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Fatty acid, triglyceride and tocopherol composition of Algerian Argan (*Argania spinosa*) fruit seed lipids

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Abstract The main focus of the present work is the analytical study of the fatty acid, triglyceride (TAG) and tocopherol composition of oil extracts from the fruit of Algerian tree *Argania spinosa*. The four dominant fatty acids (FA) found in the oil are: oleic C18:1 (52.86%), linoleic C18:2 (25.0%), palmitic C16:0 (14.65%) and stearic C18:0 (7.06%). The distribution of FA between the sn-2 and sn-1,3 positions of TAG from the oil was also determined. Unsaturated FA showed a preference for the internal position, as generally found in vegetable oils. The TAG composition was calculated using the lipase hydrolysis. The oil was found to contain trisaturated (0.47%), disaturated (9.3%), monosaturated (43.95%) and triunsaturated (45.20%) FA. Flash chromatography with solvent of increasing polarity yielded 83.42% neutral lipids, 1.56% glycolipids and 2.09% phospholipids. The oil was characterised by a relatively high amount of tocopherols (1027.8 mg/kg). The (γ+β)-tocopherols were the major isomers, with the rest being α- and δ-tocopherols.

Keywords *Argania spinosa* oil · Fatty acids · Triglycerides · Tocopherols · Polar lipids

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Introduction

The argan tree (*Argania spinosa*) is a tropical plant, which belongs to the Sapotaceae family and is distributed in the south of Morocco and Algeria. The argan tree is exploited essentially for its fruits. The endosperm seed of the fruit constitutes a good potential source of edible oil for human consumption and is endowed with important medicinal properties such as antihypercholesterolaemia. Argan oil is also widely incorporated in many cosmetic products such as anti-acne agents [1]. For these reasons, numerous studies have been carried out to determine the chemical composition of argan oil. The fat composition is 45–47.7% monounsaturated fatty acid (FA), 29.7–35% polyunsaturated FA and 20% saturated FA. The oil is rich in minor compounds such as phenolic compounds (3.3 mg/kg), carotenes, sterols (295 mg/100 g), tocopherols (637 mg/kg), alcohols, triterpenes and squalene [2–4]. The phenolic extracts from virgin argan oil give beneficial effects in protecting human low-density lipoprotein against lipid peroxidation and enhancing reverse cholesterol transport from human THP-1 macrophages [5]. Argan oil also has hypolipaemic and antioxidant properties [6]. Chemical analysis of argan fruit pulp has already shown the presence of saponins [7] and xylans [8].

In Algeria, no previous studies have been conducted on the lipids of *A. spinosa* fruit. Therefore, this study aimed to characterise the FA composition, distribution of FA in

the glycerol molecule and tocopherol composition of fruit oil of an *A. spinosa* tree growing in the South of Algeria. In this work we also determined the FA composition of polar lipids such as glycolipids and phospholipids. To our knowledge, the FA composition of the polar lipids of *A. spinosa* fruit oil has not previously been studied.

Materials and methods

The fruits of the *A. spinosa* were collected from three trees from Oued Elma in the region of Tindouf in the south-west of Algeria in July 2006. A voucher specimen was deposited in the laboratory of the Fundamental Sciences at the University of Laghouat. The results of the oil extract were the mean of three different samples randomly collected from the same region.

Boron trifluoride (10% in methanol) and pancreatic lipase were bought from Sigma-Aldrich. Hexane, diethyl ether, formic acid, hydrochloride acid, calcium chloride, acetone and all the reagents were from Fisher Scientific.

Fat extraction

The fruits were manually peeled and the almonds were air dried and milled into powder using a manual mill and extracted with hexane in a Soxhlet apparatus for 6 h.

The extract was filtered and dehydrated with anhydrous sodium sulphate. The hexane was refiltered and evaporated by rotary evaporation in bath water at 40°C and the oil was kept in a brown bottle at 6°C.

Determination of the chemical indices of the oil

Acid value, saponification value (SV), iodine value, unsaponifiable matter (UM), relative density and refractive index (RI) were determined according to the procedure described by the American Oil Chemists' Society [9].

Purification of the neutral TAG

To separate the triglyceride (TAG) fraction, 1 g of the oil was filtered through a column with 10 g of silica gel (70–230 mesh). TAG(s) were eluted with 150 ml of benzene [10].

Pancreatic lipase hydrolysis

Lipolysis of the neutral TAG was performed by the method of Luddy et al. [11]. TAG(s) (200 mg) were placed in a glass tube with 2 ml of 1.2 M NH_4Cl buffer (pH 8), 1 ml of 20% $CaCl_2$ and 100 mg of commercial porcine pancreatic lipase in 2 ml distilled water. The tube was stirred gently. After 25 min, 1 ml of 4 N 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 in 0.2 ml of chloroform and spotted on a TLC silica gel plate. The plates were developed with (hexane/diethyl ether/formic acid 50:50:1 by volume) solvent. 2-Monoglyceride (2-MG) (the positions of which were determined on TLC plate by UV Lampe) was desorbed from the plates with chloroform/methanol (9:1, v/v).

Extraction of polar lipids

Thirty grams of the powdered almond of the fruit was extracted with 200 ml of $CHCl_3/CH_3OH$ (2:1, v/v) at room temperature with a warming blender for 24 h. The lipid extract was collected in a flask and subsequently treated with Na_2SO_4. After filtration, the extract was brought to dryness on a rotary evaporator at 40°C. The extracted lipids were weighed to determine the total lipid (TL) content and stored at 4°C for further analysis.

Isolation of the lipid classes

TL in chloroform was separated into neutral lipids (NL), glycolipids (GL) and phospholipids (PL). Two grams of the TL were mixed with 10 g of powdered silicic acid (chromatography grade) in a 100-ml capacity glass Erlenmeyer flask. Chloroform (45 ml) was then added and the contents of the flask were shaken for 10 min. The chloroform solution containing the neutral lipids was filtered by suction through a sintered glass funnel and the silicic acid was washed 6 times with a 20 ml portion to remove the last traces of neutral lipids. The glycolipid fraction was eluted four times with 20 ml of acetone. The silicic acid containing the phospholipid fraction was finally eluted four times with 20 ml of methanol. Solvents were evaporated using a rotary evaporator at 40°C and the percentage of each fraction was determined gravimetrically. Residue was stored at –20°C as lipid classes.

GC analysis

Fatty acid methyl esters (FAME) of oil, TAG, 2-MG and different classes of polar lipids were prepared by an acid-catalysed esterification method using a boron trifluoride–methanol complex 12% w/v. A Delsi gas chromato-

graph, equipped with an FID detector and a Mega 10 column (25 m↔0.25 mm i.d, 0.25 μm film thickness) was used to analyse the FAME(s). 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 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.

Tocopherol analysis

The compounds were quantified by a HPLC system composed of water 2690, a quaternary pump, a thermostatted column compartment and a fluorescence detector. A licsopher RP-18 column (250↔4.6 mm, 5 μm thickness, Merck) was used with a methanol:acetonitrile (70:30 v/v) mobile phase at an isocratic elution flow with 1 ml/min. Quantification was carried out from a calibration based on the standard tocopherols.

The isomer β-tocopherol was not resolved from γ-tocopherol by RP chromatography. For this reason, we describe β- and γ-tocopherols together. All experiments were carried out in triplicate. Values of different parameters were expressed as the mean±SD.

Results and discussion

Table 1 shows the physicochemical properties and the FA composition of the fruit oil. The crude fat content of the argan fruit was 36%. This result indicated that the fruit of *A. spinosa* can be considered as an oleaginous seed, like peanuts, olives, sunflower seeds and cotton. Therefore, *A. spinosa* fruit oil could be developed into commercial products to serve as an alternative vegetable oil in the south of Algeria, the region where the *A. spinosa* tree grows.

Table 1 Physicochemical properties and fatty acid composition of *Argania spinosa* fruit oil

Chemical parameter	Argan fruit oil
Oil (%)	36 ± 0.8
AV (mg_{KOH}/g)	2.2 ± 0.2
IV (Wijis)	132.4 ± 0.7
SV (mg_{KOH}/g)	184.4 ± 0.6
η^{20}	1.4853 ± 0.01
d^{20}	0.9450 ± 0.01
UM% (w/w)	1.17 ± 0.2
C16:0	14.65 ± 0.33
C16:1	–
C18:0	7.06 ± 0.74
C18:1	52.86 ± 0.66
C18:2	25.0 ± 0.35
C18:3	0.32 ± 0.02
Total saturated	21.71
Total unsaturated	78.18
MUFA/PUFA	2.08

AV acid value, *IV* iodine value, η^{20} refractive index, d^{20} density, *UM%* unsaponifiable matter, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids

The RI and the relative density values for the argan oil were respectively 1.4853 and 0.9450. These values were comparable to those of other vegetables oils and suggest that the oil of argan contains a higher amount of unsaturated FA.

The SV was 184.4 (mg KOH/g). Because there is an inverse relationship between SV and weight of FA in the oils, it can be assumed that the oil of argan holds FA with 16–18 carbon atoms. The relatively high iodine value (132.36 mg/100 g) in the studied oil may be indicative of the presence of many unsaturated bonds and would certainly contain more unsaturated FA and can this be grouped as drying oils. The acid value for *A. spinosa* fruit oil is 2.2 mg KOH/g, which indicates that the oil contains a small quantity of free FA and the complete ripeness of the fruit. The percentage of UM was 1.7% (w/w), which is within the codex recommended maximum values for refined oils.

The values of the basic physicochemical properties of the oil as used in this study are in the range of those values reported for the majority of vegetable oils.

Gas chromatography analysis of the FAME of the argan oil (Table 1) showed that the dominant FA found in the oil were: palmitic 14.65%, stearic 7.06%, oleic 52.86%, linoleic 25.00% and linolenic 0.32%. The saturated FA in the oil are palmitic and stearic, however palmitic acid was the major saturated FA constituent in the oil. Stearic was present in lower amounts in the fruit oil of argan 7.06%. It can be seen that the total percentage of saturated FA was 21.71%.

The unsaturated FA in the argan oil were represented by oleic, linoleic and linolenic FA. Oleic acid (52.86%) is the major unsaturated FA in the argan oil, followed by linoleic (25.00%). Linolenic acid was detected in the oil in very small amounts, which did not exceed 0.32%. The unsaturated FA were predominant in the oil of argan, as confirmed by the iodine value test. These results are very different from those reported in the literature for the fruit oil of the *A. spinosa* tree grown in Morocco. It is observed that the percentage composition of oleic acid is higher in the studied argan oil than that of Morocco [3]. On the other hand, the percentage of linoleic acid is higher in the Moroccan argan oil. However, oleic acid was the main unsaturated FA in both the oil from Morocco and that in the present study. The oil studied showed a similar value for the sum of unsaturated FA (78%) as the

argan oil from Morocco (79.0%). The MUFA/PUFA ratio for the argan oil studied was higher (2.08) than that of the oil from Morocco (1.62), because it contains more monounsaturated FA (MUFA).

MUFA have great importance because of their nutritional implications and their effect on the oxidative stability of oil. The difference observed in the FA composition may be explained by the difference in altitude of the argan trees in Algeria and Morocco. This agrees with the results described by other researchers for oils from olives growing at different altitudes [12].

Overall, the FA profile of the argan oil was very similar to those of other edible vegetable oils.

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

In *A. spinosa* 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%). This result is in agreement with the general distribution pattern of the saturated FA reported for vegetable oils [13–15]. The r.p. values of linoleic and oleic acids were respectively 49% and 36%, 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 argan oil were 58.0% and 36.8%, respectively. It can be concluded that the 2-position was mainly acylated by unsaturated FA with 18 atoms of carbon. This is in good agreement with the work by Mattson and Volpenhein [14], which showed that oleic and linoleic acids are preferentially attached to the 2-position. This result is in concordance with the positional distribution theory suggested by Van der Wal [16], Coleman and Fulton [17], Gunstone [18] and Youngs [19], where 2-MG contains mostly unsaturated FA with 18 atoms of carbon. The remaining FA is randomly distributed among 1,3-positions of the TAG molecule.

The composition in TAG components of the argan 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 [20]. The results are given in Table 3.

Table 4 shows the TAG percentage in terms of four main glyceride categories: trisaturated (GS_3), disaturated (GS_2U), monosaturated (GSU_2) and triunsaturated (GU_3).

Clearly, tripalmitin (PPP) is the major component of TAG among GS_3 in the argan oil. This value is lower than expected when correlated to the content of saturated FA (21.71%). This agrees well with Khartha's restricted random distribution theory [21–23], which shows that the amount of GS_3 must not exceed a value that permits its solubility in the substrate.

Disaturated $TAG_{(s)}$ (GS_2U) have a value 9.30%. This value is due to the presence of 21.71% of the saturated FA in the original sample oil.

Generally, the GSU_2 TAG in palmitic acid forms a considerable fraction of the oil. This is due to the fact that the oil has a mean amount (8.86%) of palmito-oleolinolein (POL), which constitutes the principal TAG of the GSU_2 category.

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

The total triunsaturated TAG (GU_3) constituted 45.2%. The dioleo-linolein (OOL) TAG type has the higher percentage in the oil (21.20%), followed by the trilinolein (OOO) and the oleo-dilinolein (LLO), with 15.42% and 9.35% respectively.

The presence of oleic acid as the major unsaturated component (53.71%) in the oil and also 58.01% in the 2-MG leads to a contribution of about 84% in the total TAG, mainly as di-and monooleins. The disaturated olein (S_2O) TAG type forms 6.82% of the total TAG, whereas saturated dioleins (SO_2) have a value of 19.26% and triolein is 15.42%.

Table 2 Fatty acid composition of TAG and the proportion of each FA in the 2-position

AG	TAG%	2-MAG%	r.p.
C16:0	14.50 ± 0.35	5.20 ± 0.12	11.95
C16:1	/	/	
C18:0	7.21 ± 0.40	/	
C18:1	53.71 ± 0.66	58.01 ± 0.15	36.00
C18:2	25.00 ± 0.55	36.80 ± 0.21	49.00
TSFA	78.71 ± 0.75	5.20 ± 0.12	
TUFA	21.71 ± 1.11	94.81 ± 0.36	

r.p. relative proportion of FA esterified in the 2-position, *TSFA* total saturated fatty acids, *TUFA* total unsaturated fatty acids
(r.p.)=100↔(FA% in 2-MAG/3)↔(FA% in whole TAG),

Table 3 TAG of *Argania spinosa* fruit oil (%)

Coleman	%	Gunstone	Theorie 1 (%)
PPP	0.19 ± 0.02	P,P,P	ND
PPSt	0.11 ± 0.01	P,P,St	ND
POSt	2.38 ± 0.02	P,O,St	3.20 ± 0.21
OPSt	0.58 ± 0.01		
StOSt	0.76 ± 0.01	St,O,St	0.79 ± 0.01
StLSt	0.34 ± 0.01	St,L,St	0.37 ± 0.01
StPSt	0.06 ± 0.01	St,P,St	
OPP	1.02 ± 0.02		
POP	2.11 ± 0.03	P,O,P	3.20 ± 0.13
OOP	11.42 ± 0.32		
OPO	1.38 ± 0.02	P,O,O	13.78 ± 0.31
StOO	6.46 ± 0.25	St,O,O	6.85 ± 0.21
PLP	1.34 ± 0.01	P,P,L	1.5 ± 0.03
PPL	0.38 ± 0.01		
PLS	1.52 ± 0.02	P,St,L	1.49 ± 0.02
StPL	0.22 ± 0.01		
OOO	15.42 ± 0.31	O,O,O	14.72 ± 0.25
POL	8.44 ± 0.20		
LPO	2.04 ± 0.03	P,O,L	12.83 ± 0.41
PLO	7.24 ± 0.24		
OLSt	4.10 ± 0.20	St,O,L	6.38 ± 0.15
StOL	2.38 ± 0.10		
OOL	11.42 ± 0.30	O,O,L	20.55 ± 0.25
OLO	9.78 ± 0.42		
PLP	2.68 ± 0.12	P,L,L	2.98 ± 0.20
LPL	0.19 ± 0.02		
StLL	1.52 ± 0.01	St,L,L	1.48 ± 0.10
OLL	7.24 ± 0.14		
LOL	2.11 ± 0.02	O,L,L	9.56 ± 0.26
LLL	1.34 ± 0.02	L,L,L	1.48±

P palmitic acid, *St* stearic acid, *O* oleic acid, *L* linoleic acid

On the other hand, linoleic acid was represented mainly as saturated oleo-linoleic (SOL), with contents up to 24.20%, and saturated dilinolein (SLL), where it reached 1.71%. The third representative for linoleic acid is oleo-dilinolein (OLL), with 9.35%; trilinolein (LLL) was present in very small amounts (1.34%).

We note that all these data agree well with the "positional distribution theory" of Gunstone [18]. Moreover, the TAG structure data are in accordance with the correlation curves of Coleman and Fulton [17].

The TAG components of argan oil were also computed according to Gunstone's distribution theory [18]. It appears from Table 3 that the triacylglycerols containing three unsaturated FA (OOO, OOL, OLL and LLL) represent 46.31% of the total TAG and that other major TAG contain at least two unsaturated FA (POO, OOSt, PLL and PLO).

The calculated values for TAG containing two oleoyl and one linoleoyl chains (O, O, L) are in good agreement with the corresponding values determined by the Coleman method. Similar considerations apply to TAG containing two linoleoyl and one oleoyl chains (L,L,O) or one palmitoyl and two oleoyl chains (P,O,O). On the contrary, calculated values of the TAG containing one oleoyl, one linoleoyl and one palmitoyl chains (P,O,L) or one oleoyl, one palmitoyl and one stearoyl (P,St,O) chains (12.83% and 3.20% respectively) do not agree with the determined valuesof the Coleman method (17.72% and 2.96% respectively).

We note that most of the TAG in *A. spinosa* fruit oil result from the contribution of oleic and linoleic acids (89% of the total TAG components). This value is in the same range for many other dietary oils of vegetable origin (sunflower, soybean and groundnut).

In an earlier publication [24, 25], in the commercial oil of argan trees grown in Morocco, amounts of the principal component TAG were very different from those of the oil studied here. The same TAG types exist in large amounts in the two oils, but the values are generally very different. These differences are perhaps due to genetic factors or ecological conditions that play a role in the FA distribution in the TAG molecule. Table 5 shows the contents of lipid classes present in argan oil and their FA profile. Among the TL, significant amounts of NL were present (83.42%), whereas GL content was 1.56% and PL was 2.09%.

Table 4 The four categories of total TAG

Sample type	Coleman	Gunstone
GS_3	0.47	/
GS_2U	9.30	5.86
GSU_2	43.95	44.3
GU_3	45.20	46.31

GS_3 trisaturated glycerides, *GS_2U* disaturated glycerides, *GSU_2* monosaturated glycerides; *GU_3* triunsaturated glycerides

Table 5 Fatty acid composition of Argan crude fruit oil and its lipid classes

Fatty acids	Total lipids	Neutral lipids (83.42%)	Glycolipids (1.56%)	Phospholipids (2.09%)
C10:0	–	–	–	4.90 ± 0.24
C12:0	–	–	–	3.45 ± 0.31
C14:0	0.14 ± 0.10	–	–	1.02 ± 0.02
C16:0	14.52 ± 0.20	13.49 ± 0.50	15.04 ± 0.50	17.33 ± 0.80
C16:1	0.11 ± 0.01	–	–	–
C18:0	6.71 ± 0.30	2.51 ± 0.20	7.40 ± 0.30	5.81 ± 0.12
C18:1	51 ± 0.35	55.29 ± 0.08	53.65 ± 1.20	47.21 ± 1.10
C18:2	26.70 ± 0.24	26.88 ± 0.60	23.92 ± 0.80	17.03 ± 0.75
C20:0	0.40 ± 0.20	–	–	–
C18:3	0.40 ± 0.02	–	–	3.31 ± 0.13
C22:2	–	1.88 ± 0.22	–	–
TSFA	21.77	16.00	22.44	32.51
TUFA	78.21	82.17	77.57	67.35
TUFA/TSFA	3.59	5.13	3.45	2.07

TSFA, total saturated fatty acids, *TUFA* total unsaturated fatty acids

The FA profile of total lipid and lipid classes of *A. spinosa* fruit have oleic, linoleic, palmitic and stearic acids as the major FA. Oleic acid was the principal FA, while linoleic acid was the second main unsaturated FA. Palmitic followed by stearic were the major saturated FA. These four FA (oleic, linoleic, palmitic and stearic) constituted 87–98% of the total FAME. The ratio USFA/SFA for the lipid classes ranged from 3.45 to 5.13. Four minor FA, decanoic, dodecanoic, tetradecanoic and linolenic acids, were identified in PL fraction and constituted 12.68% of the total FAME.

Oleic acid was the prominent FA in all lipid classes, while both unsaturated FA (oleic and linoleic) constituted more than 75% of total FAME. Palmitic acid was the major saturated FA in all classes, followed by stearic acid. The total FA profile of polar lipid fractions, however, was characterised by higher palmitic acid and lower linoleic acid content than the NL.

Tocopherols (vitamin E isomers)

These are well known natural antioxidants whose presence in oils is often correlated with a relatively high abundance of unsaturated FA. Four tocopherols were clearly identified and quantified in the oil (Table 6). The major tocopherols found were (β+γ) tocopherols, presenting about 68% of the total tocopherol content. α-Tocopherol was present in a lower amount (31% of the total tocopherol) but at the same time δ-tocopherol was a minor compound (10% of the total tocopherol) in comparison to the other isomers (Table 6). The studied oil has a higher total tocopherol content (1027.80 mg/kg) than the argan oil of Morocco (637 mg/kg). The differences observed in the amounts of total tocopherol in the oils from different locations were perhaps due to the fact that the argan oil from Algeria presents a higher level of oleic acid and lower linoleic acid than the argan tree oil grown in Morocco. The relationships between these FA, and the MUFA/PUFA ratio, have been described as the main factors responsible for the oxidative stability of virgin oil [26]. On the other hand, the environmental conditions as well as genetic factors may influence the tocopherol composition.

Table 6 Tocopherol composition of the Argan oil

Tocopherols	mg/kg	mg/kg[a]
α-tocopherol	325.00 ± 2.20	35
(β+γ)-tocopherol	700.00 ± 3.55	480
δ-tocopherol	2.86 ± 1.25	122
Total	1027.80	637

[a]Argan from Morocco

A comparison with other vegetable oils with similar FA profiles permits us to say that the studied oil is rich in

tocopherol compounds and could be considered as being within the range of other oils rich in oleic acid, such as groundnut oil.

Conflict of interest The authors declare that they have no conflict of interest related to the publication of this manuscript.

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