Stereospecific Analysis of Triacylglycerols from Vegetable Oils by Two Procedures—II: Normal and High-Oleic Sunflower Oils

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ABSTRACT: Triacylglycerol stereospecific analysis of normal (NOS) and high-oleic sunflower (HOS) oils was carried out by two procedures to study the influence of variety and growing conditions. Four cultural varieties, two NOS and two HOS, were grown in seven different places of Italy. Three of the four varieties were grown both in dry conditions and with irrigation. Concerning the triacylglycerol fatty acid compositions, the results showed no significant differences between irrigated and nonirrigated samples ($P > 0.05$), between the two NOS, and between the two HOS varieties. Between NOS and HOS varieties, only stearic acid showed no significant differences ($P > 0.05$). The fatty acid compositions of the *sn*-2 position of NOS and HOS samples showed different percentage abundances ($P < 0.01$), especially for oleic and linoleic acids. Fatty acid distributions in the *sn*-1 and *sn*-3 positions indicated a certain asymmetry. The relationships between the percentage intrapositional content of each acid (one *sn*-position at a time) and its percentage content in the original triacylglycerol matrix were studied. A general regression model was used to verify if the content of each acid at the three stereospecific positions changed at the same rate as the content in the intact triacylglycerols. The interpositional compositions of all varieties of NOS and HOS oils showed analogous trends for each acid.
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KEY WORDS: Normal and high-oleic sunflower oils, stereospecific analysis, triacylglycerols.

Information concerning the structure of triacylglycerols (TAG) can play a leading role in the characterization of vegetable oils. Some procedures for stereospecific analysis of TAG have been pointed out recently (1,2), and others, based on stereospecific phosphorylation of *sn*-1,2-diacylglycerols (*sn*-1,2-DAG), have been reconsidered (3). Two methods that were previously compared (4) gave satisfactory results when applied to TAG from olive oil. Two procedures (4) were also applied to evaluate the TAG structure of sunflower oils [normal (NOS) and high-oleic (HOS)]. To determine the influence of both variety and growing conditions on TAG structure, 20 oil samples of NOS and 20 oil samples of HOS varieties, produced in seven different locations in Italy, were considered. Four varieties, two NOS and two HOS, were grown in each place. All experiments were carried out without irrigation, except for three locations where varieties were grown both under dry conditions and with irrigation. The first method for the stereospecific analysis of TAG (procedure A) was based on partial deacylation of TAG with ethyl magnesium bromide, followed by separation of *sn*-1,2(2,3)-DAG with thin-layer chromatography (TLC) on boric acid-impregnated silica and stereospecific phosphorylation of *sn*-1,2-DAG to phosphatidic acids (PA) with an *sn*-1,2-DAG kinase (*sn*-1,2-DAGK) preparation from *Escherichia coli*. The PA were then isolated by TLC on silica, and the fatty acid (FA) compositions were determined by high-resolution gas chromatography (HRGC) (3). The FA composition of the *sn*-2 position was determined by partial hydrolysis of TAG *sn*-2-monoacylglycerols (*sn*-2-MAG) *via* pancreatic lipase hydrolysis (5). The second method (procedure B) also started with partial deacylation of TAG with ethyl magnesium bromide, followed by derivatization of the total DAG fraction with (*S*)-(+)-1-(1-naphthyl)ethyl isocyanate, purification of the products on octadecylsilyl solid-phase extraction columns, and separation of the diastereomeric *sn*-1,2(2,3)-DAG urethane derivatives by high-performance liquid chromatography (HPLC) on silica. The two fractions were then transesterified and analyzed by HRGC to determine the FA compositions (2).

MATERIALS AND METHODS

The sunflower varieties used were Gloriasol (NOS), Select (NOS), Marko (HOS), and Platon (HOS). The plot locations (all in Italy) and their abbreviations are as follows: Cesa (CE), Grosseto (GR), Lonigo (LO), Papiano (PA), Pisa (PI), Rieti (RI), and S. Apollinare (SA); the SA sample of the Marko variety was not available. Trials were organized in split-plot layout 25 with five replications. Irrigation treatments were in the whole plots and varieties in the subplots. Plots were drip-irrigated twice (at the beginning of flowering and after 20 d), with 600 m^3/ha of water each time. For the chemical analy-

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sis, a combined seed sample of the five replications was used in each trial; for this reason, no statistical test could be applied to verify differences in FA compositions due to different locations. All solvents and reagents were Analar or HPLC grades (BDH Italia, Milan, Italy). Sunflower oils were extracted from each seed sample (20 g) with tetrachloroethylene (120 mL) in a Foss Electric 15310 Foss-let homogenizer–extractor apparatus (A/S N. Foss Electric, Hillerød, Denmark), which was operated under standard conditions. The TAG fractions (*ca.* 70 mg) were obtained by TLC separation as previously described (4), and the A and B procedures were also performed as previously reported (4). Only the more abundant FA (palmitic, stearic, oleic, and linoleic), representing more than 97% of the total acids, were considered. Analyses were repeated at least twice, and reported composition data are the mean values.

Student's *t* test (6) was used to evaluate the differences between FA compositions of TAG (total and positional) of the different samples (type: 3, heteroscedastic; tails: 2) and to compare the two procedures A and B (type: 1, paired; tails: 2).

RESULTS AND DISCUSSION

The FA compositions of TAG (corresponding to oil compositions) from NOS and HOS samples are shown in Table 1, and the mean values for all samples and for both irrigated and nonirrigated samples are reported in Table 2.

Slight differences were observed between irrigated and nonirrigated crops of each variety, but they were not sufficient to demonstrate, for each acid, the unequivocal behaviors in the three locations; in this regard, the *t* tests showed no significant differences ($P > 0.05$) for any of the fatty acids or considered varieties. This was in accordance with previously reported data (7,8). Some authors have reported that the FA composition of the TAG fraction depends substantially on environmental conditions (9,10). The results obtained in this research showed percentage abundances of oleic acid in the TAG fraction of NOS Gloriasol variety ranged from 26.8 to 41.8% and similarly for the NOS Select variety (27.3 to 43.9%). However, these data are not sufficient to demonstrate that the observed differences solely depend on the place of origin; no statistical analyses of the data were possible to test differences due to location. For HOS varieties, TAG oleic acid abundances were, with one exception, greater than 80% (ranges: from 74.8 to 88.1% for HOS Marko and from 80.8 to 88.5% for HOS Platon). Obviously, the comparison between TAG FA compositions of all samples of the two NOS and of the two HOS varieties showed significant differences ($P < 0.01$) for palmitic, oleic and linoleic acids, but this occurrence was not observed for stearic acid ($P > 0.05$). As regards the comparison of TAG FA compositions between Glo-

TABLE 1
Fatty Acid Compositions of Triacylglycerols (TAG) (mol% of the total) by Variety and Place of Origin in Italy[a]

NOS Gloriasol										
FA	CE	LO	RI	SA	GR	GRI[b]	PA	PAI[b]	PI	PII[b]
$C_{16:0}$	7.3[c] ± 0.1[d]	5.9 ± 0.1	7.3 ± 0.1	6.8 ± 0.1	7.5 ± 0.3	6.9 ± 0.1	7.3 ± 0.1	7.2 ± 0.7	5.3 ± 0.3	5.0 ± 0.3
$C_{18:0}$	4.7 ± 0.1	3.5 ± 0.1	3.8 ± 0.1	4.2 ± 0.1	3.5 ± 0.1	4.1 ± 0.1	4.3 ± 0.1	4.3 ± 0.1	3.3 ± 0.3	3.6 ± 0.3
$C_{18:1}$[e]	26.8 ± 0.1	41.8 ± 0.1	28.3 ± 0.1	31.8 ± 0.1	30.6 ± 0.1	32.8 ± 0.1	27.7 ± 0.1	30.6 ± 0.3	40.4 ± 0.3	39.1 ± 0.4
$C_{18:2}$	61.2 ± 0.1	48.8 ± 0.1	60.5 ± 0.1	57.2 ± 0.1	58.4 ± 0.1	56.2 ± 0.1	60.7 ± 0.1	57.9 ± 0.3	51.0 ± 0.3	52.3 ± 0.4
NOS Select										
FA	CE	LO	RI	SA	GR	GRI	PA	PAI	PI	PII
$C_{16:0}$	6.5 ± 0.1	5.9 ± 0.1	7.7 ± 1.0	6.2 ± 0.1	6.1 ± 0.1	6.1 ± 0.3	6.4 ± 0.1	6.2 ± 0.1	4.9 ± 0.1	5.2 ± 0.1
$C_{18:0}$	5.0 ± 0.1	3.8 ± 0.1	4.3 ± 0.2	4.6 ± 0.1	3.8 ± 0.1	4.1 ± 0.1	4.7 ± 0.1	4.5 ± 0.1	3.5 ± 0.1	3.5 ± 0.1
$C_{18:1}$[e]	29.4 ± 0.7	37.2 ± 0.1	27.3 ± 0.4	33.1 ± 0.1	42.1 ± 0.1	41.3 ± 0.2	31.7 ± 0.1	33.0 ± 0.1	43.9 ± 0.1	39.1 ± 0.1
$C_{18:2}$	59.1 ± 0.8	53.2 ± 0.1	60.7 ± 0.4	56.0 ± 0.1	48.0 ± 0.1	48.4 ± 0.1	57.3 ± 0.1	56.3 ± 0.1	47.8 ± 0.1	52.1 ± 0.1
HOS Marko										
FA	CE	LO	RI	GR	GRI	PA	PAI	PI	PII	
$C_{16:0}$	4.2 ± 0.1	3.8 ± 0.1	4.4 ± 0.2	4.7 ± 0.5	3.8 ± 0.1	4.0 ± 0.1	4.0 ± 0.2	3.2 ± 0.1	3.5 ± 0.2	
$C_{18:0}$	4.9 ± 0.1	4.5 ± 0.1	3.6 ± 0.1	4.3 ± 0.1	4.7 ± 0.1	4.2 ± 0.1	4.6 ± 0.2	3.4 ± 0.1	3.7 ± 0.3	
$C_{18:1}$[e]	82.9 ± 0.2	74.8 ± 0.1	84.0 ± 0.2	87.9 ± 0.3	7.1 ± 0.1	85.3 ± 0.1	81.8 ± 0.4	87.4 ± 0.1	88.1 ± 0.4	
$C_{18:2}$	8.0 ± 0.1	17.0 ± 0.1	8.1 ± 0.1	3.1 ± 0.1	4.5 ± 0.1	6.5 ± 0.1	9.6 ± 0.1	5.9 ± 0.1	4.7 ± 0.4	
HOS Platon										
FA	CE	LO	RI	SA	GR	GRI	PA	PAI	PI	PII
$C_{16:0}$	4.1 ± 0.1	3.3 ± 0.1	3.7 ± 0.2	3.7 ± 0.1	4.0 ± 0.2	3.8 ± 0.1	3.8 ± 0.1	3.6 ± 0.1	3.3 ± 0.4	3.7 ± 0.1
$C_{18:0}$	5.3 ± 0.1	3.8 ± 0.1	4.6 ± 0.1	4.6 ± 0.1	4.7 ± 0.1	4.6 ± 0.1	5.0 ± 0.1	4.8 ± 0.1	3.7 ± 0.4	3.9 ± 0.1
$C_{18:1}$[e]	80.8 ± 0.2	86.4 ± 0.1	86.6 ± 0.2	88.4 ± 0.1	88.5 ± 0.3	87.5 ± 0.1	86.1 ± 0.2	86.4 ± 0.1	87.3 ± 0.4	86.9 ± 0.4
$C_{18:2}$	9.8 ± 0.1	6.5 ± 0.1	5.1 ± 0.1	3.3 ± 0.1	2.8 ± 0.1	4.1 ± 0.1	5.1 ± 0.1	5.1 ± 0.1	5.7 ± 0.4	5.5 ± 0.4

[a]NOS, normal oleic acid; HOS, high-oleic acid; FA, fatty acid; CE, Cesa; LO, Lonigo; RI, Rieti; SA, S. Apollinaire; GR, Grosseti; PA, Papiano; PI, Pisa.
[b]GRI, PAI and PII are results from irrigated plots.
[c]Average (three experimental values).
[d]Standard deviation, %.
[e]Sum of positional isomers n-9 and n-7.

TABLE 2
Fatty Acid Composition of TAG (mol% of the total) by Variety

		NOS				HOS			
		Gloriasol		Select		Marko		Platon	
FA	Samples	$\bar{x}^b$	CV^c	$\bar{x}$	CV	$\bar{x}$	CV	$\bar{x}$	CV
$C_{16:0}$	AS	6.7	13.7	6.1	12.3	4.0	11.5	3.7	7.0
	NI	6.8		6.2		4.1		3.7	
	I	6.4		5.8		3.8		3.7	
$C_{18:0}$	AS	3.9	11.6	4.2	12.5	4.2	12.6	4.5	11.8
	NI	3.9		4.2		4.2		4.5	
	I	4.0		4.0		4.3		4.4	
$C_{18:1}$	AS	33.0	16.6	35.8	15.9	84.4	5.0	86.5	2.5
	NI	32.5		35.0		83.7		86.3	
	I	34.2		37.8		85.7		86.9	
$C_{18:2}$	AS	56.4	7.7	53.9	8.8	7.5	54.9	5.3	36.5
	NI	56.8		54.6		8.1		5.5	
	I	55.5		52.3		6.3		4.9	

[a]Data presented as averages for all samples (AS) and for nonirrigated (NI) and irrigated (I) samples.
[b]Average of the relative samples.
[c]Coefficient of variation, %; reported only for AS because of the low numbers within both NI and I groups.
[d]Sum of positional isomers n-9 and n-7. For other abbreviations see Table 1.

riasol and Select NOS varieties, no significant differences were detected ($P > 0.05$) for any of the FA; the same results were obtained for the comparison between Marko and Platon HOS varieties.

Table 3 contains the FA intrapositional compositions of all samples analyzed by procedures A and B. The FA compositions of *sn*-2-MAG obtained *via* pancreatic lipase hydrolysis, i.e., procedure A, from TAG extracted from NOS oils were in accordance with the literature data (2,3); the *sn*-2-MAG fractions obtained with procedure A from HOS oils showed FA compositions with an equally low percentage abundance of saturated FA (palmitic and stearic). Moreover, linoleic acid abundances in the *sn*-2 position of TAG from NOS were higher in comparison with TAG linoleic contents (Δ = 15.3%), while oleic acid contents did not show substantial changes (Δ = 2.9%); Δ is defined as:

$$\Delta = \frac{\bar{x}\text{MAG} - \bar{x}\text{TAG}}{(\bar{x}\text{MAG} + \bar{x}\text{TAG})/2} \bullet 100 \quad [1]$$

The TAG from HOS oils in the same *sn*-2 position had greater percentage abundances of oleic acid vs. TAG oleic percentage contents (Δ = 9.1%), while linoleic acid contents did not show substantial differences (Δ = 1.3%). This means that the percentage content of each of these acids in the *sn*-2 position increased only when it was also the most abundant in the same TAG.

The FA compositions at the *sn*-1 and *sn*-3 positions of NOS TAG, obtained *via* procedure A, were also in accordance with previously reported data (3). On the average, there was a slight relative preference for palmitic and linoleic acids for the *sn*-1 position as well as for oleic acid for the *sn*-3 position; stearic acid was present in the *sn*-3 position nearly twice as often as in the *sn*-1 position. In particular, the comparison of percentage abundances in *sn*-1 and *sn*-3 positions showed significant differences ($P < 0.01$) for all FA of the NOS Gloriasol variety for palmitic and stearic acids ($P < 0.01$) and for oleic and linoleic acids ($P < 0.05$) of the NOS Select variety.

Regarding the results of HOS TAG by procedure A, the percentage abundance of palmitic acid in *sn*-1 was greater than in the *sn*-3 position ($P < 0.01$), while the opposite situation was observed for stearic acid ($P < 0.01$); these trends reflect those of NOS TAG. Unlike NOS TAG, oleic acid showed a preference for *sn*-1 with respect to the *sn*-3 position, while linoleic acid did not show unequivocal behavior; for these acids, no significant differences were observed ($P > 0.05$).

Similar results were obtained via procedure B; in Table 4, the FA compositions of the *sn*-1,2-PA and *sn*-1,2-DAG urethane derivatives of NOS and HOS TAG oils are compared by computation of Student's *t*-test probabilities for two sets of data derived from the same samples by using the two different procedures (4). For a confidence level of 95% and 8 degrees of freedom (the analyses of the five samples used for this comparison were carried out five times), the maximum *t* value is 2.306 for the obtained values. All FA of both NOS and HOS oils gave results of no significant differences when determined as *sn*-1,2-PA or *sn*-1,2-DAG urethane derivatives (a similar test was obviously impossible for *sn*-2,3-DAG urethane derivatives because the *sn*-2,3-PA were not available as intermediate products of procedure A). The only exception was relative to stearic acid for NOS oils, which showed a *t*-test value = 2.824. The evaluations relative to the obtained results allow the conclusion that procedure A seemed to be better for stereospecific analysis of NOS TAG oils: one possible explanation of this situation was the more difficult separation of *sn*-1,2-DAG from *sn*-2,3-DAG urethane derivatives for NOS TAG oils with respect to analogous classes for HOS TAG oils (4). Moreover, procedure B can accumulate the maximum error by computation effects (2,4) and is more time-consuming than procedure A. For these reasons, the

TABLE 3
Comparison between A and B Procedures for Determination of Fatty Acid Intrapositional Compositions (mol% of the total)[a]

	FA	Procedure[b]	CE	LO	RI	SA	GR	GRI	PA	PAI	PI	PII	$\bar{x}$[c]	CV[d]
NOS Gloriasol														
sn-1	$C_{16:0}$	A[e]	13.0	10.9	12.1	13.4	13.6	13.4	14.1	12.5	10.8	8.4	12.2	14.2
		B[f]	13.1	11.6	11.6	11.5	15.7	11.0	12.7	12.5	9.1	8.3	11.7	17.7
	$C_{18:0}$	A	4.8	3.9	4.2	4.4	3.3	4.7	4.6	4.3	3.1	3.7	4.1	14.3
		B	3.9	3.6	3.2	3.4	2.9	3.4	3.2	4.2	2.5	3.2	3.4	14.4
	$C_{18:1}$[g]	A	22.9	41.4	25.7	23.1	25.1	28.5	24.6	25.6	30.8	30.8	27.9	19.9
		B	25.4	34.1	23.2	24.4	37.9	26.5	22.2	25.5	31.8	31.4	28.2	18.4
	$C_{18:2}$	A	59.3	43.8	58.0	59.1	58.0	53.4	56.8	57.5	55.3	57.1	55.8	8.2
		B	57.6	50.7	62.0	60.6	43.5	59.0	61.9	57.8	56.6	57.1	56.7	10.0
sn-2	$C_{16:0}$	A and B[h]	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.2	0.2	16.6
	$C_{18:0}$	A and B	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	35.1
	$C_{18:1}$[g]	A and B	26.8	42.3	27.7	32.2	29.7	33.0	27.5	30.9	38.1	41.1	32.9	17.3
	$C_{18:2}$	A and B	72.9	57.4	72.1	67.5	70.0	66.7	72.2	68.9	61.8	58.7	66.8	8.5
sn-3	$C_{16:0}$	A[i]	8.7	6.7	9.6	6.7	8.7	7.0	7.7	9.1	5.0	6.4	7.6	19.1
		B[j]	8.6	6.0	10.1	8.6	6.5	9.3	9.0	9.1	6.7	6.5	8.0	18.2
	$C_{18:0}$	A	9.3	6.6	7.3	8.2	7.1	7.5	8.1	8.5	6.8	7.2	7.7	11.0
		B	10.2	6.8	8.3	9.1	7.5	8.8	9.5	8.6	7.4	7.7	8.4	12.6
	$C_{18:1}$[g]	A	30.6	41.7	31.6	40.1	37.0	37.1	31.1	35.2	52.3	45.3	38.2	18.1
		B	28.2	48.90	34.2	38.8	24.3	39.0	33.4	35.3	51.3	44.8	37.8	22.8
	$C_{18:2}$	A	51.3	45.1	51.5	45.0	47.2	48.4	53.1	47.3	36.0	41.1	46.6	11.1
		B	53.0	38.2	47.4	43.5	61.6	42.8	48.0	47.0	34.7	41.0	45.7	16.8
NOS Select														
	FA	Procedure[b]	CE	LO	RI	SA	GR	GRI	PA	PAI	PI	PII	$\bar{x}$[c]	CV[d]
sn-1	$C_{16:0}$	A[e]	11.3	11.6	11.6	12.2	11.1	11.0	10.6	12.6	8.8	9.2	11.0	11.0
		Bf	11.1	9.4	11.0	10.5	10.3	10.8	11.9	10.0	9.2	9.1	10.3	8.8
	$C_{18:0}$	A	4.3	4.4	4.3	4.6	3.8	4.1	4.7	4.6	2.9	3.3	4.1	14.5
		B	4.0	2.6	4.0	3.7	2.6	4.1	3.8	3.4	3.0	3.0	3.4	16.8
	$C_{18:1}$[g]	A	22.7	37.8	22.5	24.4	41.1	38.5	27.2	27.6	28.6	29.6	30.0	22.6
		B	50.3	23.2	22.3	26.7	33.1	36.4	22.7	24.5	34.1	29.9	30.3	28.6
	$C_{18:2}$	A	61.7	46.1	61.5	58.8	44.0	46.4	57.5	55.2	59.7	57.9	54.9	12.3
		B	36.4	64.8	62.7	59.1	54.1	48.8	61.5	62.1	53.7	58.0	56.1	15.1
sn-2	$C_{16:0}$	A and B[h]	0.3	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.2	23.6
	$C_{18:0}$	A and B	0.1	0.1	0.1	0.1	0.0	0.0	0.1	0.1	0.0	0.0	0.1	86.1
	$C_{18:1}$[g]	A and B	32.3	39.9	29.5	36.2	44.5	45.0	34.8	35.8	44.9	37.4	38.0	14.3
	$C_{18:2}$	A and B	67.3	59.9	70.2	63.6	55.3	54.8	64.9	63.9	54.9	62.5	61.7	8.7
sn-3	$C_{16:0}$	A[i]	7.9	5.9	11.2	6.3	6.9	7.1	8.3	6.0	5.7	6.3	7.2	23.2
		B[j]	8.1	8.1	11.8	8.0	7.8	7.3	7.1	8.5	5.3	6.4	7.8	21.5
	$C_{18:0}$	A	10.5	6.8	8.5	9.2	7.7	8.2	9.2	8.6	7.4	7.2	8.3	13.4
		B	10.8	8.6	8.9	10.0	8.9	8.3	10.0	9.9	7.5	7.5	9.0	12.9
	$C_{18:1}$[g]	A	33.2	33.9	30.0	38.7	40.6	40.6	33.0	35.4	58.1	50.5	39.4	22.2
		B	?7.0	48.5	30.3	36.5	48.7	42.6	37.5	38.6	52.7	50.2	39.3	34.2
	$C_{18:2}$	A	48.5	53.5	50.2	45.8	44.7	44.2	49.5	49.9	28.7	36.1	45.1	16.6
		B	73.9	34.8	49.1	45.5	34.6	41.8	45.4	43.1	34.5	35.9	43.9	27.0
HOS Marko														
	FA	Procedure[b]	CE	LO	RI	GR	GRI	PA	PAI	PI	PII	$\bar{x}$[c]	CV[d]	
sn-1	$C_{16:0}$	A[e]	8.3	6.7	6.7	9.3	7.1	7.4	6.3	5.5	7.9	7.2	15.7	
		B[f]	7.1	6.1	6.8	9.8	6.4	6.8	6.6	11.3	7.2	7.6	23.3	
	$C_{18:0}$	A	4.7	4.1	3.3	4.6	4.2	4.0	4.1	3.0	3.4	3.9	14.8	
		B	3.8	3.2	5.5	3.3	3.1	3.1	4.0	3.1	2.8	3.5	23.2	
	$C_{18:1}$[g]	A	79.9	72.9	82.9	81.6	84.3	81.2	79.0	85.3	83.0	81.1	4.5	
		B	84.6	97.1	79.4	83.9	88.3	87.7	88.9	82.4	87.7	86.7	5.8	
	$C_{18:2}$	A	7.2	16.2	7.1	4.4	4.3	7.3	10.6	6.2	5.7	7.7	48.4	
		B	4.5	–6.3	8.4	2.9	2.3	2.5	0.5	3.4	2.2	2.3	171.5	
sn-2	$C_{16:0}$	A and B[h]	0.1	0.1	0.1	0.1	0.2	0.0	0.0	0.1	0.1	0.1	73.2	
	$C_{18:0}$	A and B	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	282.8	
	$C_{18:1}$[g]	A and B	92.0	82.0	92.4	97.2	94.8	93.8	90.3	94.1	95.1	92.4	4.7	
	$C_{18:2}$	A and B	7.9	17.9	7.6	2.6	5.0	6.2	9.7	5.8	4.8	7.5	58.7	
sn-3	$C_{16:0}$	A[i]	4.1	4.5	6.4	4.7	3.8	4.5	5.7	–4.2	2.5	4.5	24.7	
		B[j]	5.4	5.2	6.3	4.1	4.8	5.1	5.4	–1.6	3.2	4.2	55.8	
	$C_{18:0}$	A	10.1	9.4	7.5	8.3	9.6	8.7	9.7	7.2	7.6	8.7	12.4	
		B	10.9	10.3	5.3	9.5	10.9	9.5	9.8	7.3	8.1	9.1	20.4	

(continued)

TABLE 3 (continued)

	FA	Procedure[b]	CE	LO	RI	SA	GR	GRI	PA	PAI	PI	PII	$\bar{x}$[c]	CV[d]
HOS Marko														
	$C_{18:1}$[g]	A	76.9	69.5	76.7		84.9	77.4	80.9	76.2	82.8	86.3	79.1	6.6
		B	72.1	45.1	80.2		82.4	78.1	74.5	66.2	85.8	81.6	74.0	16.7
	$C_{18:2}$	A	8.9	16.9	9.4		2.3	3.9	6.0	8.4	5.8	3.6	7.2	60.7
		B	11.6	39.4	8.2		3.8	6.2	10.8	18.6	8.6	7.1	12.7	85.5
HOS Platon														
	FA	Procedure[b]	CE	LO	RI	SA	GR	GRI	PA	PAI	PI	PII	$\bar{x}$[c]	CV[d]
sn-1	$C_{16:0}$	A[e]	6.9	5.9	6.1	6.0	9.4	6.0	6.6	5.9	5.7	6.0	6.5	17.0
		B[f]	6.4	5.8	5.6	6.6	8.7	6.6	6.0	6.4	7.0	5.6	6.5	14.1
	$C_{18:0}$	A	4.9	3.5	4.1	3.7	4.1	4.0	4.5	4.0	2.9	3.2	3.9	15.2
		B	4.5	3.2	3.0	4.2	3.4	2.8	4.3	4.2	2.8	2.4	3.5	21.7
	$C_{18:1}$[g]	A	80.0	85.9	85.4	86.9	82.6	85.8	83.6	81.6	85.9	86.6	84.4	2.8
		B	85.8	80.9	85.9	85.5	84.7	87.0	86.1	86.0	88.2	91.5	86.2	3.1
	$C_{18:2}$	A	8.1	4.6	4.4	3.4	3.9	4.2	5.4	8.5	5.5	4.2	5.2	33.4
		B	3.3	10.1	5.5	3.7	3.1	3.6	3.6	3.4	2.0	0.5	3.9	65.2
sn-2	$C_{16:0}$	A and B[h]	0.1	0.1	0.1	0.0	0.1	0.1	0.0	0.0	0.1	0.1	0.1	69.0
	$C_{18:0}$	A and B	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.1	0.0	161.0
	$C_{18:1}$[g]	A and B	89.8	93.8	95.3	97.3	97.8	96.1	95.1	95.2	94.0	94.0	94.8	2.3
	$C_{18:2}$	A and B	10.0	6.2	4.6	2.7	2.0	3.8	4.9	4.8	5.9	5.9	5.1	43.5
sn-3	$C_{16:0}$	A[i]	5.3	3.9	4.8	5.2	2.5	5.2	4.9	5.0	4.1	5.0	4.6	18.9
		B[j]	5.8	4.1	5.3	4.5	3.2	4.6	5.5	4.5	2.8	5.4	4.6	21.7
	$C_{18:0}$	A	11.0	7.8	9.7	10.1	10.0	9.8	10.6	10.4	8.3	8.3	9.6	11.3
		B	11.4	8.1	10.8	9.6	10.7	11.1	10.7	10.1	8.3	9.1	10.0	11.7
	$C_{18:1}$[g]	A	72.5	79.7	79.1	81.0	85.0	80.7	79.5	82.6	82.0	80.3	80.2	4.0
		B	66.8	84.7	78.8	82.4	82.9	79.4	76.9	78.2	79.7	75.3	78.5	6.4
	$C_{18:2}$	A	11.2	8.6	6.4	3.7	2.5	4.3	5.0	2.0	5.7	6.4	5.6	49.9
		B	16.0	3.1	5.3	3.5	3.3	4.9	6.8	7.2	9.1	10.1	6.9	57.6

[a]For abbreviations see Tables 1 and 2. [b]Procedures A and B are described in Reference 4. [c]Average of results relative to all places. [d]Coefficient of variation %. [e]2 × 1,2 phosphatidic acid (PA)-monoacylglycerol (MAG). [f]2 × 1,2diacylglycerol-MAG. [g]Sum of positional isomers n-9 and n-7. [h]Values obtained by pancreatic lipase procedure. [i]3 × TAG – 2 × 1,2PA. [j]3 × TAG – 2 × 1,2DAG.

TABLE 4
Comparison of Fatty Acid Compositions (mol% of the total) of *sn*-1,2-PA and *sn*-1,2 DAG Urethane Derivatives for TAG of NOS and HOS Varieties[b]

NOS FA							P[c]	t Value[d]
$C_{16:0}$	*sn*-1,2-PA	5.61	7.12	4.33	6.18	5.57	0.27670	1.167
	sn-1,2-DAG	5.92	6.48	4.23	5.44	5.51		
$C_{18:0}$	*sn*-1,2-PA	2.03	2.31	1.88	2.43	2.15	0.02298	2.806
	sn-1,2-DAG	1.84	1.63	1.58	1.92	2.01		
$C_{18:1}$[e]	*sn*-1,2-PA	41.88	25.96	36.04	30.34	41.68	0.30959	1.085
	sn-1,2-DAG	38.22	24.83	36.18	31.38	40.69		
$C_{18:2}$	*sn*-1,2-PA	50.58	64.52	57.88	61.23	50.62	0.10396	1.834
	sn-1,2-DAG	54.07	67.13	57.89	61.27	51.76		
HOS FA							P[c]	t Value[d]
$C_{16:0}$	*sn*-1,2-PA	3.43	2.76	3.54	5.43	2.98	0.66731	0.446
	sn-1,2-DAG	3.13	5.74	3.18	4.43	3.27		
$C_{18:0}$	*sn*-1,2-PA	2.02	1.46	2.48	3.23	2.14	0.11653	1.760
	sn-1,2-DAG	1.64	1.48	2.33	1.68	1.43		
$C_{18:1}$[e]	*sn*-1,2-PA	77.47	89.73	84.93	86.56	90.89	0.18037	1.468
	sn-1,2-DAG	89.56	88.23	87.78	91.32	91.56		
$C_{18:2}$	*sn*-1,2-PA	16.98	6.04	9.13	4.69	4.40	0.13956	1.640
	sn-1,2-DAG	5.77	4.64	6.73	2.56	3.73		

*a*For abbreviations see Table 1 and 3. [b]Values indicated with two decimal places to minimize oversimplification introduced by rounding effect. [c]*t*-Test probablity for the two sets of data. [d]Inverse of the Student's *t* distributions. [e]Sum of positional isomers n-9 and n-7.

TABLE 5
Comparison of Fatty Acid Interpositional Distributions (Average Values) for TAG of NOS and HOS Varieties

		NOS				HOS			
		Gloriasol		Select		Marko		Platon	
FA	Position	$\bar{x}$[a]	CV[b]	$\bar{x}$	CV	$\bar{x}$	CV	$\bar{x}$	CV
$C_{16:0}$	sn-1	61.3	6.8	60.1	7.9	61.4	11.8	57.9	12.2
	sn-2	1.0	17.8	1.1	20.2	0.8	66.6	0.6	66.1
	sn-3	37.8	10.8	38.8	12.3	37.8	19.5	41.4	17.3
$C_{18:0}$	sn-1	34.5	6.4	32.8	8.7	31.1	5.6	28.7	5.5
	sn-2	0.8	35.4	0.5	71.3	0.1	282.8	0.2	154.7
	sn-3	64.7	3.3	66.7	4.5	68.8	2.5	71.1	2.3
$C_{18:1}$[c]	sn-1	28.1	8.5	27.9	12.9	32.1	1.9	32.5	2.0
	sn-2	33.2	2.8	35.5	4.0	36.6	1.0	36.5	1.0
	sn-3	38.6	6.8	36.6	11.6	31.3	2.4	30.9	2.0
$C_{18:2}$	sn-1	33.0	6.0	34.0	9.9	35.6	15.1	34.4	27.2
	sn-2	39.5	1.9	38.2	1.7	33.1	7.7	31.2	10.7
	sn-3	27.5	6.9	27.8	13.3	31.3	14.4	34.4	23.9

[a]Average of all samples. [b]Coefficient of variation, %. [c]Sum of positional isomers n-9 and n-7. For abbreviations see Table 1.

compositions of the *sn*-2-positions used for both procedures A and B were obtained *via* pancreatic lipase hydrolysis. The results reported in Table 3 show that comparable relative intrapositional trends were generally obtained by the A and B procedures, even if the cited compositions were sometimes different. Some negative or anomalous values, obtained via procedure B, can be explained by the HPLC separation problems of *sn*-1,2-DAG from *sn*-2,3-DAG urethane derivatives and/or by computation errors (4).

All of these considerations suggest that procedure A can be used for all types of oils, whereas better experimental conditions for the HPLC separation of *sn*-1,2-DAG from *sn*-2,3-DAG urethane derivatives need to be developed for an equally extensive application of procedure B.

Further considerations must regard the distribution of each FA in the three positions of TAG ("percentage interpositional compositions," obtained by using data from procedure A for the cited reasons) for all considered varieties. As shown, the CV values [coefficient of variation % = (standard deviation/mean value) · 100] were generally not higher than 20, and the trend of each of these compositions was similar (Table 5). The high values of CV in Table 5 are to be ignored because they are relative to low abundances of the saturated FA in the *sn*-2 position.

A statistical evaluation of the relationships between the percentage intrapositional content of each acid (one *sn* position at a time), obtained *via* procedure A, and its percentage content in TAG was made; this general regression model was a statistical elaboration of the percentage intrapositional content of each acid in each of the three positions and its percentage content in the total TAG of all samples (NOS and HOS varieties). This model is shown in Table 6 in which all regression parameters (intercept and slope, together with their 95% confidence limits and their *P* values, correlation coefficient—CC—and r^2) are also shown. In Figure 1, a plot of the relationship $C_{18:1sn\text{-}2}/C_{18:1TAG}$ is shown. Because the CC values

TABLE 6
General Regression Model: $X_{sn\text{-}j} = I + S \cdot X_{TAG}$[a]

			I				S					
FA	*sn-j*	X_{TAG}	Value	1.1[b]	u.l.[b]	*P*[c]	Value	1.1[b]	u.l.[b]	*P*[c]	CC[d]	r^{2e}
$C_{16:0}$	*sn*-1	$C_{16:0}$	–0.02	–1.11	1.07	0.96782	1.81	1.61	2.02	0.00000	0.947	89.65
	sn-2	$C_{16:0}$	–0.06	–0.12	–0.00	0.04348	0.04	0.03	0.05	0.00000	0.758	57.47
	sn-3	$C_{16:0}$	0.05	–1.05	1.16	0.91992	1.16	0.95	1.36	0.00000	0.881	77.66
$C_{18:0}$	*sn*-1	$C_{18:0}$	0.60	–0.40	1.61	0.23136	0.81	0.57	1.05	0.00000	0.750	56.20
	sn-2	$C_{18:0}$	*	*	*	*	*				*	
	sn-3	$C_{18:0}$	–0.60	–1.67	0.47	0.26270	2.18	1.93	2.43	0.00000	0.944	89.18
$C_{18:1}$[f]	*sn*-1	$C_{18:1}$	–7.05	–9.51	–4.58	0.00000	1.05	1.01	1.09	0.00000	0.994	98.83
	sn-2	$C_{18:1}$	–3.49	–4.72	–2.26	0.00000	1.14	1.12	1.15	0.00000	0.999	99.75
	sn-3	$C_{18:1}$	10.70	7.67	13.74	0.00000	0.81	0.76	0.86	0.00000	0.985	97.07
$C_{18:2}$	*sn*-1	$C_{18:2}$	0.16	–1.54	1.88	0.84512	1.00	0.96	1.04	0.00000	0.992	98.37
	sn-2	$C_{18:2}$	1.32	–1.94	0.70	0.00012	1.19	1.17	1.20	0.00000	0.999	99.85
	sn-3	$C_{18:2}$	1.11	–0.69	2.91	0.22058	0.81	0.77	0.86	0.00000	0.986	97.29

[a]Variables: $X_{sn\text{-}j}$, % of *X* acid in *sn-j*-positive; *I,* intercept of the representative curve; *S,* stage of the representative curve; X_{TAG}, % of *X* acid in TAG. [b]95% confidence interval (1.1 = lower limit, u.l. = upper limit); [c]Result of a *t* statistic to test whether the true value of the coefficient is equal to zero. [d]Correlation coefficient. [e]Regression coefficient, squared. [f]Sum of positional isomers n-9 and n-7. *, not determined because values not detectable.

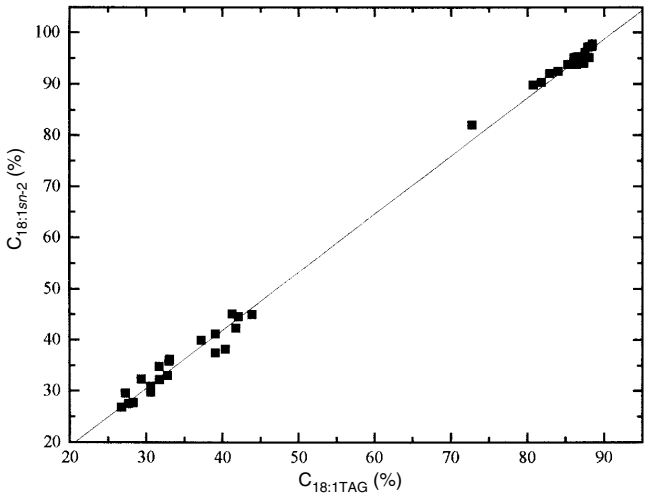

FIG. 1. Plot of the regression of the percentage in trapositional content of each acid ($C_{18:1sn\text{-}2}$) *vs.* its percentage content in triacylglycerol ($C_{18:ITAG}$).

are generally higher than 0.750, and often higher than 0.944, it is possible to conclude that this general model is valid to estimate the percentage intrapositional content of each of the three positions for each acid when only knowing its percentage content in intact TAG. With the appropriate regression model, at least the stereospecific trend of any TAG sunflower matrix could be predicted with the HRGC analysis of the TAG fraction.

These results seem to show that the stereospecificity of the biosynthethic steps leading to the TAG of sunflower oils are not influenced by variety and/or growing conditions.

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