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Fatty Acid, Sterol and Tocopherol Compositions of *P. scoparia* and *Pistacia atlantica* Seed Oil

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The present study was conducted on fatty acids, sterols and tocopherols content of *P. scoparial* and cold pressed *Pistacia atlantica* seed oils to seek the possibility of industrial applications. Tocopherols were analyzed with reversed-phase high performance liquid chromatography and fatty acids and sterols with gas chromatography. The main fatty acids from the studied samples were: oleic acid, linoleic acid and palmitic fatty acid. Oleic acid represents 51 % in *Pistacia atlantica* and 67 % in *P. scoparia* seed oils. Because of its high content of unsaturated fatty acids, it might prove to be of value in diets and may be used as edible oils. Unique amount of tocopherols and sterols may be of nutritional importance in the application of these seed oils. Total sterol content in *Pistacia atlantica* and *P. scoparia* seed oils were 2164.5 and 2084.5 mg kg^{-1} oil, respectively. High level of tocopherols in *Pistacia atlantica* cold press oil (409.97 mg kg^{-1} oil) and *P. scoparia* oil (487.92 mg kg^{-1} oil) were determined.

Key Words: Cold press oil, Fatty acid, Oleic acid, Sterol, Tocopherol.

INTRODUCTION

Search for new sources of novel oils is necessary, because no oil from any single source has been found to be appropriate for all purposes, which is due to oils from different sources generally differ in their composition. The chemical composition of edible seed oils determines the potential health benefit and industrial usages[1].

Almond and pistachio are highly nutritious and have a high fat content. They are used in food, pharmaceutical and cosmetic industries, but they also have a high cost. The genus of *Pistacia* which contains 13 or more species belongs to Anacardiaceae family. *Pistacia atlantica* is one of them which grow wild in different parts of Iran[2]. Wild populations of almond species, representing a wide range of morphological and geographical forms have evolved throughout southwest and central Asia[3]. Iran is optimally situated for growing almonds. Nearly 20 of the wild species have been reported from Iran[4]. *P. scoparia* is one of them which grow in Kerman Province of Iran. The fruit of wild almond and pistachio are used by natives as ingredient in foods and sweets. In spite of wide distribution of wild almond and pistachio with high nutritional value and low cost, they have not been used for industrial applications. Although numerous studies have been reported on the characteristics of the oil and other components of commercial almond and pistachio species, a complete study on chemical compositions of wild almond and pistachio species is not found in the literature, even though some work has been performed before, but it is the first time that cold-press *Pistacia atlantica* seed oil and fatty acids, sterol and tocopherols of *P. scoparia* seed oil composition were determined[5-9].

In the current study, oil was extracted from the fruit of *Pistacia atlantica* by cold press technique and from the fruit of *P. scoparia* by solvent extraction. The fatty acid, sterol and tocopherol composition of the oil of *P. scoparia* and *Pistacia atlantica* were determined.

EXPERIMENTAL

All chemicals used were of analytical grade from Merck. *P. scoparia* seeds were collected on October from Kerman Province in Iran. *Pistacia atlantica* seeds were picked on September from three locations in Iran including Fars, Isfahan and Kohkeloye Boyerahmad Province and were mixed equally. The outer skins of them were manually peeled and then dried in the shade.

Oil extraction: Total obtained seeds were divided into three portions and all of analytical procedures on these sub samples were repeated. Values were expressed as the mean ± standard deviation ($\bar{x}$ ± SD) of three separate contents.

100 g of *P. scoparia* samples were first ground into a fine powder and combined with 300 mL of hexane, followed by vigorous shaking for 3 h. The resulting mixture was filtered under the vacuum. The solvent was removed from the extract using a rotary evaporator at 35 ºC.

Oil of *Pistacia atlantica* seed was obtained by pressing 4 kg of seed by means of cold press machine (PR500, Germany). Fine particles in the expressed oil were separated by filtration; additionally, before each analysis these filtered crude oils were centrifuged in a centrifuge Kokusan (model H-11n, Tokyo, Japan) at 4000 rpm during 15 min.

Fatty acid composition: According to the international standard ISO5509[10], fatty acids were converted to methyl ester using boron trifluoride methanol reagent (20 %). Then, the extracted fatty acid methyl esters analyzed by a gas chromatograph, which equipped with TR-CN 100 high polar (60 m × 0.25 mm, 0.25 μm) column. The oven temperature was programmed from 100-200 ºC at 2 ºC min^{-1} and the injector and detector temperature were set at 250 and 280 ºC, respectively. Further parameters were as follows: hydrogen as carrier gas, flow rate 1 mL min^{-1}, split ratio 1:100 and injection volume 1 μL. Accompanied by flow peaks was achieved by retention times and by comparing them with authentic standards analyzed under the same conditions.

Sterol analysis: 4 g *P. scoparia* and *Pistacia atlantica* seed oils with added α-cholestanol as an internal standard were saponified with 40 mL methanolic potassium hydroxide (2 mol L^{-1}) for 1 h (under reflux) and the unsaponifiables were then extracted with diethyl ether and then submitted to thin layer chromatography. The developing solvent was hexane/diethyl ether. After drying, the spots were visualized by using 2,7-dichlorofluorescein (0.1 % in ethanol). Sterol band was identified under ultraviolet (UV) light at 232 nm. The sterol fraction was then extracted from the silica gel with pure chloroform. After solvent evaporation sterol derivatives (trimethylsilyl ethers) were synthesized at 90 ºC for 0.5 h in 100 mL anhydrous pyridine with 100 mL of a mixture of hexamethyldisilane-trimethylchlorosilane (99:1, v/v). The reaction mixture was finally evaporated to dryness and the residue was diluted in 1 mL chloroform prior to GC analyses.

The silanized sterols were analyzed by gas chromatography using Young-Lin gas chromatograph (Model 6000 South Korea) supplied with flame ionization detector (FID) and capillary column (60 m × 0.32 mm i.d.; film thickness was 0.25 μm). The carrier gas was hydrogen at 1 mL min^{-1} column flow and 1:20 split ratio. Injector and detector temperatures were at 300 and 320 ºC, respectively and oven temperature was at 250 ºC. Peak identification was carried out by comparing with the retention times of the standards.

Tocopherol analysis: Normal-phase high performance liquid chromatography (NP-HPLC) was selected to avoid extra sample treatment (*e.g.*, saponification). *P. scoparia* and *Pistacia atlantica* seed oils were dissolved in acetone (1:10), filtered by a syringe filter (0.22 μ) and 20 μL injected into a C_{18} lichrosphere RP-100 (250 mm × 4.6 mm, 5 μm) column and guard column (4.6 mm × 1.5 mm) equipped with a UV detector at 295 nm. The mobile phase was acetonitrile: methanol:water (47.5, 47.5, 5 v/v) at a flow rate of 1 mL min^{-1}. The amounts of each tocopherol were calculated by comparing with standards purchased from Sigma.

RESULTS AND DISCUSSION

Fatty acid composition: Table-1 shows the fatty acid composition of *P. scoparia* and *Pistacia atlantica* seed oils.

TABLE-1
FATTY ACID COMPOSITION OF *P. Scoparia* AND PISTACIA ATLANTICA SEED OILS (MEAN ± STANDARD ERROR (SE) (%)

Fatty acid	Value, *Pistacia atlantica*	Value, *P. Scoparia*
Myristic $C_{14:0}$	0.15 ± 0.12	–
Palmitic $C_{16:0}$	13.12 ± 0.21	6.94 ± 0.11
Palmitoleic $C_{16:1}$	2.04 ± 0.10	0.30 ± 0.03
Margaric $C_{17:0}$	0.07 ± 0.02	–
Heptadecenoic $C_{17:1}$	0.09 ± 0.02	–
Stearic $C_{18:0}$	2.78 ± 0.17	3.30 ± 0.12
Oleic $C_{18:1}$	50.65 ± 0.82	67.18 ± 0.72
Linoelaidic $C_{18:2}$	0.04± 0.15	–
Linoleic $C_{18:2}$	29.76± 0.30	22.13 ± 0.42
α-Linolenic $C_{18:3}$	0.59 ±0.08	0.15 ± 0.02
Arachidic $C_{20:0}$	0.17 ± 0.06	–
Gondoic $C_{20:1}$	0.32 ± 0.02	–
Behenic $C_{22:0}$	0.18 ± 0.04	–
Lignoceric $C_{24:0}$	0.04 ± 0.02	–
SAFA	16.51	10.24
MUFA	53.10	67.48
PUFA	30.39	22.28

[a]SAFA, saturated fatty acid; [b]MUFA, monounsaturated fatty acid. [c]PUFA, polyunsaturated fatty acid.

Major monounsaturated fatty acid (MUFA) was oleic acid. It represents 50.65 % in *Pistacia atlantica* and 67.18 % in *P. scoparia* seed oils. Linoleic acid as polyunsaturated fatty acid (PUFA) was found 29.76 % for *Pistacia atlantica* and 22.13 % for *P. scoparia* oil. The main saturated fatty acid (SFA) was palmitic acid. It was 13.12 % for *Pistacia atlantica* and 6.94 % for *P. scoparia* seed oil. Stearic acid another saturated fatty acid was found in oils was 2.87 % in *Pistacia atlantica* and 3.30 % for *P. scoparia*. Results of this study on oleic acid levels in *Pistacia atlantica* is in good agreement with those reported by Tsantili *et al.*[11] (52 %) and Arena *et al.*[12] (55 %) for commercial pistachio from Iran. Oleic acid of *P. scoparia* from current study was comparable with those reported by Moayedi *et al.*[13] (68 %), Maguire *et al.*[14] (69.2 %) and Miraliakbari and Shahidi[15] (69.9 %) for commercial and domestic almond. The PUFA and MUFA and SFA amounted to 30.39, 53.10 and 16.51 %, respectively for *Pistacia atlantica* and 22.28, 67.48 and 10.24 % for *P. scoparia* seed oil. The ratio of unsaturated/saturated fatty acid of *Pistacia atlantica* seed oil was 5.1 and of *P. scoparia* was *ca.* 8.8. Low content of saturated fatty acids and high content of monounsaturated oleic acid form *P. scoparia* and *Pistacia atlantica* is highly favorable in human nutrition. According to the results of this study, *P. scoparia* and *Pistacia atlantica* seed oil is regarded as oleic- linoleic oil because oleic acid is most abundant, followed by linoleic acid and it may be used as edible oils or for margarine manufacture[16].

Sterol composition: Phytosterols are of a great interest due to their antioxidant activity and impact on health. The analysis of the sterols provides rich information about the quality and the identity of the oil investigated and for the detection of oil and mixtures not recognized by their fatty acids profile[17]. Table-2 shows sterol composition of seed oil of *P. scoparia* and *Pistacia atlantica*. Seven sterols were identified for both oils. β-Sitosterol was the predominant component in these oils, which constituted *ca.* 87.73 % in *Pistacia atlantica* and 91.32 % in *P. scoparia* seed oils. Δ^5-Avenasterol was present in 2.28 % of *Pistacia atlantica* and 3.52 % of

TABLE-2
STEROL COMPOSITION OF PISTACIA ATLANTICA AND *P. Scoparia* SEED OIL (MEAN ± STANDARD ERROR (SE) (%)

Constituent	Value, *Pistacia atlantica*	Value, *P. scoparia*
Cholesterol	0.44 ± 0.02	0.21 ± 0.01
Campesterol	4.35 ± 0.04	3.49 ± 0.06
Stigmasterol	0.98 ± 0.02	0.41 ± 0.03
β-Sitosterol	87.73 ± 0.16	91.32 ± 0.14
Δ-5-Avenasterol	2.28 ± 0.04	3.52 ± 0.05
Δ-7-Avenasterol	1.04 ± 0.06	0.26 ± 0.06
Δ-5,24-Stigmastadienol	1.19 ± 0.02	0.29 ± 0.03
Others	1.59	0.50

P. scoparia seed oil samples. The next major component was campestrol. It was 4.35 % in *Pistacia atlantica* seed oil and 3.49 % in *P. scoparia* seed oil. Among the minor sterols stigmastadienol and $\Delta^{5,24}$-avenastrol and stigmasterol amounted to 1.19, 1.04 and 0.98 % of the total amount of sterols in *Pistacia atlantica* and about 0.29, 0.26 and 0.41 %, respectively in *P. scoparia* seed oil. Cholesterol which is specific to animal lipids is present at low levels in most vegetable oils. It was found at *ca.* 0.44 % for *Pistacia atlantica* oil and 0.21 % for *P. scoparia* oil.

In nuts such as walnuts, almonds, peanuts, hazelnuts and the macadamia nuts, β-sitosterol is the most abundant sterol. Among the different phytosterols, β-sitosterol had been most intensively investigated with respect to its beneficial and physiological effects on health.

Total sterol content in *Pistacia atlantica* and *P. scoparia* seed oils were 2164.5 and 2084.5 mg kg^{-1} oil, respectively and higher than that sterol content in almonds, Brazil nuts, hazelnuts, pecans, pine nuts, pistachios and walnuts, which oil extracted with different solvent, were ranging from 800-1600 mg Kg^{-1} oil[15-18]. Sterols are essential components of cell membranes that play a role in controlling membrane fluidity and permeability.

Tocopherol composition: Tocopherols and tocotrienols are naturally occurring constituents found in varying amounts in vegetable oils. The presence of these compounds is important in relation to oil stability and nutritional labeling and possible health effects related to the consumption of oils[19]. Because of the critical role of the tocopherols in nutrition and their relative instability, qualitative and quantitative analyses are very important. Table-3 shows the tocopherol content of *Pistacia atlantica* and *P. scoparia* seed oil. α-Tocopherol was in highest concentration in *Pistacia atlantica* and *P. scoparia* fruit oil. It was 379.68 mg kg^{-1} oil for *Pistacia atlantica* and 446.92 mg kg^{-1} oil for *P. scoparia*. α-Tocopherol not detected in commercial pistachio[20], but Maguire *et al.*[14] mentioned that α-tocopherol was the most dominant tocopherol in almonds, peanuts, hazelnuts and macadamias. It ranged from 9.4 (peanuts) to 186.4 μg g^{-1} oil (almonds). α-Tocopherol ability to act as an antioxidant and various functions at the molecular level reduce the risk of cancer and cardiovascular diseases[21,22]. However, not only α-tocopherol but also other tocopherol forms are recently considered to be of biological importance[23-26]. (β+γ)-Tocopherol and δ-tocopherol were 20.70 and 9.59 mg kg^{-1} oil in *Pistacia atlantica* and 35.40 and 5.60 mg kg^{-1} oil for *P. scoparia* seed oil, respectively.

TABLE-3
TOCOPHEROL COMPOSITION OF *Pistacia atlantica* AND *P. scoparia* SEED OIL

Tocopherols	Content (mg kg^{-1}) oil, *P. atlantica*	Content (mg kg^{-1}) oil, *P. soparia*
α-Tocopherol	379.68 ± 0.02	446.92 ± 0.08
(β+γ)-Tocopherol	20.70 ± 0.04	35.40 ± 0.10
δ-Tocopherol	9.59 ± 0.02	5.60 ± 0.04
Total	409.97	487.92

High level of tocopherols in *Pistacia atlantica* cold press oil (409.97 mg kg^{-1} oil) and *P. scoparia* oil (487.92 mg kg^{-1} oil) were determined. They were more than that Miraliakbari *et al.*[15] and Kornsteiner *et al.*[20] reported for commercial pistachio and almond which oil extracted with hexane, chloroform-methanol and petroleum ether were obtained 218.5, 236.1 and 298 mg kg^{-1} oil for pistachio and 170.6, 179.3 and 273 mg kg^{-1} oil for almond. Tocopherol content in nuts (Brazil nuts, hazelnuts, pecans, pine nuts and walnuts), which oil extracted with different solvent, were obtained from 106.8 to 321.9 mg kg^{-1} oil[15].

Conclusion

The chemical composition of edible fats and oils largely determines their stability, quality, nutritional value, sensory properties and potential health effects. A large quantity of oils and fats is presently derived from plant source and because of this; interest in newer sources of edible oils has recently grown. Nuts contain a various group of compounds that enhance the nutritional value of the human diet. Almond and pistachio oils are commercially valuable and nutritionally important. In spite of wild distribution of wild almond and pistachio with high nutritional value and low cost, they have not been used for industrial applications. Improved knowledge on the composition of *Pistacia atlantica* and *P. scoparia* seed oil would assist in efforts to achieve industrial application of these plants. *Pistacia atlantica* and *P. scoparia* seeds give a considerable yield of oil and seem to be a good source of fatty acids and lipid soluble bioactive compounds. *Pistacia atlantica* and *P. scoparia* oils are rich in monounsaturated fatty acids, predominantly oleic acid, but contain much lower amounts of polyunsaturated fatty acids, predominantly linoleic acid and small amounts of saturated lipids. Regardless of low cost and the taste, unique amount of tocopherols and sterols may be of nutritional importance in the application of these seed oils.

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