Stereospecific Analysis of TAG from Sunflower Seed Oil

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ABSTRACT: Stereospecific analysis of TAG from a sunflower seed oil of Tunisian origin was performed. The TAG were first fractionated according to chain length and degree of unsaturation by RP-HPLC. The four major diacid- and triacid-TAG fractions were palmitoyldilinoleoyl-glycerol, dioleoyllinoleoylglycerol, oleoyldilinoleoylglycerol, and palmitoyloleoyl-linoleoylglycerol, amounting to 7.2, 16.6, 29.5, and 12 mol%, respectively. The TAG of the four fractions were individually submitted to stereospecific analysis, using a Grignard-based partial deacylation, separation of *sn*-1,2(2,3)-DAG from *sn*-1,3-DAG by boric acid-impregnated silica gel TLC plates, conversion of the *sn*-1,2(2,3)-DAG to their 3,5-dinitrophenylurethane (DNPU) derivatives, fractionation of DNPU derivatives by RP-HPLC, resolution of the DNPU-DAG by HPLC on a chiral column, transmethylation of each *sn*-DNPU-DAG fraction, and analysis of the resulting FAME by GC. The data obtained were used to determine the triacyl-*sn*-glycerol composition of the main TAG of the oil. Fifteen triacyl-*sn*-glycerols were identified and quantified, representing, along with the monoacid-TAG, trilinoleoylglycerol and trioleoylglycerol, more than 90% of the total oil TAG. The two major triacyl-*sn*-glycerols were trilinoleoyl-glycerol and 1-linoleoyl-2-linoleoyl-3-oleoyl-glycerol (18.6 and 18.5% of the total, respectively). Results clearly identified linoleic acid as the major FA at the *sn*-2 position, whereas oleic and palmitic acids were the major FA at the *sn*-3 position. The *sn*-1 position was occupied to nearly the same extent by linoleic and oleic acids, and to a greater extent by palmitic acid, which was practically absent at the *sn*-2 position.

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Stereospecific analysis of TAG from seed oils can be used to establish the structure of triacyl-*sn*-glycerols (*sn*-TAG) and, from these data, to detect adulteration or alteration of oils or to tentatively elucidate the mechanisms of *sn*-TAG biosynthesis in seeds.

The first stereospecific analysis of *sn*-TAG was reported by Brockerhoff (1). It was later improved by other workers (2,3). These methods were enzymatic and involved the stereospecific lipolysis or esterification of DAG or their derivatives to discriminate between stereoisomers. New methods, based on chemical steps, have been developed more recently. These methods involve partial chemical deacylation to produce the enantiomeric *sn*-1,2(2,3)-DAG, which are separated by HPLC, either as diastereoisomers on a silica column or, more currently, as 3,5-dinitrophenylurethane (DNPU) derivatives on a chiral column (4,5). However, authors generally prefer to use the simpler method based on the separation of *sn*-1 and *sn*-3-MAG by HPLC on a chiral column (6), sometimes in combination with MS (7,8).

Using these enzymatic and chemical methods, several authors, usually working on oil total TAG, have reported the distribution of the component FA between the *sn*-1, *sn*-2, and *sn*-3 positions of oil *sn*-TAG, e.g., Damiani *et al.* (9) for sunflowerseed oils. However, when starting from less complex TAG fractions separated from total TAG, a few workers have been able to determine triacyl-*sn*-TAG composition more precisely by using either an enzymatic method (10) or a chemical method (11). In this paper, we report such a determination for a sunflower seed oil, which is more precise than our previous work (12).

EXPERIMENTAL PROCEDURES

Samples. Sunflower seeds of the Albena variety were provided by the Institut National de Recherches Agronomiques de Tunis. Chemicals were supplied by Merck (Darmstadt, Germany). Total lipids were extracted by the Soxhlet method with petroleum ether. The TAG were isolated by silicic acid column chromatography and their purities controlled by TLC on silica gel plates using hexane/ethyl ether/acetic acid (90:30:1, by vol) as developing solvent. They were analyzed by GC for FA composition.

Analytical methods. The analytical and calculation methods used in this work have been described previously (11,13). Briefly, sunflower seed oil TAG were fractionated by RP-HPLC. The four main diacid- and triacid-TAG compositions were identified by GC of their FAME on glass capillary columns coated with Carbowax 20M (Applied Science, State College, PA). They were: OLL, OOL, POL, and PLL (O: oleic, L: linoleic, and P: palmitic acids). The two monoacid-TAG, LLL and OOO, comprised of only one *sn*-TAG, were not further analyzed. TAG of the four complex fractions were partially deacylated with Grignard reagent to generate DAG. The *sn*-1,2(2,3)-DAG fraction was separated on TLC silica gel plates impregnated with boric acid and converted to DNPU derivatives. The DNPU derivatives were fractionated, according to chain length and degree of unsaturation, by preparative

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TABLE 1
Enantiomeric Composition of the *sn*-1,2(2,3)-DAG Resulting from Chemical Deacylation of Diacid- and Triacid-TAG Isolated from Sunflower Seed Oil[a]

TAG	PLL				OOL			
Mol%[b]	7.23				16.56			
sn-1,2(2,3)-DAG	LL		PL		OL		OO	
Mol%[c]	44.61		55.39		76.52		23.48	
Enantiomers	*sn*-1,2	*sn*-2,3	*sn*-1,2	*sn*-2,3	*sn*-1,2	*sn*-2,3	*sn*-1,2	*sn*-2,3
Mol%[d]	55.88	44.12	42.41	57.59	57.21	42.79	28.53	71.43

TAG	OLL				POL					
Mol%[b]	29.47				11.99					
sn-1,2(2,3)-DAG	LL		OL		OL		PL		PO	
Mol%[c]	43.52		56.48		48.52		27.53		23.94	
Enantiomers	*sn*-1,2	*sn*-2,3	*sn*-1,2	*sn*-2,3	*sn*-1,2	*sn*-2,3	*sn*-1,2	*sn*-2,3	*sn*-1,2	*sn*-2,3
Mol%[d]	72.63	27.37	33.63	66.37	40.85	59.15	54.60	45.40	64.57	35.43

[a]Analyses were not duplicated because of the good reproducibility observed with the techniques used. See Experimental Procedures section for explanation.
[b]Mol% of the total TAG.
[c]Mol% in the fraction.
[d]Mol% in the *sn*-1,2(2,3)-DAG. L, linoleic acid; O, oleic acid; P, palmitic acid.

RP-HPLC. Each fraction collected was submitted to chiral-phase HPLC to separate the *sn*-1,2- and *sn*-2,3-DAG, then identified and quantified by GC of their component FA as methyl esters. A calculation method was applied to these data to determine the *sn*-TAG composition of the four analyzed TAG fractions.

The analytical methods used in this work (13) were developed using a great variety of TAG mixtures from commercial and natural origins. Results obtained showed great reproducibility. These methods, first applied to peanut oil and cottonseed oil TAG, are directly applicable to TAG mixtures extracted from other oils. This was verified by several authors for other stereospecific analyses of oil TAG (1–5).

RESULTS

Our results (12), obtained on sunflower seed oil, showed that the total TAG FA were L (56.4 mol%), O (32.0 mol%), P (5.7%), and stearic (4.2%) acids.

Table 1 reports the percentage in total TAG of the four TAG fractions submitted to stereospecific analysis, the composition of *sn*-1,2(2,3)-DAG formed by chemical deacylation, and the enantiomer composition of these DAG. OLL is the major TAG, amounting to nearly 30% of the total, reflecting the high level of O and L in the oil (together representing near 90% of the total FA). This is followed by OOL (16.6%), also reflecting the high content of O and L.

The reported molar percentages of the two or three enantiomeric DAG formed from each TAG fraction show that marked differences exist in the second fraction between OL and OO and also in the fourth one between OL and the other two, PL and PO. Great differences between enantiomers, such as OO, LL, OL and PO, reveal a pronounced asymmetry in the distribution of FA at the three positions of the glycerol moiety in TAG.

In Table 2 is reported the triacyl-*sn*-glycerol composition of the four fractions analyzed. Obviously, the three or six triacyl-*sn*-glycerols present in the four fractions of TAG were found in very different proportions. In the three first fractions containing diacid-glycerols, the major stereoisomer had L in the central (*sn*-2) position of the glycerol moiety. In the triacid-TAG fraction (POL), the preference for the *sn*-2 position was shared by L and O. In this fraction, the two triacyl-*sn*-glycerols with P at the *sn*-2 position were present at <2%. These data indicate a clear preference for the *sn*-2 position of L, followed to a lesser extent by O.

In Table 3 are listed the 17 triacyl-*sn*-glycerols found in the oil TAG studied, taking into account the monoacid-TAG

TABLE 2
Triacyl-*sn*-glycerol Composition of the Four Fractions of TAG Analyzed[a]

TAG	PLL		OOL		OLL		POL	
Mol%[b]	7.23		16.57		29.47		11.99	
Triacyl-*sn*-glycerols	PLL	37.67	OOL	33.55	OLL	24.10	POL	28.40
	LLP	51.55	LOO	13.41	LLO	62.94	LOP	15.06
Mol%[c]	LPL	10.78	OLO	53.04	LOL	12.96	PLO	29.04
							OLP	24.54
							OPL	1.17
							LPO	1.79

[a]Analyses were not duplicated because of the good reproducibility observed with the techniques used. See Experimental Procedures section for explanation.
[b]Mol% in total oil TAG.
[c]Mol% in the TAG fraction. For TAG abbreviations see Table 1.

TABLE 3
Major Triacyl-*sn*-glycerols of Sunflower Seed Oil[a,b]

Triacyl-*sn*-glycerols	Mol% in the oil
LLL: trilinoleoyl-*sn*-glycerol	18.6
LLO: 1-linoleoyl-2-linoleoyl-3-oleoyl-*sn*-glycerol	18.5
OLO: 1-oleoyl-2-linoleoyl-3-oleoyl-*sn*-glycerol	8.8
OLL: 1-oleoyl-2-linoleoyl-3-linoleoyl-*sn*-glycerol	7.1
OOL: 1-oleoyl-2-oleoyl-3-linoleoyl-*sn*-glycerol	5.5
OOO: trioleoyl-*sn*-glycerol	4.9
LOP: 1-linoleoyl-2-oleoyl-3-palmitoyl-*sn*-glycerol	4.8
LOL: 1-linoleoyl-2-oleoyl-3-linoleoyl-*sn*-glycerol	3.8
LLP: 1-linoleoyl-2-linoleoyl-3-palmitoyl-*sn*-glycerol	3.7
PLO: 1-palmitoyl-2-linoleoyl-3-oleoyl-*sn*-glycerol	3.5
POL: 1-palmitoyl-2-oleoyl-3-linoleoyl-*sn*-glycerol	3.4
OLP: 1-oleoyl-2-linoleoyl-3-palmitoyl-*sn*-glycerol	2.9
PLL: 1-palmitoyl-2-linoleoyl-3-linoleoyl-*sn*-glycerol	2.7
LOO: 1-linoleoyl-2-oleoyl-3-oleoyl-*sn*-glycerol	2.2
LPL: 1-linoleoyl-2-palmitoyl-3-linoleoyl-*sn*-glycerol	0.8
LPO: 1-linoleoyl-2-palmitoyl-3-oleoyl-*sn*-glycerol	0.2
OPL: 1-oleoyl-2-palmitoyl-3-linoleoyl-*sn*-glycerol	0.1

[a]Analyses were not duplicated because of the good reproducibility observed with the techniques used. See Experimental Procedures section for explanation.
[b]For abbreviations see Table 1.

(LLL and OOO). The two major triacyl-*sn*-glycerols, present in nearly the same percentage, were LLL and LLO, with L at the *sn*-2 position. They together represent more than one-third of the total. Twelve stereoisomers were found in percentages of 1–10%. Their total amounted to a little more than 50%. The three last stereoisomers, with P at the *sn*-2 position, were present at <1% each.

The percentage distribution of the three major FA are reported in Table 4 for each position, along with the percentage distribution of each FA between the three positions. L was the major FA at each position. O was found more frequently at the two external positions, with a slight preference for the *sn*-3 position. P was almost equally distributed between the *sn*-1 and *sn*-3 positions and practically absent from the *sn*-2 position. Data in the second part of Table 4 confirm the preferential location of L at the *sn*-2 position. O and P showed a slight preference for the *sn*-3 position as compared to the *sn*-1 position. These data should be considered in comparison with the FA composition of total TAG (Table 4, last column), in particular the high L content and to a lesser extent the O content.

DISCUSSION

Few publications have dealt with the composition in triacyl-*sn*-glycerols of seed oils. Generally, the stereospecific studies have been concerned solely with determination of FA composition at *sn*-1, *sn*-2, and *sn*-3 positions of the total oil TAG. This type of study is much less time consuming and provides insight into the structure of the oil triacyl-*sn*-glycerols, but it does not permit a complete knowledge of the triacyl-*sn*-glycerol composition of the oil.

A work similar to that presented in this paper has been published concerning peanut oil (11). It also reported the percentages of 17 triacyl-*sn*-glycerols. But the data obtained in these two studies cannot easily be compared because of the very different FA compositions of peanut oil, rich in O, and sunflower oil, rich in L. The major triacyl-*sn*-glycerol of peanut oil was trioleoyl-*sn*-glycerol, whereas trilinoleoyl-*sn*-glycerol was the major stereoisomer of sunflower oil. Both studies found that triacyl-*sn*-glycerols with P were in very low percentages at the *sn*-2 position.

Comparison of data obtained in this study with those for cottonseed oil (11) is more instructive because of the relative similarity in the FA compositions of both oils, namely, a high L content (56.4 and 46.4%, respectively). In previous works, only the cottonseed oil TAG fraction POL was analyzed by an enzymatic method (10) and by a chemical method (11), together with peanut oil POL. The three sets of data were relatively similar. In particular the two major stereoisomers (PLO, OLP) were the same in the three cases, as were the minor ones (OPL, LPL) with P at the *sn*-2 position.

The minor differences observed can be due to differences in FA compositions between the two types of seed oils.

Although few works have determined the triacyl-*sn*-glycerol composition of seed oils, a number of publications have reported the FA composition at the three positions of the glycerol moiety in total TAG. As pointed out above, comparisons between data can be undertaken only when FA compositions are similar. This is obviously the case with data obtained by Damiani *et al.* (9) on a "normal" seed oil of the sunflower variety Gloriasol. The FA compositions of both oils were very similar. The distribution of L among the three positions was practically the same, with a decided preference first for the *sn*-2 position, then for the *sn*-1 position. Oleic acid was, in both cases, preferentially found at the *sn*-3 position, and then at the *sn*-1. The preferential location of O at the *sn*-1 or the *sn*-3 positions was very marked in both oils. P, which was present in very low percentages in both oils, was nearly absent from the *sn*-2 position, confirming the well-known property of plant lipids that saturated FA are practically absent at the *sn*-2 position of plant TAG (1). In both oils, the preferen-

TABLE 4
Distribution of the Three Major FA Between the Three Positions of Triacyl-*sn*-glycerols of Sunflower Seed Oil[a,b]

	FA composition (mol%) of the three positions			% Distribution of FA between the three positions			FA composition
FA	*sn*-1	*sn*-2	*sn*-3	*sn*-1	*sn*-2	*sn*-3	of total oil TAG (mol%)
L	52.6	65.8	42.0	32.8	41.0	26.2	56.4
O	29.3	24.6	38.1	31.8	26.7	41.5	32.0
P	9.6	1.1	11.4	43.4	5.0	51.6	5.7

[a]Analyses were not duplicated because of the good reproducibility observed with the techniques used. See Experimental Procedures section for explanation.
[b]For abbreviations see Table 1.

tial location of P between the *sn*-1 or *sn*-3 position was not very pronounced. However, the *sn*-1 position was favored in oil of Italian origin, whereas the reverse was true in this work for Tunisian oil. The different geographic origins may explain this slight difference.

Other seed oils rich in L have been analyzed stereospecifically to determine FA intrapositional composition. In the past, analyses were carried enzymatically, using in particular Brockerhoff's method (1). Results concerning several vegetable oils have been reported (13). Soybean oil and corn oil have FA compositions close to that sunflower oil. The intrapositional compositions of the FA are similar, L being preferentially esterified at the *sn*-2 position and O at the *sn*-3 position, with P being equally distributed between the two external positions. The preferential esterification of L at the *sn*-2 position was recently confirmed by regiospecific analysis of corn and soybean oils (14), and of grapeseed and cottonseed oils (15), along with the relatively uniform distribution of O between the internal and the two external positions. The near absence of P at the *sn*-2 position was also confirmed.

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