NPC Natural Product Communications

2015
Vol. 10
No. 10
1723 - 1728

Effect of Maturation Degree on Composition of Fatty Acids and Tocopherols of Fruit Oil from *Pistacia atlantica* Growing Wild in Algeria

Hamid Guenane[a], Isabelle Bombarda[b], Mohamed Didi OuldElhadj[c] and Mohamed Yousfi[a,*]

[a]*Laboratoire des Sciences Fondamentales, Université Amar Télidji, Laghouat (03000), Algeria*

[b]*Aix Marseille Université, LISA, EA4672, Equipe METICA, 13397 Marseille cedex 20, France*

[c]*Laboratoire de Protection des Ecosystèmes en Zones Arides et Semi arides, Université KasdiMerbah, Ouargla (30000), Algeria*

yousfim8@gmail.com

Received: June 3rd, 2015; Accepted: July 10th, 2015

Pistacia atlantica fruit oil has been used for a long time by local populations for culinary and medicinal purposes. In this study, the fatty acid composition and tocopherol content were determined in twelve samples of *P. atlantica* fruit oil at three stages of maturation (immature, intermediate maturity and mature) collected in three different sites from the region of Laghouat. The results indicated a significant difference between the oil of mature fruits (green and black) and the immature ones (light red), which were distinguished by richness in unsaturated fatty acids and tocopherols. The oil from fruits of intermediate maturity (dark red) seems to combine these properties with those of the mature group, including oil yields. Such data emphasize the value of this oil, which needs further investigation.

Keywords: *Pistacia atlantica*, Fruit oil, Fatty acids, Tocopherols, Maturity.

Fruits of *Pistacia atlantica* Desf. are widely consumed by the local population as a nutriment. The oil extracted from the fruit is usually used in traditional medicines against several diseases, such as inflammation [1]. Several works have revealed that plants constitute a major source of natural bioactive compounds, including essential oil [2, 3]. Among these plants, *P. atlantica* has a great value because of the antioxidant activity of its extracts [4, 5], the composition of its fruit oil [6, 7], its flavonoid content [8] and its anti-cancer activities [9]. All these virtues explain its diverse uses by local populations.

There are no data available on the variation of the composition of *P. atlantica* fruit oil, according to the degree of maturation. The aim of this work was the study of the fatty acid and tocopherol composition of the oil extracted from *P. atlantica* fruits, stemming from several sites in Laghouat, according to the degree of maturation. Such an approach could supply useful and exploitable information for possible applications.

Oil obtained from *P. atlantica* mature fruits has a clear yellow color with a pleasant smell, whereas, oil of immature fruits gives a green color with a very pleasant smell. Results of oil content are shown in Table 1. The oil content varies considerably according mainly to the maturation degree. Indeed, the highest yields (from 27.7% to 46.1 %) were obtained from mature fruits (green or black) compared with immature ones ($p<0.001$). Fruits of intermediate maturity have medium oil content (20.1% and 35.0%). Our results are similar to those reported for *P. lentiscus* [10, 11]. It seems from our results that the accumulation of oil in *P. atlantica* fruits occurs during maturation.

Nine fatty acids commonly found in vegetable oils were detected in the oil of *P. atlantica* fruits (Table 2). Palmitic acid (C16:0) was the most predominant saturated fatty acid. It represents between 13.9 % and 21.9 % for the immature fruits, and between 22.1 % and 25.7 %

Table 1: Oil content according to the maturation stage.

Sample	Oil (% w/w)	Mean of immature samples	Mean of intermediate samples	Mean of mature samples
G1	27.7			
DR1	20.1			
LR1	16.6			
G2	37.7			
DR2	35.0			
LR2	16.8	13.8±4.6**	27.5±10.5	37.8±6.2
G3	37.7			
LR3	7.0			
G4	41.7			
LR4	14.9			
B1	46.1			
B2	35.7			

**$p<0.001$; (compared with mature samples).

for the mature fruits. Stearic (C18:0), myristic (C14:0) and arachidic (C20:0) acids were found, but in a lower proportion. Concerning the unsaturated fatty acids, palmitoleic, oleic, linoleic and linolenic acids were found in all the oils. They represent a total percentage from 72.7 to 75.8 for the mature fruits, and from 75.9 to 83.7 for the immature fruits. Oleic acid, and in second place linoleic acid, were the most predominant unsaturated fatty acids. Their proportions were slightly higher in the oil of immature fruits. It should be noted that the ratio of unsaturated fatty acids to saturated fatty acids was significantly higher in immature fruit oil compared with the mature ones ($p<0.05$). This ratio was also elevated in samples with intermediate maturity (Table 2). These results are comparable with those reported for *P. lentiscus*, in particular concerning linoleic acid [10, 11]. The high level of unsaturated fatty acids in *P. atlantica* fruit oil makes it a dietary nutriment.

In spite of the nutritional importance of tocopherols [12, 13], their content and composition in *P. atlantica* seed oils remain unknown. Tocopherols analysis was performed for eleven samples. Figure 1 shows chromatograms of three samples (with different maturation degrees) from the same tree. Peaks corresponding to tocopherol

Table 2: Fatty acids composition of *P. atlantica* samples obtained by CG (%).

Fatty acids	Samples											
	G1	**DR1**	**LR1**	**G2**	**DR2**	**LR2**	**G3**	**LR3**	**G4**	**LR4**	**B1**	**B2**
C14:0	0.09	0.08	0.1	0.07	0.07	0.1	Tr	0.1	0.1	0.1	0.1	0.08
C16:0	25.1	17.5	13.9	25.0	21.9	14.8	25.7	17.3	24.3	21.1	22.6	22.1
C16:1ω7	0.5	0.7	0.3	0.4	0.4	0.4	0.8	0.3	0.7	0.9	1.8	3.1
C18:0	1.9	2.2	2.2	1.8	2.1	2.1	1.6	2.4	2.6	2.0	1.5	2.0
C18:1ω9	49.6	53.1	51.7	47.4	48.6	51.2	41.7	48.0	40.4	48.3	50.1	40.21
C18: 2	22.0	25.6	30.8	24.4	26.2	30.8	29.4	30.8	30.8	26.6	23.1	32.9
C 18 :3w3	0.8	0.7	0.8	0.7	0.7	0.6	0.8	0.9	1.0	0.9	0.8	1.1
C20:0	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr
C20:1	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr
ΣSFA	27.1	19.7	16.2	26.9	24.0	17.0	27.2	27.3	27.1	23.2	24.2	24.2
ΣMUFA	50.1	53.8	52.0	47.8	49.0	51.6	42.5	48.4	41.2	49.2	51.9	41.9
ΣPUFA	22.8	26.3	31.7	25.1	27.0	31.4	30.2	31.7	31.80	27.5	23.9	33.9
ΣUFA	72.9	80.2	83.7	73.0	75.9	83.0	72.7	80.1	73.0	76.8	75.8	75.8
ΣUFA/ΣSFA	2.7	4.6	5.1	2.8	4.65	4.9	2.7	2.9	2.7	3.3	3.1	1.7

Mean of ΣUFA/ΣSFA	Immature samples	Intermediate maturity samples	Mature samples
	4.06±1.11*	4.65±0.01	2.85±0.22

SFA saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids, *UFA* unsaturated fatty acids, *Tr* trace. *p<0.05 (compared with mature samples).

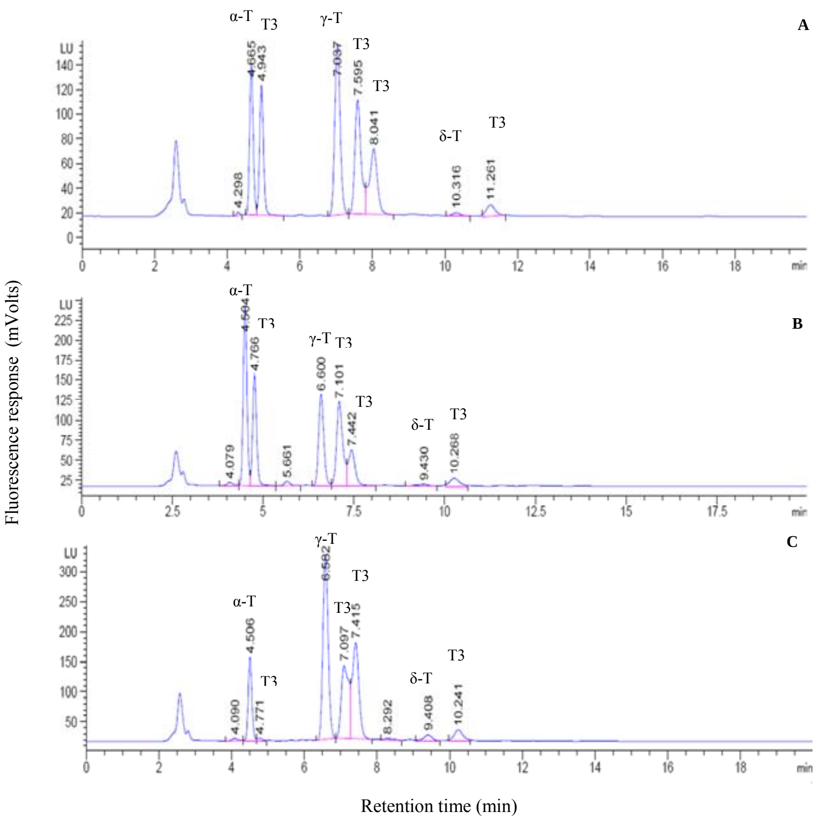

Figure 1: FLD-HPLC tocopherols chromatograms of 3 samples of different maturation degree issued from the same tree. A: mature sample (G2), B: sample of intermediate maturity (DR2) and C: immature sample (LR2). Tocopherols (T) were identified by co-injection with commercial standards. T3 means tocotrienols which were attributed by comparison with chromatograms obtained under similar conditions by Kamal-Eldin *et al*. [14] and Nielsen and Hansen [15]

isomers were identified by co-injection with commercial standards. α-Toc, γ-Toc and δ-Toc were detected, but β-Toc only in trace amounts. The other peaks most probably correspond to tocotrienols (more polar than tocopherols), which were attributed by comparison with chromatograms obtained under similar conditions by Kamal-Eldin *et al.* [14] and Nielsen and Hansen [15].

The quantification of tocopherols by liquid chromatography was based on an external standard method using α-tocopherol. The results are expressed as mg α-tocopherol equivalents per 100 g of oil. Results are presented in Table 3. Total tocopherol content shows differences between individuals and groups (immature fruits, fruits with intermediate maturity and mature fruits). We note a significant difference between the two main groups (mean value

Table 3: Total tocopherols content and relative composition of tocopherols of *P. atlantica* fruits oil (mg/100g oil).

Sample	Total tocopherols	Relative composition[a]		
		α-Toc	γ-Toc	δ-Toc
G1	51.5	22.5 (43.6%)	27.3 (52.9%)	1.8 (3.4%)
DR1	50.0	16.0 (31.9%)	31.8 (63.6%)	2.2 (4.5%)
LR1	nd	nd	nd	nd
G2	30.6	11.1 (36.2%)	17.9 (58.5%)	1.6 (5.32)
DR2	38.6	21.4 (55.4%)	15.4 (39.9%)	1.8 (4.7%)
LR2	49.4	11.8 (24.0%)	34.8 (70.5%)	2.7 (5.5%)
G3	22.61	7.5 (33.2%)	13.4 (59.2%)	1.7 (7.6%)
LR3	59.2	32.6 (55.1%)	23.0 (38.9%)	3.6 (6.0%)
G4	36.2	19.6 (53.9%)	15.5 (42.9%)	1.1 (3.2%)
LR4	43.5	29.0 (66.8%)	12.7 (29.2%)	1.7 (3.9%)
B1	23.8	11.8 (49.8%)	10.1 (42.7%)	1.8 (7.5%)
B2	39.9	14.6 (36.5%)	23.5 (59.0%)	1.8 (4.5%)
Mean of immature samples	50.7±7.9*	24.5±11.1 (48.6±22.1%)	23.5±11.0 (46.2±21.6%)	2.7±0.9* (5.1±1.1%)
Mean of intermediate samples	44.3±8.1	18.7±3.8 (43.66±16.6%)	23.6±11.6 (51.8±16.7%)	2.0±0.3 (4.6±0.1%)
Mean of mature samples	34.1±10.9	14.5±5.6 (42.2±8.3%)	18.0±6.4 (52.5±7.9%)	1.6±0.24 (5.2±1.9%)

[a] relative composition of tocopherols determined by HPLC, nd not determined. *p<0.05 (compared with mature samples).

50.7±7.9 mg/100 g for the immature fruits *versus* a mean value 34.1±10.9 mg/100 g for the mature group, $p<0.05$). Intermediate maturity samples had an intermediate value of 44.3±8.1 mg/100 g.

The predominant tocopherols in *P. atlantica* fruit oil were α- and γ-tocopherol with values ranging from 7.5 to 32.6 mg/100 g and from 10.1 to 34.8 mg/100 g, respectively. Their amounts were slightly higher in immature fruit oil. δ-Tocopherol was detected in all samples, but in low amounts. Its amount was significantly elevated in immature fruit oil ($p<0.05$). The proportion of γ-tocopherol was slightly elevated in mature fruits and the one of intermediate maturity (52.5±7.9% and 51.8±16.7%, respectively). To our knowledge, this is the first time that all tocopherols of *P. atlantica* fruit oil have been analyzed on a polar silica column, which allows for a good separation not only for the four isomers but also the corresponding tocotrienols (Figure 1). It seems that there is a difference between the studied groups concerning tocotrienol isomers, but a study of tocotrienol composition is necessary to confirm these observations

Saber-Tehrani *et al.* studied the tocopherol composition of Iranian *P. atlantica* cold pressed oil using a C-18 Lichrospher RP-100 apolar column and they reported that α-tocopherol was largely the most predominant [16]. Ours results on *P. atlantica* seem to be similar to those of Matthäus and Özcan for *P. terebinthus* [17]. *P. atlantica* fruit oil appears to be richer in tocopherols (particularly in the immature stage) than other food vegetable oils such as sunflower oil (67 mg/100 g) and olive oil (20 mg /100 g) [18]. This richness would supply to the oil beneficial properties. Indeed, tocopherols, notably α-tocopherol, not only have a protective effect on cells against oxidative stress, inflammatory response, lipid peroxidation and cancer, but contribute to the natural protection and conservation of the oil against oxidative deterioration [12, 13, 17, 19]. We should not neglect the anticancer, antidiabetic and cardioprotective effect of tocotrienols, which are now clearly demonstrated [20]. Thus, the study of these compounds in *P. atlantica* fruit oil is of a great interest.

The results of both studies, *i.e*., fatty acids and tocopherols, allowed us to establish the dissimilarity dendrograms for these compounds using the XLStat software and literature data reported for other oils [18, 21-25]. Dendrograms are shown in Figure 2.

The fatty acids composition of *P. atlantica* fruit oil showed a similarity with vegetable oils of high dietary value such as olive oil and peanut oil (Figure 2A). Concerning tocopherols (Figure 2B), the dendrogram indicated two main groups: the first contains oils where α-Toc was largely the most predominant, such as olive oil, sunflower oil and palm oil; the second includes oils with substantial amounts of both α- and γ-Toc, such as rapeseed oil and corn oil. *P. atlantica* fruit oil seems to belong to this latter group.

According to the maturation stage, the dendrogram showed that our oil could be divided into two distinct subgroups. One subgroup was represented by the immature fruit oil, which was characterized by richness in both α- and γ-Toc. The second was composed of mature fruit oils and those of intermediate maturity that was slightly dominated by γ- over α-Toc. This observation should be confirmed by further investigations.

Finally, we can say that our work has brought very interesting and original preliminary results supporting the fact that *P. atlantica* fruit oil before full maturity might be a healthy diet and a valuable source of non-toxic natural bioactive molecules.

However, further studies are needed for the assessment of the biological activities of these compounds.

Experimental

Plant material: *Pistacia atlantica* fruits were collected in August and September 2011 at 3 different sites from the region of Laghouat. After air-drying in the shade at room temperature for one week, 12 samples were used for this study. These samples were divided into 3 groups according to the maturation degree revealed by the skin color. It is important to note that fruit maturation does not take place in an identical way for the same tree. Indeed, there are fruits which finish their maturation prematurely and others later. In that case it is not surprising to find mature fruits at the beginning of August and immature fruits at the end of September. The characteristics of samples are shown in Table 4.

Reagents and standards: Chemicals were purchased from Sigma (USA), Aldrich (Milwaukee, USA), Fluka Chemie (Buchs, Switzerland) and Merck (Germany).

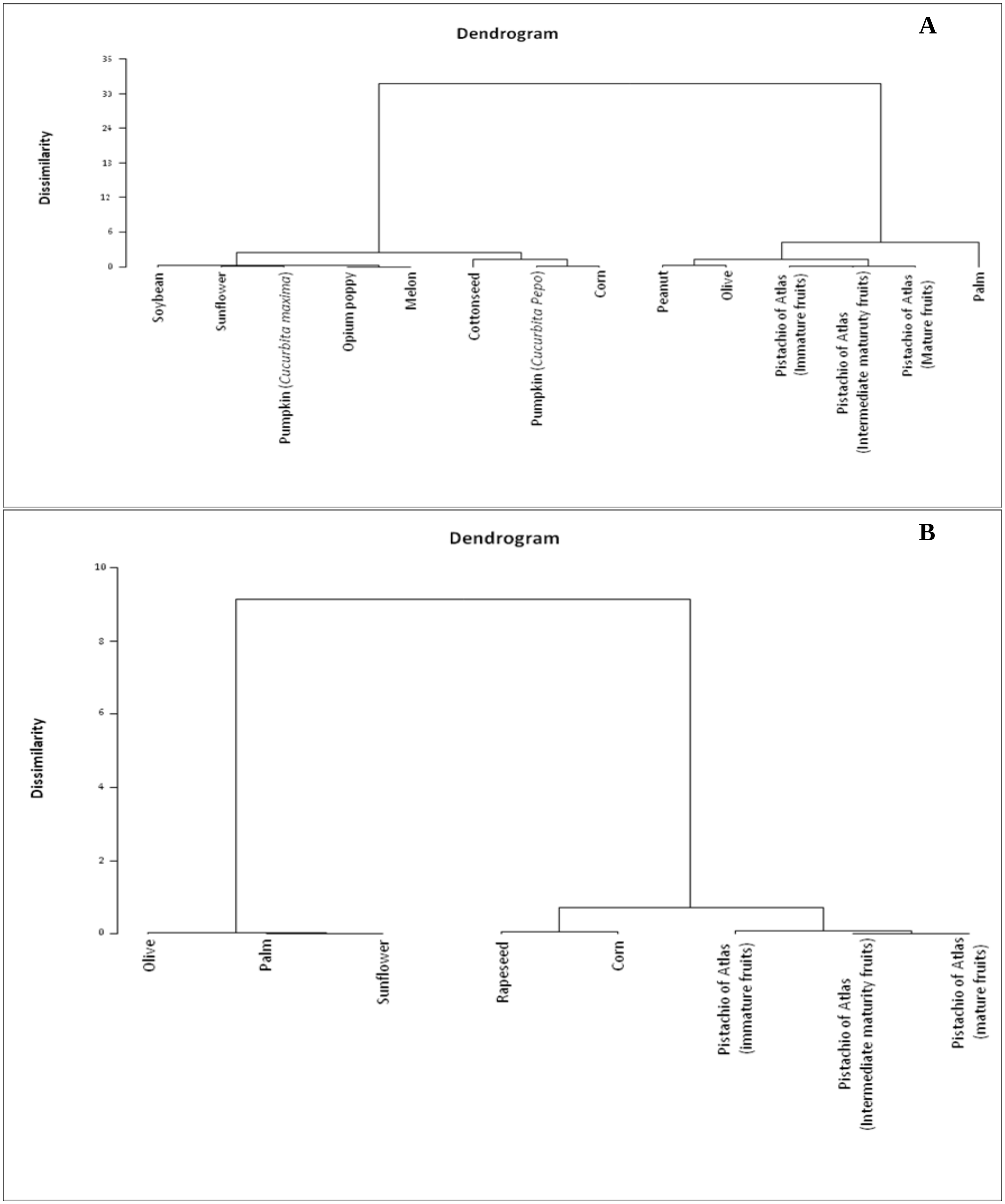

Figure 2: Dissimilarity dendrograms of different vegetable oils including *P. atlantica* fruit oil (with three stages of maturation; immature, intermediate maturity and mature) according to their fatty acids (A) and tocopherols composition (B).

Table 4: Characteristics of *P. atlantica* fruits samples.

Sample	Color of the skin	Degree of maturation	Month of harvest	Site of harvest
G1[a]	Green	Mature	August	Site 1
DR1[a]	Dark red	Intermediate maturity	August	Site 1
LR1[a]	Light red	Immature	August	Site 1
G2[a]	Green	Mature	September	Site 1
DR2[a]	Dark red	Intermediate maturity	September	Site 1
LR2[a]	Light red	Immature	September	Site 1
G3[b]	Green	Mature	September	Site 2
LR3[b]	Light red	Immature	September	Site 2
G4	Green	Mature	September	Site 3
LR4	Light red	Immature	September	Site 3
B1	Black	Mature	September	Site 3
B2	Black	Mature	September	Site 3

[a] and [b] mean that the fruits were collected from the same tree in sites 1 and 2, respectively.
Site 1: 2°59’32,58"E, 33°30’37,07"N, 873m; Site 2 : 2°39’44,19"E, 33°41’23,50"N, 836 m; site 3 : 3°21’16,07"E, 33°09’17,84"N, 736m.

Oil extraction: The fruits (100 g) were milled into powder using a manual mill and extracted with *n*-hexane (150 mL) by stirring at room temperature for 48 h. Samples were then dehydrated with anhydrous sodium sulfate and filtered. The solvent was evaporated under reduced pressure using a rotary evaporator at 40°C. The dried crude oils were stored at +4°C until use.

Fatty acids methyl ester preparation and gas chromatography (GC) analysis: Fatty acid methyl esters (FAMEs) were prepared by refluxing 0.5 g of oil samples with 25 mL of 0.5% sodium methylate solution for 20 min. After cooling, 50 mL of distilled water and 25 mL of *n*-hexane were added to the mixture. The organic phase that contains FAMEs was then recovered, washed and dried over anhydrous sodium sulfate. After filtration and evaporation of *n*-hexane, FAMEs were purified by flash chromatography over silica gel as stationary phase and chloroform as the mobile phase.

FAMEs were analyzed on a Chrompack CP 9002 gas chromatograph equipped with a split-splitless mode injection system, flame ionization detector (FID) and a DB23 capillary column (30 m x 0.32 mm i.d., 0.32 µm film thickness). Oven temperature was in isotherm mode (250°C). Injector and detector temperatures were set at 250°C. Nitrogen was used as carrier gas at a flow rate of 1 mL/min. The injection volume was 1 µL. The fatty acids were identified by comparison of their retention times with those of pure standards.

Tocopherol analysis: Prior to HPLC analysis, 35 mg of seed oil was diluted with 1 mL *n*-hexane and 20 µL of the solution was injected. Tocopherols were analyzed by HPLC using an Agilent 1290 Infinity apparatus equipped with a Quaternary Pump Model 1260, an automatic liquid sample and a fluorimetric detector (FLD 1260). The detection was set at 295 nm for excitation wavelength and at 330 nm for emission wavelength. The separation column was a SI60 (250 mm x 4.6 mm i.d., 5 µm, Lichrosorb, Merck KGaA Darmstadt, Germany). The mobile phase was *n*-hexane/isopropanol, 99/1, v/v, in isocratic mode, with a flow rate set at 1 mL/min, during 20 min. Column compartment was set at 25ºC. Commercial standard (α-Toc) and a mixt (α-Toc, β-Toc, γ-Toc and δ-Toc) were co-injected with samples for the identification of tocopherol isomers.

Statistical analysis: Student's test was used for the statistical comparisons. Differences were considered to be significant at $p<0.05$, $p<0.01$ or $p<0.001$. The dissimilarity dendrograms to compare *P.atlantica* fruit oil with various vegetable oils were made using XL Stat software.

References

[1] Benabderrahmane M, Benali M, Aouissat H, Jordan Buesso M-J. (**2009**) Activité antimicrobienne des huiles essentielles de *Pistacia atlantica* Desf. de l’Algérie. *Phytothérapie*, ***7***, 304-308.

[2] (a) Djeridane A, Yousfi M, Nadjemi B, Boutassouna D, Stocker P, Vidal N. (**2006**) Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. *Food Chemistry*, ***97***, 654–660; (b) Atmani D, Chaher N, Berboucha M, Ayouni A, Lounis H, Boudaoud H, Debbache N, Atmani D. (**2009**) Antioxidant capacity and phenol content of selected Algerian medicinal plants. *Food Chemistry*, ***112***, 303–309.

[3] Gourine N, Bombarda I, Yousfi M, Emile M. Gaydou EM. (**2010**) Chemotypes of *Pistacia atlantica* leaf essential oils from Algeria. *Natural Product Communications*, ***5***, 115-120.

[4] Gourine N, Yousfi M, Bombarda I, Nadjemi B, Stocker P, Gaydou E-M. (**2010**) Antioxidant activities and chemical composition of essential oil of *Pistacia atlantica* from Algeria. *Industrial Crops and Products*, ***31***, 203–208.

[5] Peksel A, Arisan I, Yanardag R. (**2013**) Radical scavenging and anti-acetylcholinesterase activities of aqueous extract of wild pistachio (*Pistacia atlantica* Desf.) leaves. *Food Science and Biotechnology*, ***22***, 515-522.

[6] Yousfi M, Nedjmi B, Bellal R, Ben Bertal D, Pallad G. (**2002**) Fatty acids and sterols of *Pistacia atlantica* fruit oil. *Journal of the American Oil Chemists' Society*, ***79***, 1049-1050.

[7] Yousfi M, Nadjemi B, Belal R, Bombarda I, Gaydoud E-M. (**2005**) Triacylglycerol composition of oil from *Pistacia atlantica* fruit growing in Algeria. *Journal of the American Oil Chemists' Society*, ***82***, 93-96.

[8] Mosharrafa SAM, Kawashty SA, Saleh NAM. (**1999**) Flavonoids of *Pistacia atlantica* Desf. *Bulletin of the National Research Centre (Egypt)*, ***24***, 109–114.

[9] Rezaei PF, Fouladdel Sh, Hassani Sh, Yousefbeyk F, Ghaffari SM, Amin Gh, Azizi A. (**2012**) Induction of apoptosis and cell cycle arrest by pericarp polyphenol-rich extract of Baneh in human colon carcinoma HT29 cells. *Food and Chemical Toxicology*, ***50***, 1054–1059.

[10] Charef M, Yousfi M, Saidi M. (**2008**) Determination of the fatty acid composition of acorn (*Quercus*), *Pistacia lentiscus* seeds growing in Algeria. *Journal of the American Oil Chemists' Society*, ***85***, 921-924.

[11] Trabelsi H, Cherif OA, Sakouhi F, Villeneuve P, Renaud J, Barouh N, Boukhchina S, Mayer P. (**2012**) Total lipid content, fatty acids and 4-desmethylsterols accumulation in developing fruit of *Pistacia lentiscus* L. growing wild in Tunisia. *Food Chemistry*, ***131***, 434-440.

[12] Kamal-Eldin A, Appelqvist L. (**1996**) The chemistry and antioxidant properties of tocopherols and tocotrienols. *Lipids*, ***31***, 671-701.

[13] Hussain N, Irshad F, Jabeen Z, Haider-Shamsi I, Li Z, Jiang L. (**2013**) Biosynthesis, structural, and functional attributes of tocopherols in planta; past, present, and future perspectives. *Journal of Agricultural and Food Chemistry*, ***61***, 6137-6149.

[14] Kamal-Eldin A, Görgen S, Pettersson J, Lampi AM. (**2000**) Normal-phase high-performance liquid chromatography of tocopherols and tocotrienols. Comparison of different chromatographic columns. *Journal of Chromatography A*, ***881***, 217–227.

[15] Nielsen MM, Hansen A. (**2008**) Rapid high-performance liquid chromatography determination of tocopherols and tocotrienols in cereals. *Cereal Chemistry*, ***85***, 248–251.

[16] Saber-Tehrani M, Givianrad MH, Aberoomand-Azar P, Waqif-Husain S, Jafari Mohammadi SA. (**2013**) Chemical composition of Iran's *Pistacia atlantica* cold-pressed oil. *Journal of Chemistry*, *Volume* ***2013***, 1-6.

[17] Matthäus B, Özcan MM. (**2006**) Quantification of fatty acids, sterols and tocopherols in Turpentine (*Pistacia terebenthus* Chia) growing wild in Turkey. *Journal of Agricultural and Food Chemistry*, ***54***, 7667-7671.
[18] Karlenskind A. (**1992**) Manuel des corps gras. Tome 1 Technique et documentation. Lavoisier, Paris, France, 120-207.
[19] Cardeno A, Sanchez-Hidalgo M, Aparicio-Soto M, Alarcon-de-la-Lastra C. (**2014**) Unsaponifiable fraction from extra virgin olive oil inhibits the inflammatory response in LPS-activated murine macrophages. *Food Chemistry*, ***147***, 117-123.
[20] Wong RSY, Radhakrishnan AK. (**2012**) Tocotrienol research: past into present. *Nutrition Reviews*, ***70***, 483-490.
[21] Rezanka T. (**1999**) Characterization of fatty acids and triacylglycerols in vegetable oils by gas chromatography and statistical analysis. *Analytica Chimica Acta*, ***398***, 253-261.
[22] Feeter DK. (**1974**) Determination of tocopherols, sterols, and steryl esters in vegetable oil distillates and residues. *Journal of the American Oil Chemists' Society*, ***51***, 184-187.
[23] Changbumrung S, Buavatana T, Migasena P. (**1980**) Alpha-tocopherol in vegetable oils. *International Journal for Vitamin and Nutrition Research*, ***50***, 242-246.
[24] Giacometti J. (**2001**) Determination of aliphatic alcohols, squalene, alpha-tocopherol and sterols in olive oils: direct method involving gas chromatography of the unsaponifiable fraction following silylation. *Analyst*, ***126***, 472-475.
[25] Schwartz H, Ollilainen V, Piironen V, Lampi A.M. (**2008**) Tocopherol, tocotrienol and plant sterol contents of vegetable oils and industrial fats. *Journal of Food Composition and Analysis*, ***21***, 152-161.