sn*-3 TAG positions ranges from 0.26 to 0.36, being lower in those lines with higher oleic acid content.

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

INTRODUCTION

Seed commodity oils predominantly consist of linoleic acid and sometimes oleic acid, with a lower content of linolenic, palmitic, and stearic acids. One of the main goals in the study of oilseed storage lipids is to understand how the fatty acid and triacylglycerol (TAG) biosynthetic pathways are controlled. Achieving this should make it possible to modify the fatty acid composition of seeds, enabling us to improve the physical or chemical properties of the oil used in food products or industrial commodities. Several oilseed crop variants have been selected for modifications in their fatty acid composition. These include rapeseed and soybean with low linolenic acid content, which improves their oxidative stability when stored; some sunflower mutants with an elevated oleic acid content, which can reach 90%; soybean and rapeseed with a high oleic acid content; and, finally, new lines with increased saturated fatty acid content, mainly stearic, such as some soybean and sunflower mutants or genetically engineered rapeseed lines (*1*).

In sunflower, we have obtained a collection of lines by mutagenesis (*2*), and some of these lines have higher stearic acid content than standard sunflower seeds. Genetic studies of the control of the high stearic acid phenotype in sunflower lines have shown that at least two genes are needed for the acquisition of this characteristic (*3*). Moreover, biochemical analysis of these lines has shown that two enzymatic activities are involved in the accumulation of high levels of stearic acid in the seeds of these mutant lines (*4*). Thus, on the one hand, there is less desaturation of stearic acid by the stearoyl-ACP desaturase, producing an increase in the intraplastidial content of stearoyl-ACP. On the other hand, there is enhanced thioesterase activity toward saturated acyl-ACPs. As a consequence of these two enzymatic modifications, the total amount of stearoyl-CoA increases in the acyl-CoA pool, thereby increasing the stearic acid content of the storage lipids. Therefore, if we also take into account that isoenzymes for these two activities might exist and produce the different biochemical properties found in the high-stearic sunflower mutants (*4*, *5*), new lines with altered seed fatty acid composition could be selected through genetic recombination after crossing. In this way, lines have been generated with increased stearic acid content such as the line CAS-29 or CAS-30. Other lines have also been established that carry only one of the mutated genes, such as CAS-19 and CAS-20 (*6*), or high-stearic lines on a high-oleic background such as CAS-15 and CAS-33.

The TAG species in the oil determine its physical, chemical, and nutritional properties. One of the main chemical properties of vegetable oils is that they have low saturated fatty acid content in the *sn*-2 TAG position, even in oils with high-saturated fatty acid content such as that of cocoa. The saturated fatty acid distribution on the stereochemical *sn*-1 and *sn*-3 TAG positions in most vegetable oils is not random, as originally proposed by Vander Wal (*7*). For example, the oleic acid content is higher at the *sn*-1 than at the *sn*-3 position in cocoa butter TAG (*8*, *9*), but it is virtually the same in olive oil (*10*, *11*). Furthermore, stearic acid is predominantly found in the *sn*-3 position of sunflower (*12*) and olive oil TAGs (*10*). A coefficient has been proposed (*13*), denominated the α coefficient, to calculate the

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

10.1021/jf0503412 CCC: $30.25
Published on Web 05/18/2005

Table 1. Phenotype and Origin of the Control and Novel Sunflower Lines

line	phenotype[a]	origin	ref
CAS-3	HS	mutagenesis	*2*
CAS-19	MS	CAS-3 × standard line	*6*
CAS-20	MS	CAS-3 × standard line	*6*
CAS-29	VHS	CAS-3 × CAS-4	IG[b]
CAS-30	VHS	selection from CAS-3	IG
CAS-9	HO	HA-OL9 × standard line	IG
CAS-15	HS HO	CAS-3 × RHA-345	IG
CAS-33	MS HO	CAS-3 × RHA-345	IG

[a] HO, high-oleic; HS, high-stearic; MS, medium-stearic; VHS, very high-stearic. [b] IG, Sunflower Collection of Instituto de la Grasa, CSIC.

asymmetry of saturated fatty acids between the *sn*-1 and *sn*-3 TAG positions. An α value equal to 0.5 indicates a symmetrical distribution, in agreement with the Vander Wal hypothesis (*7*). A coefficient smaller than 0.5 implies that there are more saturated fatty acids at *sn*-1 than at the *sn*-3 position, or vice versa. When the saturated fatty acid content of the oil is very high, α tends to be near 0.5 because the whole enzymatic system is completely filled with saturated fatty acids.

In this paper, we describe the lipid characterization of the oils from some new sunflower lines with medium, high, and very high stearic acid contents. These lines were generated on both a normal and a high-oleic background, by crossing and recombining the original mutant lines between themselves or with standard and high-oleic sunflower lines.

MATERIALS AND METHODS

Plant Material. The sunflower lines (*Helianthus annuus* L.) studied in this work, their phenotype, and origin are shown in **Table 1**. As control lines, we used the standard line RHA-274 from the U.S. Department of Agriculture (USDA) that has a normal fatty acid composition, the high oleic acid line CAS-9, and the high stearic acid line CAS-3 (*2*). The new sunflower lines were obtained by crossing these lines with other mutant lines, such as the medium-stearic CAS-4 (*2*) or the high-oleic RHA-345 from the USDA, and selection (see **Table 1**). The lines CAS-19 and CAS-20 were selected after crossing the high-stearic CAS-3 with a standard line (*6*). CAS-19 has only the es_1 allele that encodes the stearate desaturase (*4, 14*), and CAS-20 has only the es_2 allele that encodes for the thioesterase with enhanced activity toward stearoyl-ACP (*4, 15*). CAS-29 was obtained from the cross of CAS-3 with CAS-4, selecting for elevated stearic acid content. The other very high stearic acid content line CAS-30 was selected from the CAS-3 line. Two lines with high and medium stearic acid contents on a high-oleic background were selected by crossing CAS-3 with RHA-345, and these lines are CAS-15 and CAS-33, respectively.

Plants were cultivated in growth chambers at 25/15 °C (day/night), with a 16-h photoperiod and a photon flux density of 300 μmol·m^{-2}·s^{-1}. For each experiment three samples from three different plants were collected, each sample was analyzed independently, and mean and standard deviation were calculated. The *t* test for unpaired observation was used to determine significant differences between means.

Lipid Extraction and Separation. Seeds were peeled, and ~500 mg was ground in a screw-cap glass tube (10 × 13 mm) with a pestle and sand. Total lipids were extracted (*16*) and separated on TLC silica gel plates of 0.25 mm thickness, and these were developed with hexane/ethyl ether/formic acid (75:25:1 v/v/v). TLC plates were partially covered with a glass plate and exposed to iodine vapors. The unexposed TAG fractions were scraped off the plates and eluted from silica with chloroform/methanol (1:2 v/v).

Lipase Hydrolysis. 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 buffer, pH 8, with 0.1 mL of $CaCl_2$ (22%) and 0.25 mL of deoxycholate (0.1%). After ~60% of the TAGs were 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 of ethyl ether, and the reaction products were separated by TLC as indicated above. The free fatty acids and *sn*-2-monoacylglycerols bands, representing the positions 1+3 and 2 of TAG, respectively, were scraped off the plate and transmethylated. The procedure was checked by comparing the fatty acid composition of the original TAGs with those remaining after the partial hydrolysis.

Table 2. Fatty Acid Composition of the Seed Oil from the New Sunflower Lines Studied Here and the Controls RHA-274 (Standard Fatty Acid Composition), CAS-9 (High-Oleic), and CAS-3 (High-Stearic) Lines[a]

	fatty acid composition (mol %)					
line	16:0	18:0	18:1	18:2	20:0	22:0
RHA-274	7.4	5.8	37.3	48.3	0.3	0.9
CAS-3	7.2	26.3	18.6	45.1	1.4	1.4
CAS-19	7.2	21.5	19.9	48.0	1.8	1.6
CAS-20	6.0	10.0	38.3	43.7	0.4	1.6
CAS-29	7.2	34.5	13.0	41.7	1.8	1.8
CAS-30	6.6	30.3	10.2	49.6	1.5	1.8
CAS-9	3.1	5.2	86.2	3.3	0.7	1.5
CAS-33	6.1	17.4	64.1	9.7	1.1	1.7
CAS-15	5.4	24.9	57.8	8.2	1.8	1.9

[a] Mean of three independent experiments; SD < 10% of mean value.

Lipid Analysis. Fatty acid methyl esters were obtained from the isolated lipids by heating the samples at 80 °C for 1 h in a 3 mL solution of methanol/toluene/H_2SO_4 (88:10:2 v/v/v). After cooling, 1 mL of heptane was added and mixed. The fatty acid methyl esters were recovered from the upper phase and then separated and quantified using a Hewlett-Packard 5890A gas chromatograph 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. The fatty acids were identified by comparison with known standards and quantified by flame ionization detection (FID).

TAG species were separated by gas−liquid chromatography (GLC) and quantified by FID, using a Hewlett-Packard 6890 gas chromatograph with a Quadrex alumninum-clad bonded methyl 65% phenyl silicone 15M, 400-65HT-15-0.1F column (New Haven, CT). The TAGs were identified and the data corrected for the relative response of the FID (*17*). After a 5 min hold, the oven temperature was ramped from 340 to 355 °C at 1 °C/min, the detector and injector temperatures were 400 °C, and the split ratio was 1:100. Hydrogen was used as the carrier gas at a linear rate of 31 cm/s.

The coefficient of asymmetry, α, was calculated as the α of SUS/SUU as recommended for vegetable oils with a low saturated fatty acid content in the *sn*-2 position (*13*).

RESULTS AND DISCUSSION

The fatty acid composition of the mutant lines analyzed in this work can be found in **Table 2**. The stearic acid contents of the oils and purified lipids from the new high-stearic lines CAS-15, CAS-19, CAS-20, CAS-29, CAS-30, and CAS-33 are significantly different from the stearic contents of their parental lines CAS-3, RHA-274, and RHA-345. The stearic acid content of the medium-stearic lines ranged from 10% in CAS-20 to 21.5% in CAS-19. In contrast, the new very high stearic acid mutant lines had stearic acid contents >30%, ranging from 30.3 to 34.5% in the lines CAS-29 and CAS-30, respectively. This represents at least 5% more than the previous mutant lines (*18*). The contents of the two lines selected from a high-oleic background are 17.4 and 24.9% in CAS-33 and CAS-15, respectively, and this is twice the stearic content previously described in high-oleic background (*12*). It is important to note that except for CAS-20, in the standard high linoleic acid lines the increase in stearic acid corresponded to a reduction of oleic acid. There was a large variation in oleic acid, from 10.2% in

Table 3. Analysis of the Fatty Acid Distribution in the *sn*-2 and *sn*-1+3 of the TAGs in the Seed Oils of the New Sunflower Lines Studied in This Work and in the Control Lines RHA-274 (Standard Fatty Acid Composition), CAS-9 (High-Oleic), and CAS-3 (High-Stearic)[a]

		fatty acid composition (mol %)					
line		16:0	18:0	18:1	18:2	20:0	22:0
RHA-274	*sn*-1+3	10.1	7.6	38.7	42.0	0.5	1.1
	sn-2	0.6	0.6	40.8	58.0	–[b]	–
CAS-3	*sn*-1+3	10.6	38.2	15.2	32.3	1.9	1.8
	sn-2	0.7	1.6	32.4	65.4	–	–
CAS-19	*sn*-1+3	8.8	35.4	15.5	36.7	1.8	1.9
	sn-2	1.3	1.3	25.1	72.2	–	–
CAS-20	*sn*-1+3	7.8	19.2	36.7	33.7	1.1	1.6
	sn-2	0.5	0.6	50.1	48.8	–	–
CAS-29	*sn*-1+3	8.6	45.3	7.8	33.8	2.2	2.3
	sn-2	1.1	1.4	23.8	73.8	–	–
CAS-30	*sn*-1+3	7.8	39.8	9.2	40.0	1.9	1.4
	sn-2	1.0	2.1	14.6	82.3	–	–
CAS-9	*sn*-1+3	4.2	6.4	84.3	3.1	0.5	1.4
	sn-2	0.5	0.6	96.6	2.3	–	–
CAS-33	*sn*-1+3	8.2	24.5	50.2	13.9	1.2	1.7
	sn-2	1.0	1.2	71.3	26.5	–	–
CAS-15	*sn*-1+3	7.2	33.1	46.9	7.3	2.7	2.9
	sn-2	1.7	1.9	87.4	9.0	–	–

[a] The results shown are the mean of three independent experiments; SD < 10% of mean value. [b] – signifies <0.5.

Table 4. TAG Composition of the Lines with Medium Stearic Acid Content, CAS-19 and CAS-20[a]

	TAG (mol %)		
TAG species[b]	RHA-274	CAS-19	CAS-20
PLP	0.7	0.5	–[c]
POS	–	1.5	1.2
POO	3.0	0.7	2.8
PLS	1.0	4.8	1.4
POL	7.3	3.1	4.9
PLL	5.8	4.4	3.0
SOS	–	3.1	1.3
SOO	2.3	3.2	6.8
SLS	–	10.2	1.2
OOO	7.7	–	8.3
SOL	5.5	14.7	11.8
OOL	20.2	3.8	18.6
SLL	5.0	23.3	7.8
OLL	26.4	10.1	18.8
LLL	13.6	11.0	9.4
SLA	0.1	1.2	–
OLA	0.2	0.8	0.7
LLA	0.3	1.1	–
OOB	0.3	–	0.8
SLB	–	0.7	*d*
OLB	0.7	0.8	1.2
LLB	0.3	1.1	–

[a] As a control, the TAG composition of the standard RHA-274 line is shown. Mean of three independent experiments; SD < 10% of mean value. [b] P, palmitic; S, stearic; O, oleic; L, linoleic; A, arachidic; B, behenic. [c] – signifies <0.2%. [d] These peaks are not separated and are included in the upper TAG type.

CAS-30 to 19.9% in CAS-19, whereas the linoleic acid contents were quite similar in these lines, ranging from 41.7 to 49.6% as in the parental lines (*2*). The lines of a high-oleic background have a more or less constant and reduced proportion of linoleic acid because of the mutation in the oleate desaturase. Like other lines, the increase in stearic acid content was at the expense of oleic acid. The other saturated fatty acids (palmitic, arachidic, and behenic) did not suffer important modifications in terms of their relative content.

One of the main characteristics of vegetable oils, except for some tropical oils such as palm and coconut (*9*), is the low saturated fatty acid content at the *sn*-2 position of the TAG molecule. This characteristic is due to the specificity of the acyl transferases that synthesize the TAGs. In vegetable oil synthesis, the lysophosphatidic acid acyl-transferase (LPAAT) has a very low affinity for saturated fatty acids. Thus, when we increased the total stearic content up to 6 times, such as in these mutants, this increase in stearic acid might be reflected at the *sn*-2 position. Moreover, what happens if at the same time the amount of oleic acid is also increased? Taking into account the fact that the substrates available for TAG synthesis have been completely modified, the fatty acids at the *sn*-2 position of the TAGs and, specifically, the stearic acid content at this position, have been analyzed to address these questions (**Table 3**). Stearic acid was found mainly in the *sn*-1+3 positions in all TAGs, and its proportions, ranging from 19.2% in CAS-20 to 45.3% in CAS-29, did not increase significantly. Therefore, the specificity of the acyl-transferases is maintained even when the stearic acid content is very high. It is noteworthy that the percentages of stearic acid at the *sn*-2 position of the control low-stearic lines RHA-274 and CAS-9 are 7.9 and 9.4% of the stearic content at *sn*-1+3, respectively. This value is always higher than that found in the new high-stearic lines, which is as low as 3.1% for CAS-20 and CAS-29. Hence, it appears that LPAAT specificity is maintained even in the high-stearic mutant lines. The other fatty acids have a similar behavior in normal and high-stearic lines, oleic and linoleic acids being those fatty acids mainly found at *sn*-2.

To further characterize and discriminate between these oils, it is necessary to analyze the TAG profile, to determine if the modification in fatty acid synthesis affects the fatty acid distribution in TAGs. The TAG composition of medium stearic acid mutant lines CAS-19 and CAS-20 is shown in **Table 4**, where it is compared with that of standard line RHA-274. The main difference that can be observed between these medium-stearic lines and the standard line is the increase in TAG molecular species with two saturated fatty acids such as POS, SOS, and SLS. These species constitute a total of 14.8% of the TAGs in CAS-19 and are not found in the standard line. In general, the TAGs contain some increase in saturated fatty acids, reducing the content of triunsaturated TAGs such as OLL and LLL. This was similar to the TAG composition found in the CAS-29 and CAS-30 mutants when compared to the TAG composition of the original stearic mutant CAS-3 (**Table 5**). In this case, there is an increase in the TAGs with two saturated fatty acids. The SLS content was 20.5 and 17.0% in CAS-29 and CAS-30, respectively, whereas in CAS-3 it was only 11.0%. Likewise, the SOS content was 5.6 and 2.9% in CAS-29 and CAS-30, respectively, compared to 3.3% for CAS-3 (**Table 5**). This high SLS TAG content came about at the expense of OOL, which is not found in the mutant oils, and POL and OLL, which show a noticeable decrease. Another important disaturated TAG species is the PLS TAG that comprise 6.8 and 5.8% in CAS-29 and CAS-30, respectively.

The TAG compositions of the high-stearic and high-oleic mutant lines CAS-33 and CAS-15 and the high-oleic control CAS-9 are shown in **Table 6**. The TAG composition of these oils is completely different from previous ones, because SOO and OOO are the main TAG species, and the amount of TAGs containing linoleic acid is negligible. In the oil from CAS-15, which is derived from CAS-3 but on a high-oleic background, the disaturated SOS content is 10.4%. This is similar to the

Table 5. TAG Composition of the Lines with High Stearic Acid Content, CAS-29 and CAS-30[a]

	TAG (mol %)		
TAG species[b]	CAS-3	CAS-29	CAS-30
PLP	0.8	0.6	0.5
POS	2.0	2.0	1.0
PLS	6.8	6.8	5.8
POL	3.4	1.7	1.4
PLL	5.1	3.7	4.4
SOS	3.3	5.6	2.9
SOO	3.9	2.1	1.8
SLS	11.0	20.5	17.0
SOL	14.0	13.5	11.5
OOL	2.8	–[c]	–
SLL	25.0	28.9	34.0
OLL	6.7	3.1	4.4
LLL	8.3	5.3	9.4
SLA	1.2	2.0	1.2
OLA	0.8	0.7	0.8
LLA	1.3	1.1	1.4
SLB	0.8	1.0	0.6
OLB	0.8	0.4	0.5
LLB	0.9	0.8	1.2

[a] The TAG composition of the stearic mutant CAS-3 is shown as a control. Mean of three independent experiments; SD < 10% of mean value. [b] P, palmitic; S, stearic; O, oleic; L, linoleic; A, arachidic; B, behenic. [c] – signifies <0.2%.

Table 6. TAG Composition of the High Oleic and Stearic Acid Lines CAS-33 and CAS-15 with the TAG Composition of the High-Oleic Mutant CAS-9 Shown as a Control[a]

	TAG (mol %)		
TAG species[b]	CAS-9	CAS-33	CAS-15
POP	–[c]	0.5	0.5
POS	0.4	3.0	4.5
POO	6.4	8.7	6.2
PLS	–	0.9	0.6
POL	–	0.8	1.4
PLL	–	0.4	–
SOS	0.5	4.3	10.4
SOO	11.7	29.5	37.7
SLS	–	1.4	*d*
OOO	70.9	32.5	14.3
SOL	*d*	5.1	7.7
OOL	6.6	2.8	4.0
SLL	–	3.0	1.2
OLL	–	1.1	0.8
SOA	–	0.4	1.5
OOA	0.9	1.5	2.8
OLA	–	–	0.5
SOB	–	0.7	1.4
OOB	2.5	2.8	3.7
OLB	–	–	0.7

[a] Mean of three independent experiments; SD < 10% of mean value. [b] P, palmitic; S, stearic; O, oleic; L, linoleic; A, arachidic; B, behenic. [c] – signifies <0.2%. [d] These peaks are not separated and are included in the upper TAG type.

SLS content in CAS-3 but differs from the SOS content of 4.3% in the CAS-33 mutant. The other important disaturated TAG is POS, which represents 4.5% in CAS-15 and 3.0% in CAS-33.

As a consequence of the modified fatty acid composition of high-stearic mutants, the pattern of TAG molecular species has also been modified. Not only is the relative abundance of the TAG species altered, but new species of TAG were also found in these oils. The TAG molecular species found in these oils are defined by the acyl-CoAs available and reflect the specificity of the TAG biosynthetic enzymes. Thus, these new TAG patterns can be used to fingerprint the oil. The TAG profile of these oils (**Table 7**) resembles some tropical oils with high disaturated TAG content, such as aceituno oil, in which this type of TAG represents 34% of the total content (*9*). Nevertheless, it is lower than in other oils such as cocoa and illipe butter, which have ~70−80%. Some genetically modified oilseeds, such as high-stearic soybean and sunflower (*12*), also have high saturated fatty acid contents. However, the genetically engineered high-stearic sunflower lines had only 11% stearic acid and 7.4% disaturated TAGs, much less than the mutant lines described here. The high-stearic soybean has 24.7% stearic acid and 27.7% disaturated TAGs, as well as a high proportion of linoleic and linolenic acids and a small amount of oleic acid in these TAGs. These values are also lower than in the very high stearic acid mutants CAS-29 and CAS-30.

Table 7. Subclasses of TAG Species, Total Saturated Fatty Acid Content, and α Coefficient as the α SUS/SSS from the New Medium and High Stearic Acid Sunflower Lines[a]

	CAS-19	CAS-20	CAS-29	CAS-30	CAS-15	CAS-33
SUS	22.0	5.1	38.5	29.0	18.4	11.3
SUU	53.2	39.8	52.9	57.0	61.9	51.2
UUU	24.9	55.1	8.4	13.8	19.1	37.5
oleic	19.9	38.3	13.0	10.2	57.8	64.1
total sat.	32.1	18.0	45.3	40.2	34.0	26.3
α sat.	0.36	0.27	0.36	0.32	0.28	0.26

[a] SUS, disaturated TAG; SUU, monosaturated TAG; UUU, triunsaturated TAG; oleic, oleic acid content; total sat., total saturated fatty acids content; α sat., asymmetry coefficient α calculated as α SUS/SUU.

The distribution of saturated fatty acids between positions *sn*-1 and *sn*-3 in vegetable oils is not totally symmetric. An asymmetry coefficient, α, of SUS/SUU has been proposed to calculate this asymmetry of the fatty acids and TAG composition, taking into account the saturated content at *sn*-2 (*13*). The α value, as well as the data used to calculate it, the total disaturated TAG (SUS), monosaturated TAG (SUU), triunsaturated TAG (UUU), and the total saturated fatty acid content of these oils are shown in **Table 7**. As can be seen, there is no logical relationship between the total saturated fatty acid content and the disaturated and monosaturated TAGs, and this is due to the α asymmetry coefficient of each oil. When asymmetry increases, the total amount of disaturated TAGs is reduced, producing a small α value. For example, CAS-19 and CAS-15 have similar total saturated contents but very different α coefficients, 0.36 and 0.28, respectively. Indeed, the content of SUS is higher in CAS-19 even though it has slightly lower saturated fatty acid content. This asymmetry α coefficient has been calculated in other high-saturated oilseed TAGs, and it is normally small, suggesting a strong asymmetric distribution of the saturated fatty acids in the TAG molecules (*13*). It is noteworthy that the lines with more oleic acid have the smallest α values, probably because the substrate selectivity in TAG biosynthesis is different in the case of oleic and linoleic acids.

In conclusion, a new set of high stearic acid containing oils on standard and, for the first time, on high-oleic backgrounds, is presented in this paper. Due to their higher stearic acid contents, these oils could have more desirable physical and chemical properties for the food industry.

ACKNOWLEDGMENT

Thanks are due to M. C. Ruiz and D. Cabrera for skillful technical assistance.

LITERATURE CITED

(1) Martínez-Force, E.; Garcés, R. New oilseed varieties with modified fatty acid composition in the oil. *Trends Agron.* **1999**, *2*, 13−21.

(2) Osorio, J.; Fernández-Martínez, J. M.; Mancha, M.; Garcés, R. Mutant sunflower with high concentration of saturated fatty acids in the oil. *Crop Sci*. **1995**, *33*, 739−742.

(3) Perez-Vich, B.; Garcés, R.; Fernández-Martínez, J. M. Genetic control of high stearic acid content in the seed oil of sunflower mutant CAS-3. *Theor. Appl. Genet.* **1999**, *99*, 663−669.

(4) Cantisán, S.; Martínez-Force, E.; Garcés, R. Enzymatic studies of high stearic acid sunflower seed mutants. *Plant Physiol. Biochem.* **2000**, *38*, 377−382.

(5) Martínez-Force, E.; Garcés, R. Dynamic channeling during de novo fatty acid biosynthesis in *Helianthus annuus* seeds. *Plant Physiol. Biochem.* **2002**, *40*, 383−391.

(6) Perez-Vich, B.; Muñoz-Ruz, J.; Fernandez-Martinez, J. M. Developing midstearic acid sunflower lines from a high stearic acid mutant. *Crop Sci.* **2004**, *44*, 70−75.

(7) 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.

(8) Takagi, T.; Ando, Y. Stereospecific analysis of triacyl-*sn*-glycerols by chiral high performance liquid chromatography. *Lipids* **1995**, *26*, 542−547.

(9) Padley, F. B. Major vegetable fats. In *The Lipid Handbook*; Gunstone, F. D., Harwood, J. L., Padley, F. B., Eds.; Chapman and Hall: London, U.K., 1994; pp 53−146.

(10) Santinelli, F.; Daminani, P.; Christie, 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.

(11) Damiani, P. L.; Cossignani, M. S.; Simonetti, B.; Campisi, L.; Favretto, L. G. Stereospecific analysis of the triacylglycerol fraction and linear discriminant analysis in a climatic differentiation of Umbrian extra virgin olive oils. *J. Chromatogr. A* **1997**, *758*, 109−116.

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

(13) Martínez-Force, E.; Ruiz-López, N.; Garcés, R. The determination of the asymmetrical stereochemical distribution of fatty acids in triacylglycerols. *Anal. Biochem.* **2004**, *334*, 175−182.

(14) Perez-Vich, B.; Fernandez-Martinez, J. M.; Grondona, M.; Knapp, S. J.; Berry, S. T. Stearoyl-ACP and oleoyl-PC desaturase genes cosegregate with quantitative trait loci underlying high stearic and high oleic acid mutant phenotypes in sunflower. *Theor. Appl. Genet.* **2002**, *104*, 338−349.

(15) Perez-Vich, B.; Knapp, S. J.; Leon, A. J.; Fernandez Martinez, J. M.; Berry, S. T. Mapping minor QTL for increased stearic acid content in sunflower seed oil. *Mol. Breed.* **2004**, *13*, 313−322.

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

(17) Fernández-Moya, V.; Martínez-Force, E.; Garcés, R. Identification of triacylglycerol species from high-saturated sunflower (*Helianthus annuus* L.) mutants. *J. Agric. Food Chem*. **2000**, *48*, 764−769.

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

Received for review February 15, 2005. Revised manuscript received April 8, 2005. Accepted April 28, 2005. This work was supported by CICYT Spanish government and Advanta Seeds B.V.

JF0503412