Triacylglycerol Composition of Oil from *Pistacia atlantica* Fruit Growing in Algeria

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ABSTRACT: The distribution of FA between the *sn*-2 and *sn*-1,3 positions of TAG from *Pistacia atlantica* fruit oil of Algeria has been determined. Unsaturated FA showed a preference for the internal position. Linoleic and oleic acids occurred predominantly in the *sn*-2 position with lesser amounts evenly distributed between the *sn*-1 and *sn*-3 positions, as generally found in vegetable oils. The oil was found to contain TAG that were trisaturated (0.93%), disaturated (15.06%), monosaturated (44.64%), and triunsaturated (38.10%). The distribution of the TAG calculated using the lipase hydrolysis technique is slightly different from that determined with HPLC. This is particularly true for trioleoyl and trilinoleoylglycerols. In contrast, the agreement between theory and experiment is good for TAG containing two palmitoyl and one oleoyl, one oleoyl and two linoleoyl, and one palmitoyl and two oleoyl chains.

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KEY WORDS: Fatty acids, HPLC, lipase, *Pistacia atlantica* oil, stereospecific analysis, triacylglycerols.

No investigation of the TAG components of *Pistacia atlantica* fruit oil has been reported. The aim of this study is to elucidate the distribution of FA attached to the glycerol molecule and determine the amount of TAG in the oil of *P. atlantica*. This study is an extension of previous work by the authors to evaluate the fruits of the *P. atlantica* tree as a potential source of vegetable oil, with special emphasis on their FA and sterols (1).

The main FA of many species of pistachio are oleic, linoleic, and palmitic. β-Sitosterol is the main sterol of *P.* and *P. vera* fruit oils (1–4).

The FA composition can indicate the stability levels and the nutritional quality of oils, but not their functional properties. Therefore, the type and amount of the various TAG species present in the oil are determinants of the physical and functional properties of the oil.

EXPERIMENTAL PROCEDURES

Pistacia atlantica fruits were obtained from the Tadjrouna region of the Laghouat department in southern Algeria. Boron trifluoride (12.5% in methanol) and pancreatic lipase were purchased from Sigma-Aldrich (St. Louis, MO). Hexane, diethyl ether, acetic acid, hydrochloric acid, calcium chloride, acetone, and acetonitrile were purchased from Fisher Scientific (Fairlawn, NJ).

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Fat extraction. The seeds were milled into powder through a 0.5-mm sieve using a hammer mill (IKA-Werke MF 10, Selangor, Malaysia) and extracted with hexane by agitation at room temperature for 24 h. The hexane was removed by rotary evaporation in a water bath at 40°C.

Preparation of TAG. To separate the TAG fraction, 1 g of the oil was filtered through a column made with 20 g of silica gel. TAG were eluted with 150 mL of benzene.

GC analysis. FAME were prepared by the AOCS Official Method Ce 2-66 (5). A Delsi gas chromatograph, equipped with an FID and a Mega 10 column (25 m × 0.25 mm i.d., 0.25 μm film thickness; Mega, Legnano, Italy) was used to analyze the FAME. The GC conditions were as follows: initial oven temperature (150°C), heating rate (2°C/min), final temperature (200°C), injection port temperature (250°C), detector port temperature (250°C), hydrogen gas flow (30 mL/min), air flow (300 mL/min), and helium gas carrier flow (1.0 mL/min). The injection volume was 0.1 μL. The FA were identified by comparing their retention times with those of pure standards purchased from Sigma-Aldrich.

RP-HPLC. The AOCS Official Method (Ce 5b-89) (5) was used to determine the TAG composition. The analyses were carried out isocratically with a mobile phase consisting of 75:25 (vol/vol) acetone/acetonitrile.

Oil samples were dissolved in HPLC-grade acetone, and 20-μL aliquots were injected into the column (Superspher C18, 25 cm × 4.2 μm; Merck, Darmstadt, Germany) and eluted at a flow rate of 1 mL/min. The column was equilibrated at 30°C, and the effluent was monitored with a Shimadzu LC 10AS refractive index detector. The TAG were identified by comparing retention times with those of pure standards purchased from Sigma-Aldrich.

Pancreatic lipase hydrolysis. Lipolysis of the neutral TAG was performed by the method of Luddy *et al.* (6). TAG (200 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 100 mg of commercial porcine pancreatic lipase in 2 mL distilled water. The tube was sealed and stirred gently. After 10 min, 1 mL of 6 M HCl was added, and the reaction mixture was extracted using diethyl ether. The extract was dehydrated over Na_2SO_4, and the solvent was evaporated. Lipids were dissolved with 0.2 mL of chloroform and spotted on a TLC silica gel plate. The plates

were developed with hexane/diethyl ether/acetic acid (70:30:1, by vol) solvent. The 2-MAG (the positions of which were determined on the TLC plate by UV lamp) were desorbed from the plates with diethyl ether/methanol (9:1, vol/vol). The methyl esters of the separated FA of the 2-MAG as well as those of the original TAG were prepared by AOCS Official Method Ce 2-66 (5) and analyzed in triplicate by GC. All compositions were converted to molar percentages of FA in the calculated 1,3-position and in that of the isolated 2-position, then the composition in TAG components was computed according to Coleman (7) and Gunstone (8).

RESULTS AND DISCUSSION

The FA composition of the 2-MAG obtained by lipase hydrolysis in comparison with those present in the original TAG and the relative proportion (r.p.) values of each FA in question are reported in Table 1. The FA detected in the original TAG were oleic, linoleic, palmitic, palmitoleic, and stearic. The FA constituents of the 2-MAG were oleic, linoleic, and palmitic acids.

In *P. atlantica* fruit oil, palmitic acid is preferentially esterified at the 1- and 3-positions in the whole TAG since the r.p. values are lower than the random value (33.3%) (9). This result is in agreement with the general distribution pattern of the saturated FA reported for vegetables oils (10–12). The r.p. values of linoleic and oleic acids were, respectively, 44.42 and 41.20%, indicating that these acids generally show a preference for the 2-position.

The proportions of oleic and linoleic acids in the 2-position of the *P. atlantica* fruit oil were 56.8 and 33.8%, respectively. It can be concluded that the 2-position was mainly acylated by unsaturated FA. This is in a good agreement with work by Mattson and Volpenhein (10), which showed that oleic and linoleic acids are preferentially attached at the 2-position (10). This finding is in accordance with the positional distribution theory suggested by Vanderwal (13), Coleman and Fulton (14), Gunstone (8), and Young (15). According to this theory, 2-MAG contain mostly C_{18} unsaturated FA. The remaining FA are randomly distributed among the 1,3-positions of the TAG molecule.

The composition in TAG components of the oil was calculated from the FA found in the 1,3-positions and those determined in the 2-position for the oil, according to the distribution theory (7). The results are given in the Table 3.

TABLE 1
FA Composition of TAG, 2-MAG, and the Proportion of Each FA in the 2-Position

FA	TAG%	2-MAG	2-Mono[a]
C16:0	25.20	6.87	9.10
C16:1	1.00	0.63	
C18:0	1.84	0.92	—
C18:1	45.80	56.86	41.20
C18:2	25.40	33.85	44.42
C18:3	0.84	0.84	—
Saturated FA	27.00	7.80	—
Unsaturated FA	73.00	92.20	—

[a]Relative proportion (r.p.) of FA esterified in the 2-position: (r.p.) = 100 × (FA% in 2-MAG)/3 × (FA% in whole TAG)

TABLE 2
The Four Categories of Total TAG

Sample type[a]	Coleman (7)	Gunstone (8)	HPLC (5)
GS_3	0.93	1.04	1.50
GS_2U	15.06	15.96	13.07
GSU_2	44.64	45.95	39.83
GU_3	38.12	37.06	39.90

[a]GS_3, trisaturated glycerides; GS_2U, disaturated glycerides; GSU_2, monosaturated glycerides; GU_3, triunsaturated glycerides.

Table 3 shows the glyceride percentages in term of the four main glyceride categories: trisaturated (GS_3), disaturated (GS_2U), monosaturated (GSU_2), and triunsaturated (GU_3). Clearly, tripalmitin (PPP) is the major component TAG among GS_3 deduced in the oil sample. This value is lower than expected when correlated to the content of saturated FA (27%). This result agrees well with Khartha's restricted random distribution theory (16–18), which shows that the amount of GS_3 must not exceed a value that permits its solubility in the substrate.

Disaturated TAG (GS_2U) had a value of 15.06%. This considerable amount is undoubtedly due to the presence of 27% of the saturated FA in the original oil sample (Table1).

Generally, the GSU_2 TAG in palmitic acid forms a considerable fraction of the oil. In our opinion, this is because the oil

TABLE 3
Component TAG[a] (%)

Sample	%	TAG	%	TAG	%	Total
PPP	0.80					0.80
PPS	0.13					0.13
PPO	6.70	POP	1.90			8.60
PPL	4.00	PLP	1.00			5.00
PPLn	0.03					0.03
SPO	0.15	SOP	1.07			1.22
SPL	0.08	SLP	0.64			0.72
OPO	1.11	POO	15.68			16.80
OPLn	0.08	LnOP	0.48			0.56
LPL	0.32	PLL	4.90			5.22
POL	9.36	PLO	8.26	LPO	1.06	18.70
LPLn	0.03	LnLP	0.28			0.31
SLL	0.42					0.42
SOO	1.26					1.26
SOL	0.75	OLS	0.66			1.41
OOO	9.20					9.20
OOL	9.66	OLO	5.46			15.12
OOLn	0.56					0.56
LLO	5.76	LOL	2.54			8.30
LOLn	0.30	OLLn	0.33			0.60
LLL	1.51					1.51
LnLL	0.18					0.18
PoLO	0.80	PoOL	0.40			1.20
LLPo	0.24					0.24
OOPo	1.20					1.20
Other						0.71

[a]P, palmitic acid; S, stearic acid; O, oleic acid; L, linoleic acid; Ln, linolenic acid, Po, palmitoleic acid.

TABLE 4
TAG of *Pistacia atlantica* Fruit Oil (%)

Classes	nΔ^a	nC^b	HPLC (5)	%	Coleman (7)	%	Gunstone (8)	%
000	0		P,P,P	1.50	PPP	0.80	P,P,P	
			P,P,S		PPS	0.13	P,P,S	
001	1	52	P,O,S		POS	1.07	P,O,S	1.28
					OPS	0.15		
	1	54	S,S,O		SOS	Trace		
							S,O,S	0.06
		50	P,P,O	8.00	OPP	1.90		
					POP	6.70	P,P,O	8.82
011	2	52	P,O,O	16.70	OOP	15.68		
					OPO	1.11	P,O,O	17.70
	2	54	S,O,O		SOO	1.26	S,O,O	1.26
002	2	50	P,P,L	5.07	PLP	1.00	P,P,L	4.97
					PPL	4.00		
	2	52	P,S,L		PLS	0.64	P,S,L	0.71
					SPL	0.08		
111	3	54	O,O,O	12.40	OOO	9.20	O,O,O	8.72
012	3	52	P,O,L	17.01	POL	9.36		
					LPO	1.06	P,O,L	19.60
					OLP	8.26		
	3	54	S,O,L		OLS	0.66	S,O,L	1.42
					SOL	0.75		
112	4	54	O,O,L	16.50	OOL	9.66	O,O,L	14.50
					OLO	5.46		
022	4	52	P,L,L	6.12	PLL	4.90	P,L,L	5.44
					LPL	0.32		
	4	54	S,L,L		SLL	0.42	S,L,L	0.40
122	5	54	O,L,L	8.41	OLL	5.76	O,L,L	8.05
					LOL	2.54		
222	6	54	L,L,L	2.60	LLL	1.51	L,L,L	1.50
Other				5.69		0.71		4.57

[a]nΔ, total number of double bonds.
[b]nC, total number of carbon atoms of FA. For abbreviations see Table 3.

has a high amount (18.70%) of palmito-oleolinolein (POL), which constitutes the principal TAG of the GSU_2 category. The high proportions of oleic, linoleic, and palmitic acids in the oil sample lead to the predominance of POL TAG over the other TAG types.

The amount of the various TAG types with respect to the type of acid was clearly manifested by the fact that the percentage of palmitodiolein (POO) TAG is higher than the palmitodilinolein (PLL) because the content of oleic acid is higher than that of linoleic acid in the first one.

The total triunsaturated TAG (GU_3) constituted about 38%. The dioleolinolein (OOL) TAG type has the higher percentage in the oil. The OOO and LLO TAG make up a considerable amount of the oil sample.

The presence of oleic acid as the main unsaturated component (45% in the oil and 56% in the 2-MAG) leads to its contribution of about 84% in the total TAG, mainly as di- and monooleins. The disaturated olein (S_2O) TAG type forms about 9.82% of the total TAG, whereas saturated dioleins (SO_2) have a value of 18.06%, and triolein is 9.20%.

On the other hand, linoleic acid was represented mainly as saturated oleo-linoleic (SOL), with levels up to 18.70%, and saturated dilinolein (SLL), where it reached 5.64%. The third representative TAG for linoleic acid is oleo-dilinolein (OLL), with 8.30%; trilinolein was present in a very low percentage, 1.51%.

The present data agree well with the "positional distribution theory" as presented in graphs published by Gunstone (8). The glyceride structure data also are in accordance with the correlation curves of Coleman (7).

The TAG of *P. atlantica* oil also have been separated by HPLC according to their degree of saturation and their total number of carbon atoms in the three fatty acyl chains. The results are reported in Table 4, where the dominant TAG are POL, OOL, POO, and OOO. It is important to observe that calculated values for TAG containing two palmitoyl chains and one oleoyl chain (PPO) or one palmitoyl and two oleoyl chains (POO) agree well with the corresponding values estimated by HPLC. The same observation can be made for TAG containing one oleoyl and two linoeloyl chains (OLL).

In contrast, calculated values of trioleoyl (OOO) and trilinoleoyl glycerols (LLL) (9.20 and 1.51%, respectively) do not agree with the observed values (12.40 and 2.6%, respectively) obtained by HLPC. For the other TAG (OOL and LLP), the calculated values are very near to those estimated by HPLC.

We note that most of the TAG in *P. atlantica* oil result from

the combination of oleic and linoleic acids (73% of the total, TAG components). A similar preponderance is noted for the TAG distribution in soybean, olive, and peanut oils. According to HPLC analysis, TAG containing three unsaturated FA constituted 40% of the total TAG. This is in good accordance with the values calculated by using the distribution theory (7,8). However, some discrepancies appear when considering individual species, in particular in the case of trioleoyl and trilinoleoyl glycerols.

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