Positional Analysis and Determination of Triacylglycerol Structure of *Argania spinosa* Seed Oil

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The distribution of fatty acids between the sn-1, sn-2 and sn-3 positions of triacylglycerols from *Argania spinosa* seed oil of Morocco has been determined. Saturated fatty acids showed a preference for external positions. The sn-1 position contained slightly more palmitic acid than the sn-3 position, whereas stearic acid was preferentially esterified at the sn-3 position. Linoleic acid occurred predominantly in the sn-2 position with lesser amount evenly distributed between the sn-1 and the sn-3 positions, as generally found in vegetable oils. Oleic acid was distributed with a slight preference shown for the internal position, whereas the distribution between the external positions revealed a slight preference for the sn-1 position. The distribution of the triacylglycerols determined from high-performance liquid chromatography (HPLC) is at variance with that calculated from the 1-random 2-random 3-random distribution theory. This is particularly true for trioleoyl and trilinoleoylglycerols. In contrast, the agreement between theory and experiment is good for triacylglycerols containing two oleoyl and one linoleoyl chains, one oleoyl, one linoleoyl and one palmitoyl chains or one oleoyl, one palmitoyl, and one stearoyl chains.

KEY WORDS: Argan oil, *Argania spinosa*, positional analysis, stereospecific analysis, triacylglycerol.

Oil extracted from seeds of *Argania spinosa* L., Sapotaceae (or Argan oil) is used commonly as a food oil in southern Morocco. This oil contains significant amounts of tocopherol (1), and oleic and linoleic acids (1–3). Analyses of the triacylglycerol classes of Argania seed oil performed by gas-liquid chromatography (GLC) and high-performance liquid chromatography (HPLC) have been reported (1–3).

In this study, additional results on the triacylglycerol composition of this oil are reported, and the positional distribution of fatty acids in the triacylglycerol species is presented. A comparative discussion between the composition of triacylglycerol determined by HPLC and that calculated according to the 1-random, 2-random, 3-random distribution theory is also presented.

EXPERIMENTAL PROCEDURES

Samples of *Argania* oil used for this study were prepared in the laboratory. Seeds (100 g) were dried at 40°C, crushed in a coffee mill and extracted with hexane in a Soxhlet apparatus. Hexane was evaporated and the residual material (46 g) was solubilized in 700 mL of chloroform.

To separate the triacylglycerol fraction, an aliquot of the chloroform solution (15 mL) containing 1 g of extracted material was filtered through a column made with 30 g of silica gel with 5% moisture. Triacylglycerols were eluted with 250 mL of benzene. Further purification of the triacylglycerols was achieved by thin-layer chromatography on 0.5-mm silica plates (20 × 20 cm), which were developed with hexane-diethylether-formic acid (65:33.5:1.5, v/v/v). Triacylglycerols were located by spraying the plate with 2,7-dichlorofluorescein (0.1%) in ethanol. Identified fractions were extracted with 15 mL of hexane. To avoid autoxidation, storage and handling of all samples were done, whenever possible, under nitrogen.

The method introduced by Brockerhoff (4) was used to determine the composition of fatty acids esterified at the sn-1, sn-2 and sn-3 positions of triacylglycerols (4–9). According to this method (4,8,9), 1,2- and 2,3-diacylglycerols and 2-monoacylglycerols are prepared by pancreatic lipase deacylation of triacylglycerols. Fatty acid composition at the internal position is obtained by transmethylation of the 2-monoacylglycerols followed by gas chromatography (GC) analysis of the methyl ester derivatives. In the mixture of phosphatidylphenols obtained from the diacylglycerols (8–10), the 1,2-diacylphosphatidylphenols are hydrolyzed by use of pancreatic phospholipase A_2, which allows determination of the fatty acids at position sn-1 and provides additional information about fatty acids at the sn-2 position. Fatty acids at the sn-3 position are determined by difference from the known fatty acid composition of the triacylglycerol fraction.

Deacylation of triacylglycerols by pancreatic lipase was performed according to the method of Deroanne *et al.* (11). Triacylglycerols (20 mg) were placed in a glass tube with 2 mL of 1.2 M NH_4Cl buffer (pH 8), 1 mL of 5% $CaCl_2$ and 40 mg of commercial porcine pancreatic lipase (Merck Inc., Darmstadt, Germany) in 1 mL of distilled water. The tube was sealed and stirred gently. After 5 min, 1 mL of 6M HCl was added and the reaction mixture was extracted with $CHCl_3$-CH_3OH (2:1). The extract was dried over Na_2SO_4 and the solvent was evaporated. Lipids were dissolved with 0.1 mL of chloroform and streaked on a 0.5-mm thin-layer chromatographic (TLC) silica plate. The plate was developed with the following solvent system: petroleum ether-diethylether-formic acid (60:38.5:1.5, v/v/v). The 2-acylglycerols (located by spraying 2,7-dichlorofluorescein in ethanol) and the mixture of sn-1,2 and sn-2,3 diacylglycerols (located by transmitted light) were extracted from the plate with diethylether-methanol (9:1, v/v). The 2-monoacylglycerols were transesterified with methanolic sodium methoxyde (1N), and the resulting methyl esters were analyzed by GLC in a Hewlett-Packard (Palo Alto, CA) chromatograph equipped with a capillary column impregnated with butanediol succinate (BDS) 8%, 45 m in length and 0.5 mm internal diameter. The analysis conditions were: 180°C oven, 250°C injector, 250°C detector, nitrogen gas vector, flow rate 1.1 mL/min. Identification of the GLC peaks was achieved by the methods described by Mallet *et al.* (12), and yielded the fatty acid composition at the sn-2 position of the original triacylglycerols. The fraction containing sn-1,2 and sn-2,3 diacylglycerols was dissolved in 1 mL

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TABLE 1

Distribution of Fatty Acids in *Argania spinosa* Oil Triacylglycerols and Comparison with Soybean, Maize, Olive and Peanut Oils

		Fatty acid distribution (mole %)										
		C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C22:0	C24:0
Argan	Total[a]	<0.1	12.5	0.4	6.0	42.8	37.4	0.1	0.5	0.1		
	sn-1		20.2	0.3	3.5	42.7	33.1	<0.1	0.2	0.1		
	sn-2	<0.1	3.5	0.1	0.3	51.0	44.8	0.1	0.1	0.1		
	sn-3		13.7	0.8	14.2	34.7	34.2	<0.1	1.2	0.1		
Soya[b]	Total[a]		9.3		3.9	24.4	54.4	8.2				
	sn-1		13.8		5.9	22.9	48.4	9.1				
	sn-2		0.9		0.3	22.4	69.7	7.1				
	sn-3		13.1		5.6	28.0	45.2	8.4				
Maize[c]	Total[a]		11.3	1.7	2.1	28.2	57.3	1.0				
	sn-1		17.9	0.3	3.2	27.5	49.8	1.2				
	sn-2		2.3	0.1	0.2	26.5	70.3	0.7				
	sn-3		13.5	0.1	2.8	30.6	51.6	1.0				
Olive[b]	Total[a]		10.5	0.7	2.3	76.2	9.6	0.9				
	sn-1		13.1	0.9	2.6	71.8	9.8	0.6				
	sn-2		1.4	0.7		82.9	14.0	0.8				
	sn-3		16.9	0.8	4.2	73.9	5.1	1.3				
Peanut[b]	Total[a]		5.4	2.3	3.3	58.3	22.3	1.3[d]	1.6		2.1	1.3
	sn-1		13.6	0.3	4.6	59.2	18.5	1.1	0.7		1.3	0.7
	sn-2		1.6	0.1	0.3	58.3	38.6	0.3			0.2	0.5
	sn-3		11.0	0.3	5.1	57.3	10.0	2.7	4.0		5.7	2.8

[a]Total fatty acid of the triacylglycerol fraction.
[b]From Reference 14.
[c]From Reference 8.
[d]Including C 20:1.

TABLE 2

Distribution of Individual Fatty Acids of *Argania spinosa* Oil at the sn-1,2,3 Positions

Fatty acids	sn-1	sn-2	sn-3
C16:0	54.0	9.4	36.6
C16:1	25.0	8.3	66.7
C18:0	19.4	1.7	78.9
C18:1	33.3	39.7	27.0
C18:2	29.5	40.0	30.5
C20:0	13.3	6.7	80.0

of anhydrous diethylether and treated with phenyldichlorophosphate (0.25 mL in 1 mL of anhydrous diethylether) according to the method of Brockerhoff (8,9) as modified by Weber *et al.* (10). The mixture of phosphatidylphenols was analyzed on a 0.3-mm TLC silica plate with the following solvent system: $CHCl_3$-CH_3OH-NH_4OH (85:13:2). Phosphatidylphenols (Rf 0.5) were located by transmitted light and extracted with $CHCl_3$-CH_3OH (50:50). The solvent was evaporated and the phosphatidylphenols were dissolved in 2 mL of ether, 1.5 mL of 0.1 M triethylamine and 0.1 mL of 0.05 M $CaCl_2$. One mg of porcine pancreatic phospholipase A_2 (Sigma Chemical Co., St. Louis, MO) was added to the mixture, which was then stirred overnight under a nitrogen atmosphere. The mixture containing unmodified 2,3-diacyl-*sn*-glycero-1-phosphatidylphenols, 1-acyl-*sn*-glycero-3-phosphatidylphenols and fatty acids originally at the sn-2 position was finally extracted with $CHCl_3$-CH_3OH (50:50), and the extract was analyzed on a 0.5-mm TLC silica plate (20 × 20 cm). The TLC plate was developed with diethyl ether-petroleum ether (35:65, v/v), dried, and placed in an ammonia atmosphere for 10 min. The plate was further developed with methanol-ammonia-chloroform (10:2:88, v/v/v). The 1-acyl-*sn*-glycero-3-phosphatidylphenols (Rf 0.1) were extracted from the plate with diethyl ether, and the methyl esters of fatty acids originally at the sn-1 position were obtained by transesterification (13). Free fatty acids originally at the sn-2 position were also extracted (Rf 0.5), converted to methyl esters, and analyzed by GLC. Analyses were carried out as described above.

RESULTS AND DISCUSSION

The fatty acid distribution in triacylglycerols of argan oil is reported in Table 1 and compared with data already published for soybean, maize, olive and peanut oils (8,14).

The distribution of each fatty acid at the three positions of the triacylglycerols of Argan oil was calculated from the data of Table 1 according to Mattson and Beck (15), and results are presented in Table 2.

Data of Tables 1 and 2 show that oleic, linoleic and palmitic acids are the three predominant fatty acyl chains and represent 42.8, 37.4 and 12.5% of the total fatty acids of argan oil, respectively. A similar predominance is noted for the triacylglycerol distribution in soybean, maize, olive or peanut oils.

The relative amount of linoleic acid esterified at the sn-2 position is significantly higher than that at the external positions as is also observed in soybean and olive oils (14), maize (8) and peanut (14,16,17) oils. Oleic acid, the most abundant fatty acid, is more evenly distributed among the three positions, with a preference for the internal position as in olive oil.

Previous reports (16,18,19) state that the symmetry

TABLE 3

Triacylglycerols of *Argania spinosa* Oil (mole %)

Classes	nΔ[a]	nC[b]	HPLC		1-random 2-random 3-random		c	
000	0		—	—	—			
001	1	52	P,S,O	1.8	POS	1.5	P,S,O	1.9
					SOP	0.2		
					OPS	0.2		
	1	54	S,S,O		SOS	0.3	S,S,O	0.3
	1	50	P,P,O	3.2	PPO	0.2	P,P,O	1.8
					OPP	0.2		
					POP	1.4		
011	2	52	P,O,O	11.5	POO	3.6	P,O,O	7.1
					OOP	3.0		
					OPO	0.5		
	2	54	S,O,O (+ S,S,L)	3.9	OOS	3.1	S,O,O	
					SOO	0.6	(+S,S,L)	3.9
002	2	50	P,P,L	1.6	PLP	1.2	P,P,L	1.6
					PPL	0.2		
					LPP	0.2		
	2	52	P,S,L	1.6				
	2	54	S,S,L (+ S,O,O)	3.9	SLS	0.2	S,S,L	
							(+S,O,O)	3.9
111	3	54	O,O,O	12.8	OOO	7.6	O,O,O	7.6
012	3	52	P,O,L	13.6	PLO	3.1	P,O,L	12.4
					POL	3.5		
					LOP	2.3		
					OLP	2.6		
					LPO	0.4		
					OPL	0.5		
	3	54	S,O,L	3.0	OLS	2.7	S,O,L	6.2
					SLO	0.5		
					LOS	2.4		
					SOL	0.6		
112	4	54	O,O,L	19.5	OOL	7.4	O,O,L	19.9
					OLO	6.6		
					LOO	5.9		
022	4	52	P,L,L	6.3	PLL	3.1	P,L,L	5.5
					LLP	2.0		
					LPL	0.4		
	4	54	S,L,L	1.8	SLL	0.5	S,L,L	2.6
					LLS	2.1		
122	5	54	O,L,L	13.6	OLL	6.5	O,L,L	17.3
					LOL	5.8		
					LLO	5.1		
222	6	54	L,L,L	7.4	LLL	5.1	L,L,L	5.1

[a]nΔ: Total number of double bonds.
[b]nC: Total number of carbon atoms of the fatty acyl chains.
[c]Values of this column represent the total content of triacylglycerol species calculated from the 1-random-2-random-3-random data irrespective of the positional location of the fatty acids.

of distribution of certain fatty acids between the sn-1 and the sn-3 positions depends on their respective content in total triacylglycerol, and that these differences are specific for each species (18). In argan oil, the distribution of linoleic acid is symmetric on the sn-1 and the sn-3 positions. Oleic acid is preferentially incorporated at the sn-1 position compared to the sn-3 position. The distribution of stearic acid is significantly asymmetric with preferential incorporation at the sn-3 position. Palmitic acid is preferentially esterified at the sn-1 position as it is in other fats such as maize (8) and peanut (14,16,18) oils. It should be noted that palmitic and stearic acids are present predominantly at external positions in *Argania spinosa* oil as in the other vegetable oils compared in this study. Previous studies have shown that arachidic acid is seldom detected at the sn-1 position (17), and in all cases esterified preferentially at the sn-3 position (16,18). This is confirmed for argan oil where 80% of this acid is located at the sn-3 position.

Triacylglycerols of some dietary oils have been separated by HPLC according to their degree of saturation and total number of carbon atoms in the three fatty acyl

TABLE 4

Comparative Distribution of the Major Triacylglycerols of *Argania spinosa* Oil and Selected Vegetable Oils

TG	Olive[a]	Soybean[a]	Peanut[b]	*Argania*
SPP	0.00	0.00		
PPP	0.00	0.00		
POP	1.60	1.61		1.4
OPP	1.10	0.10		0.2
PPO				0.2
			P,P,O 1.4	
SOP	0.60	0.40		0.2
POS				1.5
OPS	0.00	0.00		0.2
			S,P,O 1.2	
SOS	0.00	0.00		0.3
OOS	3.20	0.60		3.1
SOO				0.6
			O,O,S 4.8	
OPO	0.10	0.10		0.5
POO				3.6
OOP	20.30	1.90		3.0
			P,O,O 11.7	
PLP	0.20	2.50		1.2
LPP	0.10	0.20		0.2
PPL				0.2
SLS				0.2
OOO	43.50	1.50	O,O,O 24.6	7.6
OLP	2.73	6.90		2.6
LOP	1.72	4.30		2.3
PLO				3.1
POL				3.5
OPL				0.5
LPO				0.4
			P,O,L 7.1	
LOS	0.00	0.00		2.4
SLO				0.5
SOL				0.6
OLS				2.7
			L,O,S 2.5	
OLO	6.36	4.60		6.6
OOL				7.4
LOO	5.76	5.82		5.9
			O,O,L 17.2	
LLS	0.00	4.50		2.1
LLP	0.32	13.21		2.0
PLL				3.1
LPL				0.4
			L,L,P 1.5	
SLL				0.5
OLL				6.5
LOL	1.70	5.61		5.8
LLO	7.70	17.80		5.1
			O,L,L 5.5	
LLL	2.20	17.20		5.1

[a]From Reference 21.
[b]From Reference 22.

chains by using a Philips Pye Unicam PU 4003 system (Cincinnati, OH) equipped with a Waters differential refractometer (Milford, MA) and a Delsi integrating recorder (Argenteuil, France) (J.C. Bouteiller and R. Maurin, unpublished results). Two successive Nucleosil (Macherey Nagel Co., Düren, Germany) C18, 5 μm columns (25 cm length for each one) were used. The mobile phase was acetonitrile-acetone (40:60, v/v), 0.9 mL/min flow rate. Results for argan oil are reported in Table 3, which also includes composition of triacylglycerols calculated according to 1-random-2-random-3-random distribution theory (9,20).

It appears from data of Table 3 that 1-random-2-random-3-random triacylglycerols containing three unsaturated fatty acids (OOO, OOL, OLO, LOL, OLL, LOO, LLO and LLL) account for 50% of the total triacylglycerols (each one >5%), and that other major triacylglycerols (each one >3%) contain at least two unsaturated fatty acids (POO, OOP, OOS, PLL, POL and PLO).

It is of interest to observe that calculated values for triacylglycerols containing two oleoyl and one linoleoyl chains (O,O,L) agree well with the corresponding values estimated from HPLC. Similar observation can be made for triacylglycerols containing one oleoyl, one linoleoyl and one palmitoyl chains (P,O,L) or one oleoyl, one palmitoyl,

and one stearoyl (P,S,O) chains. On the contrary, calculated values of trioleoyl (O,O,O) and trilinoleoyl glycerols (L,L,L) (7.6 and 5.1%, respectively) do not agree with the observed values (12.8 and 7.4%, respectively) according to HPLC analysis.

Similarly, triacylglycerols containing one palmitoyl and two oleoyl chains (P,O,O) represent 11.5% of the triacylglycerols separated by HPLC, while according to calculation they should represent only 7.1% of the total triacylglycerol species. The percentage of triacylglycerols containing one oleoyl and two linoleoyl chains (O,L,L) determined by HPLC is slightly lower than the calculated values. HPLC analysis confirmed that no saturated triacylglycerol was present in amounts higher than 0.1%. Comparison of the general pattern for Argania oil with other vegetable oils is shown in Table 4.

In conclusion, the fatty acid composition of *Argania spinosa* seed oil shows that oleic and linoleic acids account for 80.2% of the total fatty acids. This value is in the same range as for many other dietary oils of vegetable origin. Of particular interest is the finding that the content in linoleic acid (37.4%) is markedly higher than that of olive oil (10%). According to HPLC analysis data, triacylglycerols containing three unsaturated fatty acids account for 50% of the total triacylglycerols. This is in good accordance with the content in the same triacylglycerols calculated by using the 1-random-2-random-3-random distribution theory. However, some discrepancies appear when considering individual species, in particular in the case of trioleoyl and trilinoleoyl glycerols. Further work is needed to know whether similar observations can be made from the analysis of other vegetable oils.

ACKNOWLEDGMENTS

The authors are indebted to Prof. L. Sarda and Dr. E. Ucciani for their interest and help in the course of this study and in the preparation of the manuscript.

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[Received April 5, 1991; accepted October 29, 1991]