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Thermoxidative Stability of Triacylglycerols from Mutant Sunflower Seeds

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ABSTRACT: Thermoxidative stability was evaluated in triacylglycerols (TAG) from the oils of the mutant sunflower lines CAS-3, CAS-4, and CAS-8 (with a high percentage of stearic acid), CAS-5 (with a high percentage of palmitic acid), all from standard high-linoleic genetic backgrounds, and the mutant sunflower line CAS-12 (with a high percentage of palmitic acid), from a high-oleic genetic background. These oils contained unusually high contents of TAG molecular species with one or two saturated fatty acids at the *sn*-1,3 positions. Purified total TAG devoid of tocopherols were subjected to controlled thermoxidative treatment at 180ºC. Polymerized TAG were determined at 2-h intervals for 10 h. After this time, total polar compounds, oxidized TAG monomers, TAG dimers, and TAG oligomers were determined. TAG from highly saturated sunflower oils with levels of linoleic acid similar to those found in conventional sunflower oils (40–50%) showed enhanced thermal stability. In these TAG, the amount of polar compounds formed during the thermoxidative treatment was similar to that formed in the high oleic acid line. Excellent results were obtained for the TAG of the CAS-12 oil, which had the highest thermal stability, producing half the amount of polar compounds as the conventional line and less than two-thirds that of the high-oleic line.

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Vegetable fats and oils used for deep-frying and other applications of the food industry require high thermal stability. Palm oil, partially hydrogenated, and high-oleic vegetable oils are commonly used to fulfill this requirement. However, partially hydrogenated fats contain *trans* isomers of fatty acids that are considered nutritionally undesirable (1). Also, palm oil and partially hydrogenated fats contain higher levels of saturated fatty acids at the *sn*-2 position of triacylglycerols (TAG) than do most vegetable oils, such as soybean, sunflower, and rapeseed oils (2). The location of saturated fatty acids in the *sn*-2 position has been suggested to have negative biological effects and to be involved in the atherogenic process (3,4).

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High-oleic sunflower oil has been shown to be more resistant to oxidation and polymerization under frying conditions at high temperatures than conventional sunflower oil (5). Investigations on changes of specific molecular species of TAG in conventional and high-oleic sunflower oils and palm oil during frying operations (6) indicated that linoleate-containing species were oxidized more rapidly than those containing oleate. Nevertheless, all species degraded and those containing oleate were oxidized more rapidly than might be expected. It was suggested that linoleate could be the primary reactant initiating oxidation, but the oxidation reactions could propagate readily to all unsaturated species. A detailed study on the formation of polar compounds (7) indicated that linoleic acid was preferentially involved in the formation of dimeric fatty acyl residues, and in turn TAG polymers, although the participation of oleic acid became very important at low linoleic acid content levels.

Recently, new sunflower mutant lines were developed (8,9) that contain high levels of palmitic (16:0) or stearic (18:0) acids, almost exclusively at the *sn*-1,3 positions, and various concentrations of oleic acid (18:1n-9) and linoleic acid (18:2) (10). Thus, oils from a single species differing in the content of only one major fatty acid are now available for analysis. It is of great interest to study the thermal stability of oils with unique TAG structures, particularly those containing molecular species with one or two saturated fatty acids at the *sn*-1,3 positions.

In this paper, the thermoxidative stability of purified TAG from the new sunflower mutants is explored. The evolution of polymerized TAG was followed during heating; and a complete analysis of polar compounds, including total level and distribution in oxidized TAG monomers, TAG dimers and TAG oligomers, was carried out at the end point.

EXPERIMENTAL PROCEDURES

Plant material. Mature seeds were used from the mutant sunflower (*Helianthus annuus* L.) lines CAS-3, CAS-4, and CAS-8, with high 18:0 content, and CAS-5, with high 16:0 content, all from a standard high 18:2 genetic background (8), and the mutant line CAS-12, with high 16:0 content in high 18:1n-9 genetic background (9), obtained from plants grown in southern Spain during the spring of 1997. Seeds from a

conventional sunflower line (SO) and a high 18:1n-9 sunflower line (HOSO) were supplied by Advanta (Marchena, Spain) and used as check lines.

Lipid extraction. The seeds were ground, mixed with anhydrous sodium sulfate, and extracted with petroleum ether (bp 50–70°C) in a Soxhlet apparatus, for 5–7 h, following IUPAC Standard Method 1122 (12).

Purification of TAG. Tocopherol-free TAG were obtained according to a modified procedure of Yoshida *et al.* (13). Total seed lipids (3 g) were mixed with 3 mL petroleum ether and passed over aluminum oxide, which had been activated at 200°C for 3 h immediately before use. The alumina (1.5 g × 2) was placed into two pipette tips connected by a piece of silicone tube, thus forming a double-body column. The lipid solution was placed on the top and allowed to filter through the alumina. The column was washed further with 6 mL petroleum ether. The solvent was evaporated and the purified TAG were flushed with nitrogen and stored at −20°C.

Analytical methods. The purity of the purified TAG was checked by thin-layer chromatography (TLC) on silica gel plates developed with petroleum ether/ethyl ether (9:1, vol/vol). Tocopherol content was determined by high-performance liquid chromatography (HPLC) following IUPAC Standard Method 2432 (12). Fatty acid methyl esters were obtained by transesterification of the TAG sample with methanol/toluene/H_2SO_4 (88:10:2, vol/vol) at 80ºC for 1 h, according to Garcés and Mancha (14). The fatty acid composition of TAG was analyzed by gas–liquid chromatography (GLC) of the corresponding methyl esters using an SP-2380 (Supelco, Bellefonte, PA) capillary column (60 m × 0.25 mm i.d.), 0.2 mm film thickness, hydrogen as the carrier gas, and flame-ionization detector. The fatty acid composition in the *sn*-2 and *sn*-1,3 positions of TAG was determined after partial hydrolysis with pancreatic lipase followed by preparative TLC on silica gel, according to IUPAC Standard Method 2210 (12). Monoacylglycerols and free fatty acid bands were isolated and transmethylated, and their fatty acid composition was determined as described above. Calculations of the composition of TAG molecular species were carried out on the basis of the 1,3-random-2-random distribution model (15), using a macro worksheet for Microsoft Excel 97 (developed by E. Martínez-Force). Polymerized TAG were quantified by high-performance size exclusion chromatography (HPSEC) following IUPAC Standard Method 2508 (12). Total polar compounds (TPC) and their distribution in oxidized TAG monomers, TAG dimers, and TAG oligomers were determined through a combination of adsorption chromatography and HPSEC (16). Conditions applied for HPSEC in both methods were as follows: A Rheodyne 7725i injector (Cotati, CA) with a 10-mL sample loop, a Waters 510 HPLC pump (Waters Associates, Milford, MA), two 100 Å and 500 Å Ultrastyragel columns (Waters Associates) connected in series and operated at 35°C, and a refractive index detector (Hewlett-Packard, Palo Alto, CA) were used. The columns were 25 × 0.77 cm i.d. and packed with a porous, highly cross-linked styrenedivinylbenzene copolymer (<10 μm). HPLC-grade tetrahydrofuran served as the mobile phase with a flow of 1 mL/min. Sample solutions of 50 mg oil/mL and 15 mg polar compounds/mL in tetrahydrofuran were used for the analysis of polymerized TAG and polar compounds distribution, respectively.

Thermal oxidation of TAG samples. Thermoxidative treatment of the samples was carried out in duplicate under strictly controlled conditions with a Rancimat (Metrohm Ltd., Herisau, Switzerland) apparatus (11). Briefly, 2 ± 0.01 g of purified TAG were weighed out in standard glass tubes (13 × 1 cm i.d.), introduced into Rancimat reaction vessels containing 6 g of glycerol to facilitate heat transfer, and inserted in the heating block previously heated to 180 ± 1°C. Single samples of 50 mg were withdrawn at 2-h intervals for the analysis of polymerized TAG. After heating for a total period of 10 h, final samples were taken out and analyzed for polar compounds and their distribution in oxidized TAG monomers, TAG dimers, and TAG oligomers. Rancimat instructions were carefully observed for the cleaning of vessels and for temperature correction. No bubbling of air was applied during heating and the vessels were left open. This procedure closely resembled the usual conditions for discontinuous frying, such as temperature, surface/oil volume ratio, and presence of air. It was described in detail, with data reproducibility information in a recent publication (11).

Statistical methods. Data of total level and distribution of polar compounds, and data of polymerized TAG for thermoxidized samples, are expressed as the mean of two experiments and the standard error of the mean. Differences between samples were assessed with Student's *t* test, with probability values of 5% being statistically different (17).

RESULTS AND DISCUSSION

Characterization of TAG samples. Following purification of the TAG of mutant sunflower seed oils, TLC analysis confirmed the absence of free fatty acids and polar lipids; the only impurities observed were hydrocarbons and waxes normally found in sunflower oils. No traces of tocopherols were observed by HPLC analysis. The fatty acid composition was identical to that of the total seed lipids, indicating that the purification procedure caused no selective loss of TAG species.

The fatty acid compositions of TAG from the mutant seed oil compared to the check lines were very different from each other (Table 1). SO contained 35.4% 18:1n-9 and 51.0% 18:2, HOSO had 87.4% 18:1n-9 and 2.5% 18:2. The high 18:0 mutants (CAS-3, CAS-4, and CAS-8) had high values of 18:0 (from 9.6 to 23.8%, depending on the line), at the expense of 18:1 and 18:2. The two high 16:0 lines (CAS-5 and CAS-12) contained relatively high percentages of palmitoleic acid (16:1) and octadec-11-enoic acid (18:1n-7) compared with the other lines (18). These fatty acids are minor components (0.1 to 3%) of conventional seed oils (2,19). The high 16:0 mutant line from a high 18:2 background (CAS-5) had increased percentages of 16:0 (35.7%), 16:1 (6.3%), hexadec-9,12-dienoic acid (16:2) (1.3%), and 18:1n-7 (4.3%), and a

TABLE 1
Fatty Acid Percentages in Total Triacylglycerols[a]

	Fatty acid composition (mol%)									
Line	16:0	16:1	16:2	18:0	18:1n-9	18:1n-7	18:2	20:0	18:3	22:0
SO	6.3	—[b]	—	4.9	35.4	0.4	51.0	0.3	—	0.8
HOSO	3.7	—	—	3.8	87.4	—	2.5	0.4	0.3	1.1
CAS-3	7.2	—	—	23.8	15.9	0.2	49.5	1.4	—	1.5
CAS-4	6.2	—	—	12.5	35.0	0.3	42.8	0.8	—	1.2
CAS-8	6.9	—	—	9.6	38.9	0.3	41.3	0.7	—	1.5
CAS-5	35.7	6.3	1.3	2.1	5.5	4.3	43.1	0.3	—	0.8
CAS-12	30.2	6.4	—	2.0	52.6	4.2	1.9	0.3	—	1.4

[a]Percentages are for total triacylglycerols of conventional sunflower oil (SO), high-oleic sunflower oil (HOSO) check lines, and high-stearic (CAS-3, CAS-4, and CAS-8), high-palmitic (CAS-5), and high-palmitic, high-oleic (CAS-12) mutant sunflower lines.
[b]Dashes represent values less than 0.2%.

reduced percentage of 18:1n-9 (5.5%). The high 16:0 mutant line from a high 18:1n-9 background (CAS-12) contained increased percentages of 16:0 (30.2%), 16:1 (6.4%), 18:1n-7 (4.2%), and a very low percentage of 18:2 (1.9%).

The fatty acid distribution between the *sn*-2 and *sn*-1,3 positions is shown in Table 2. In all lines, despite the very different fatty acid composition, the saturated fatty acids were nearly absent from the *sn*-2 position (less than 2%), but were located at the *sn*-1,3 positions. The lines with the highest contents of 18:0 (CAS-3) or 16:0 (CAS-5) contained 46.0 and 57.8% saturated fatty acids, respectively, in the *sn*-1,3 positions, as already reported in a previous paper on the characterization of lipid classes of these sunflower mutants (10).

The calculated theoretical content of TAG molecular species in sunflower mutant oils and check line oils is shown in Table 3. These data were calculated according to the 1,3-random-2-random distribution model (15) from the fatty acid composition in the *sn*-2 and *sn*-1,3 positions (Table 2). Thus, each TAG species is given as the sum of the expected isomers (e.g., OLL included LLO and LOL but POP did not include PPO, where L, O, P, Po, and S stand for 18:2, 18:1n-9 and 18:1n-7, 16:0, 16:1, and 18:0, respectively). Previous studies showed that calculation of TAG species through the distribution model applied was in good agreement with the direct determination by GLC (20).

Great differences were observed in the TAG composition of the different mutants and the check lines. In SO, the main TAG species were OLL, OOL, and LLL, whereas OOO was particularly abundant in high-oleic sunflower oil (HOSO) (72.2%). SLL was present in increased amounts in the high 18:0 lines, especially in CAS-3 (20.0%). PLL, PLP, and POL were greater in the high 16:0 line (CAS-5), as compared with the check lines. The greatest levels of POO and POP were found in the high 16:0 from the high 18:1n-9 background (CAS-12), which also contained the unusual species POPo (9.1%) and OOPo (7.5%) and a low concentration of OOO (14.5%), in spite of the high 18:1n-9 percentage of this line (Table 1). The unique TAG composition of the mutant lines

TABLE 2
Fatty Acid Percentages in the *sn*-2 and *sn*-1,3 Positions of Triacylglycerols[a]

Line	Position	16:0	16:1	18:0	18:1n-9	18:1n-7	18:2
SO	*sn*-2	0.8	—[b]	0.6	38.7	—	59.9
	sn-1,3	9.3	—	6.6	37.2	0.6	45.9
HOSO	*sn*-2	0.8	—	0.5	96.1	—	2.6
	sn-1,3	5.3	—	4.9	86.7	—	2.7
CAS-3	*sn*-2	0.5	—	0.9	22.8	—	75.8
	sn-1,3	10.6	—	35.4	14.7	—	37.3
CAS-4	*sn*-2	0.7	—	0.8	45.1	—	53.4
	sn-1,3	9.4	—	19.4	35.4	—	34.5
CAS-8	*sn*-2	0.5	—	0.4	44.9	—	54.2
	sn-1,3	9.8	—	11.3	38.2	—	39.7
CAS-5	*sn*-2	1.8	4.4	—	9.6	1.7	81.2
	sn-1,3	54.4	8.0	3.4	4.7	5.7	22.0
CAS-12	*sn*-2	1.2	2.9	0.2	89.3	3.5	2.8
	sn-1,3	45.7	9.6	3.0	35.3	4.2	1.5

[a]Values were obtained after partial enzymatic hydrolysis, as described in the Experimental Procedures section. See Table 1 for abbreviations.
[b]Dashes represent values less than 0.2%.

TABLE 3
Major Triacylglycerol Molecular Species (%) from Seeds of Standard and Mutant Sunflower Lines, as Calculated from Data in Table 2

	SO	HOSO	CAS-3	CAS-4	CAS-8	CAS-5	CAS-12
POP[a]	0.3	0.3	0.3	0.4	0.4	3.3	19.8
POPo	—[b]	—	—	—	—	1.5	9.1
PLP	0.5	—	0.9	0.5	0.5	24.4	0.6
PLPo	—	—	—	—	—	8.2	0.2
POS	0.5	0.5	1.7	1.6	1.0	0.4	2.5
POO	2.7	8.8	0.7	3.0	3.4	1.3	33.5
PLS	0.7	—	5.7	1.9	1.2	3.0	—
OOPo	—	—	—	—	—	—	7.5
POL	7.8	0.5	4.2	6.5	7.6	11.9	2.3
PLL	5.1	—	6.0	3.5	4.2	21.4	—
LLPo	—	—	—	—	—	2.9	—
SOS	—	—	2.9	1.7	0.6	—	—
SOO	1.9	8.6	2.4	6.2	3.9	—	2.2
SLS	0.3	—	9.5	2.0	0.7	—	—
OOO	5.5	72.2	0.5	5.7	6.6	—	14.5
SOL	5.3	0.5	13.9	13.3	8.7	0.6	—
OOL	22.0	6.5	4.1	17.7	21.5	1.4	1.5
SLL	3.6	—	20.0	7.1	4.9	1.2	—
OLL	29.0	—	11.5	18.4	23.5	4.2	—
LLL	12.6	—	10.5	6.4	8.5	3.9	—

[a]P, 16:1; O, 18:1, n-9 and n-7; Po, 16:1; S, 18:0; and L, 18:2. For other abbreviations see Table 1.
[b]Dashes represent values less than 0.2%.

was reflected in the fatty acid distribution in TAG molecules and, particularly, in the high content of saturated acids in the *sn*-1,3 position.

Thermoxidative stability of TAG samples. The thermoxidative treatment of the purified TAG, heated at 180°C for 10 h, gave rise to the formation of the different types of polar compounds shown in Table 4. In starting TAG, polar compounds were not found in detectable amounts, as expected, after purification through the alumina column (data not shown). Given that samples were devoid of the natural antioxidants of sunflower oils (tocopherols), the results obtained after the thermoxidative treatment can be directly correlated with the fatty acid composition and, more specifically, with the composition of TAG molecular species of the different lines. As expected, SO contained the highest content of TPC (24.6%), mainly as TAG dimers and oligomers, which corresponded to the more advanced steps of oxidation–polymerization. The TAG of this line contained 51% 18:2 and 35.8% 18:1n-9 + 18:1n-7, the main TAG species being OLL, OOL, and LLL. These molecules are susceptible to oxidation in more than one of the three fatty acyl residues and thus are easily polymerized.

The HOSO line, with 87.4% 18:1n-9 and only 2.5% 18:2 and OOO as the main TAG, contained significantly less TPC (21.3%), mainly because of the significantly decreased content of TAG oligomers. However, in general, total levels of polar compounds and, specifically, oxidized TAG monomers, of the lines with highly saturated fatty acid composition from a high 18:2 background (CAS-3, CAS-4, CAS-8, and CAS-5) did not significantly differ from those of either SO or HOSO. Overall, there was a lower tendency for polymerization in the

TABLE 4
Quantitation of Total Polar Compounds (TPC) in Triacylglycerols (TAG)[a]

	Polar compounds (wt%)			
Line	Total	Ox-TAG monomers	TAG dimers	TAG oligomers
SO	24.6 ± 0.4^{a}	9.7 ± 0.1^{a}	10.2 ± 0.1^{a}	4.7 ± 0.4^{a}
HOSO	21.3 ± 0.1^{b}	10.7 ± 0.2^{b}	7.8 ± 0.1^{b}	2.9 ± 0.2^{b}
CAS-3	$21.6 \pm 0.7^{a,b}$	$9.2 \pm 0.5^{a,b,c}$	$8.7 \pm 0.2^{c,d}$	3.7 ± 0.1^{a}
CAS-4	$21.4 \pm 1.0^{a,b}$	$8.9 \pm 0.7^{a,b,c}$	$8.5 \pm 0.5^{a,b,c,d}$	$3.7 \pm 0.2^{a,b}$
CAS-8	$22.3 \pm 0.7^{a,b}$	9.5 ± 0.1^{a}	9.3 ± 0.1^{c}	3.7 ± 0.1^{a}
CAS-5	21.2 ± 0.4^{b}	$8.9 \pm 0.7^{a,b,c}$	8.6 ± 0.2^{d}	3.7 ± 0.5^{ab}
CAS-12	12.9 ± 0.6^{c}	6.8 ± 0.5^{c}	4.7 ± 0.2^{a}	1.5 ± 0.1^{c}

[a]Oils from conventional and mutant sunflower lines were heated at 180°C for 10 h. Polar compounds are expressed as wt% (mean ± SEM, $n = 2$ thermoxidation experiments). Values in columns with different superscript roman letters differ significantly ($P < 0.05$). Ox-TAG, oxidized TAG; see Table 1 for other abbreviations.

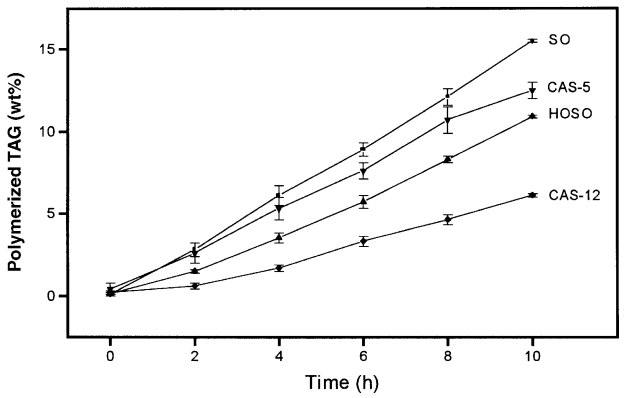

FIG. 1. Evolution of polymerized triacylglycerol (TAG) formation in purified TAG heated to 180°C. Abbreviations for sunflower lines: ■, conventional sunflower oil (SO); ▲, high-oleic sunflower oil (HOSO); ▼, high-palmitic (CAS-5); ◆, high-palmitic, high-oleic (CAS-12). Results are expressed as wt% (means ± SEM; n = 2 thermoxidation experiments).

former, indicating that the saturated fatty acid content of these lines compensated for the relatively high levels of 18:2 (41.3–49.5%). The double mutant CAS-12, with 32.2% saturated acids (mainly 16:0), 56.8% 18:1n-9 and 18:1n-7, and only 1.9% 18:2, contained half the TPC (12.8%) of SO and also had significantly lower amounts than those of HOSO. This substantial decrease in polar compounds was mainly accounted for by the lowest content of all altered groups, especially dimers and oligomers.

The time course for formation of polymerized TAG (dimers + oligomers) during thermoxidation is shown in Figure 1 for samples SO, HOSO, CAS-5, and CAS-12. Results of CAS-4, CAS-8, and CAS-3 were very similar to those of CAS-5 and were therefore omitted. A linear accumulation of polymers was observed in all lines up to 10 h. The results for polymerized TAG were in agreement with those for dimers plus oligomers (Table 4), thus confirming that independent determinations resulted in similar response factors. Clearly, SO was most susceptible to polymerization, whereas the evolution of polymers in CAS-12 differed significantly from the others at any point in the heating treatment, and showed by far the lowest levels. Also, the lines with a high saturated fatty acid percentage from a conventional background and HOSO behaved similarly throughout the heating period.

The resistance of TAG to polymerization was primarily attributable to the fatty acid composition of TAG. As a rule, the more unsaturated the fatty acid composition of TAG, the higher the susceptibility to polymerization. The greatest content of polar compounds, especially polymers, was found in SO, which contained 18:2 and 18:1n-9 as the main fatty acids. High 18:1n-9 contents gave rise to a reduction of polar compounds, mainly polymers, and the presence of saturated fatty acids also increased the thermoxidation resistance. Thus, behavior was similar for HOSO and the lines containing greater amounts of saturated fatty acids and of the highly unsaturated 18:2 (CAS-3, CAS-4, CAS-8, and CAS-5).

In CAS-12, the combination of a high saturated fatty acid content and a high 18:1n-9 background considerably enhanced resistance to the formation of polar compounds. A low amount of oxidized TAG monomers was formed in CAS-12, but the greatest reduction was observed in polymerized TAG, mainly in oligomers.

The oils from the highly saturated fatty acid sunflower lines would be appropriate for specific applications in the food industry that require considerable thermal stability. Particularly, the excellent characteristics of CAS-12 oil, liquid at room temperature and highly stable at frying temperature, make it a promising alternative for high-performance frying operations.

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REFERENCES

1. Willet, W.C., and A. Ascherio, *Trans* Fatty Acids: Are the Effects Only Marginal? *Am. J. Public Health 84*:722–724 (1994).
2. Gunstone, F.D., J.L. Harwood, and F.B. Padley, *The Lipid Handbook*, Chapman & Hall, London, 1994, pp. 123–124.
3. Bracco, U., Effect of Triglyceride Structure on Fat Absorption, *Am. J. Clin. Nutr. 60*, 1002s–1009s (1994).
4. Renaud, S.C., J.C. Ruf, and D. Petithory, The Positional Distribution of Fatty Acids in Palm Oil and Lard Influences Their Biologic Effect in Rats, *J. Nutr. 125*:229–237 (1995).
5. Dobarganes, M.C., G. Márquez-Ruiz, and M.C. Pérez-Camino, Thermal Stability and Frying Performance of Genetically Modified Sunflower Seeds (*Helianthus annuus* L.) Oils, *J. Agric. Food Chem. 41*:678–681 (1993).
6. Dobson, G., W.W. Christie, and M.C. Dobarganes, Changes in Molecular Species of Triacylglycerols During Frying, *Grasas Aceites 47*:34–37 (1996).
7. Jorge, N., L. Ap. Guaraldo Goncalvez, and M.C. Dobarganes, Influence of Fatty Acid Composition on the Formation of Polar Glycerides and Polar Fatty Acids in Sunflower Oils Heated at Frying Temperatures, *Grasas Aceites 48*:17–24 (1997).
8. Osorio, J., J. Fernández-Martínez, M. Mancha, and R. Garcés, Mutant Sunflower with High Concentration of Saturated Fatty Acids in the Oil, *Crop Sci. 35*:739–742 (1995).
9. Fernández-Martínez, J.M., M. Mancha, J. Osorio, and R. Garcés, Sunflower Mutant Containing High Levels of Palmitic Acid in High Oleic Background, *Euphytica 97*:113–116 (1997).
10. Álvarez-Ortega, R., S. Cantisán, E. Martinez-Force, and R. Garcés, Characterization of Polar and Nonpolar Seed Lipid Classes from Highly Saturated Fatty Acid Sunflower Mutants, *Lipids 32*: 833–837 (1997).
11. Barrera-Arellano, D., G. Márquez-Ruiz, and M.C. Dobarganes, A Simple Procedure to Evaluate the Performance of Fats and Oils at Frying Temperatures, *Grasas Aceites 48*:231–235 (1997).
12. *International Union of Pure and Applied Chemistry (IUPAC) Standard Methods for the Analysis of Oils, Fats and Derivatives*, 1st Supplement to the 7th Revised and Enlarged edn., Blackwell Scientific Publications, Oxford, 1992, Methods 1122, 2432, 2210, and 2508.
13. Yoshida, H., I. Kondo, and G. Kajimoto, Participation of Free Fatty Acids in the Oxidation of Purified Soybean Oil During Microwave Heating, *J. Am. Oil Chem. Soc. 69*:1136–1140 (1992).

14. Garcés, R., and M. Mancha, One Step Lipid Extraction and Methyl Esters Preparation from Fresh Plant Tissues, *Anal. Biochem. 211*:139–143 (1993).
15. 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. 37*: 18–20 (1960).
16. Dobarganes, M.C., M.C. Pérez-Camino, and G. Márquez-Ruiz, High-Performance Size Exclusion Chromatography of Polar Compounds in Heated and Non-Heated Fats, *Fat Sci. Technol. 90*:308–311 (1988).
17. Yamane, T., *Statistics*, 3rd edn., Harper and Row Publishers, New York, 1973, pp. 647–675.
18. Martínez-Force, E., R. Álvarez-Ortega, and R. Garcés, Enzymatic Characterization of High-Palmitic-Acid Sunflower (*Helianthus annuus* L.) Mutants, *Planta 207*:527–532 (1999).
19. Kleiman, R., and K.L. Payne-Wahl, Fatty Acid Composition of Seed Oils of the Meliaceae, Including One Genus Rich in *cis*-Vaccenic Acid, *J. Am. Oil Chem. Soc. 61*:1836–1838 (1984).
20. Carelli, A.A., and A. Cert, Comparative Study of the Determination of Triacylglycerol in Vegetable Oils Using Chromatographic Techniques, *J. Chromatogr. 630*:213–222 (1993).

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