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Research Article

Chemical Composition of Iran's *Pistacia atlantica* Cold-Pressed Oil

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The lipid fraction of *Pistacia atlantica* seeds was extracted for the first time by means of cold-press technique and analyzed for its chemical composition. The fatty acids, sterols, triacylglycerols (TAG), tocopherols, polyphenols, and pigments were identified and their concentrations were determined by means of reversed-phase high-performance liquid chromatography (RP-HPLC) and gas chromatography (GC). Because of its high content of unsaturated fatty acids, it might prove to be of value in diets and it may be used as edible cooking or salad oils or for margarine manufacture. *Pistacia atlantica* seed oil has the unique sterols and tocopherols content providing source of natural antioxidants. The main triacylglycerols were SLL + PLO, SOL + POO, OOLn + PLL, OOO, and SOO. This paper examined the phenolic fraction of *Pistacia atlantica* seed oil. Moreover, caffeic acid followed by cinnamic acid, pinoresinol, vanillin, *p*-Coumaric acid, ferulic acid, and *o*-Coumaric acid was also determined. This paper presents the first investigation of chlorophyll's and carotene's composition in *Pistacia atlantica* seed oil. Furthermore, pheophytin a was the major component, followed by luteoxanthin, neoxanthin, violaxanthin, lutein, lutein isomers, chlorophyll a, chlorophyll a′, and pheophytin a′ were also determined.

1. Introduction

The genus of *Pistacia* which contains 13 or more species belongs to *Anacardiaceae* family [1]. One of the most widely distributed species of *Pistacia* is *Pistacia atlantica* which is called "Baneh" in Iran and is the most economically important tree species in many rural areas. The resin of wild pistachio, called Saqez, is used for a variety of industrial and traditional uses, including food and medicine [2]. The fruit of wild pistachio is used by natives as flavor in food after grinding it and it is used for its oil, although the fruit is small and not commercially valuable.

Tree nuts and their oils contain several bioactive and health-promoting components. Dietary consumption of tree nut oils may have even more beneficial health effects than consumption of whole tree nuts, possibly due to the replacement of dietary carbohydrates with unsaturated lipids and/or other components present in the oil extracts [3]. Unrefined oils contain a number of minor components such as tocopherols, carotenoids, and phenols. Tocopherols and carotenoids improve oil stability and thus oils naturally rich in these constituents are preferred [4, 5]. Tocopherols and phenols can act as antioxidants and reduce the risk of cancer and cardiovascular diseases [6–8]. Phytosterols have been used as blood cholesterol-lowering agents for the last half century. They have been shown to be effective and safe [9].

Conventional vegetable oil extraction is carried out by pressing or solvent extraction. Solvent oil extraction is the most efficient method; however, its application presents some industrial disadvantages such as emissions of volatile organic compounds into the atmosphere, high operation costs, and poor quality products caused by high processing temperatures [10].

In this work, oil was extracted from the fruit of *Pistacia atlantica* by cold press technique and the fatty acid, sterol, triacylglycerol, tocopherol, phenolic, and pigment composition of the oil of *Pistacia atlantica* was determined. Although some work has been performed before to determine the sterol

and triacylglycerol composition of *Pistacia atlantica* fruit oil from Algeria [11–13] and total phenol and total tocopherol of *Pistacia atlantica* from Iran [14], it is the first time *Pistacia atlantica* cold press oil from Iran was investigated and phenol, pigment, and tocopherol fractions of *Pistacia atlantica* seed oil were determined.

2. Experimental

2.1. Samples. *Pistacia atlantica* seeds were collected in September from three locations in Iran including Fars, Isfahan, and Kohkeloye Boyerahmad province. Samples were mixed equally and the outer skin was manually peeled and then dried in the shade.

2.2. Reagents and Standards. All chemicals and solvents used in this study were of analytical grade and were purchased from Sigma, Fluka, BDH and Merck chemical companies.

2.3. Oil Extraction. Oil was obtained by pressing 5 kilograms of *Pistacia atlantica* seed by means of cold press machine (PR500, Germany). This operation was carried out three times and then fine particles in the expressed oil were separated by filtration; before each analysis, these filtered crude oils were centrifuged in a centrifuge Kokusan (model H-11n, Tokyo, Japan) at 4000 rpm during 15 minutes. Experiments were carried out in triplicate after oil extraction as soon as possible; otherwise extracted oil samples were stored at 3°C for a short time. The yield of seed oil was calculated as 15.32 ± 0.6% in dry weight.

2.4. Fatty Acid Analysis. The oil was converted to methyl ester using boron trifluoride methanol reagent (20%) and fatty acids were converted into the methyl ester derivatives. Then, the extracted fatty acid methyl esters (FAMEs) dissolved in $CHCl_3$ and analyzed by a gas chromatograph [15]. A Young-Lin gas chromatograph (model, 6000 South Korea), equipped with a split-splitless mode injection system, flame-ionization detector, and TR-CN 100 High polar (60 m × 0.25 mm, 0.25 μm) column was used for FAME analysis. The gas chromatographic conditions were injection port temperature (250°C), initial oven temperature (100°C), heating rate (2°C/min), final temperature (200°C), detector port temperature (280°C), hydrogen gas flow rate (30 mL/min), air flow rate (300 mL/min), and hydrogen gas carrier flow rate (1 mL/min). The injection volume was 1 μL. The FAMEs were identified by comparing retention times to pure standards purchased from Fluka (Germany).

2.5. TGA Analysis. TAGs were separated by RP-HPLC (model Young-Lin Acme 9000). Oil samples were dissolved in HPLC-grade acetone (5%) and after filtration by a syringe filter (0.22 μ), 20 μL were injected into C18 Column (25 cm × 4.6 mm i.d., 5 μm particle size, Tracer Excel 120 ODSA) and were eluted with acetonitrile and acetone (40 : 60) at a flow rate of 1.5 mL/min. The column was equilibrated at room temperature and the effluent monitored with a refractive index detector. The TAGs were identified by comparing retention times to pure standards (Lardon AB Limhamnsgardens Alle 16 Malmo, Sweden).

2.6. Sterol Analysis. For determination of sterols, 5 g of sample were saponified with 50 mL of 2 N ethanolic KOH at 85°C for 30 min (the solution becomes clear) in the presence of 2 mg α-cholestanol as internal standard. After addition of 50 mL of water, unsaponifiables were extracted three times with 100 mL diethyl ether. The pooled extracts were washed three times with 50 mL of deionized water and the solvent was removed under reduced pressure with a rotary evaporator. The unsaponifiable was diluted in chloroform and then submitted to thin layer chromatography (TLC) on silica gel previously immersed in the 0.2 N ethanolic potassium hydroxide solution and then placed in stove at 100°C for 1 hour. The developing solvent was hexane/diethyl ether (65 : 35, (v/v)). After drying, the spots were identified by spraying 2,7-dichlorofluorescein (0.1% in ethanol). Sterol band was observed under ultraviolet (UV) light at 232 nm by comparing its rate of flow (RF) value with standard. The sterol fraction was then extracted from the silica gel with hot pure chloroform. After solvent evaporation sterol derivatives (trimethylsilyl ethers, TMS) were synthesized at 90°C for 30 min in 10 mL anhydrous pyridine with 10 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 column flow and 1 : 20 split ratio. Injector and detector temperatures were 300 and 320°C, respectively. Oven temperature was at 250°C. Peak identification was carried out by comparing with the retention times of the standards (cholesterol (99%), stigmasterol (95%), b-sitosterol (95%), campesterol (98%), and sitostanol (96.7%) were purchased from Sigma Chemical Co. (St. Louis, MO, USA)).

2.7. Tocopherol Analysis. Tocopherols were measured by RP-HPLC and UV-detection at 295 nm. extracted oil was diluted with acetone (1 : 10) and 20 microliter was injected to HPLC after filtration by a syringe filter (0.22 μ) with a C-18 lichrospher RP-100 (250 mm × 4.6 mm, 5 μm) column and guard column (4.6 × 1.5 mm). The mobile phase was acetonitrile, methanol, and water (47.5, 47.5, 5 v/v) at a flow rate of 1 mL/min. The amounts of each tocopherol were calculated by comparing with standards purchased from Sigma.

2.8. Phenol Analysis. 2 g anhydrous oil sample in 10 mL screw-cap test tube was stirred with 1 mL of syringic acid (0.003 g/mL) as internal standard for 30 sec and then was extracted with 5 mL methanol and water (80 : 20) solution by homogenization for 1 min and was sonicated in water bath for 20 min at room temperature. The sample was then centrifuged at 5000 rev/min for 25 min and the supernatant

phase was filtered through a 0.22 μm syringe filter prior to HPLC analysis. Phenolic compounds were determined using RP-HPLC (model Young-Lin Acme 9000) with a UV detector at 280 nm. The chromatographic separation was obtained at 25°C by a C18 column (25 cm × 4.6 mm i.d., 5 μm particle size., Tracer Excel 120 ODSA) under gradient conditions with solvent A (water with 0.2% phosphoric acid), B (methanol), and C (acetonitrile) as follow: 0–40 min, 96% A, 2% B, 2% C; 40–45 min, 50% A, 25% B, 25% C; 45–60 min, 40% A, 30% B, 30% C; 60–72 min, 0% A, 50% B, 50% C; 72–82 min 96% A, 2% B, 2% C with 1 mL/min flow rate. The sample injection volume was 20 μL and identification of compounds was achieved by comparing their retention time with standards (caffeic acid, vanillin, *p*-coumaric acid, ferulic acid, pinoresinol, and cinnamic acid from Sigma-Aldrich Chemie GmbH). Total phenolics were determined spectrophotometrically by using Folin-Ciocalteu's reagent and the results are expressed as gallic acid equivalents. (Folin-Ciocalteau's reagent and standard gallic acid were purchased from Sigma-Aldrich GmbH (Sternheim, Germany)).

2.9. Pigment Analysis. Oil samples were extracted by liquid-phase distribution between N,N-dimethyl-formamide (DMF) and hexane. 25 g of *Pistacia atlantica* oil samples were dissolved in 150 mL DMF and treated with five 50 mL portions of hexane in decanting funnel. The DMF phase contained xanthophylls, chlorophylls and chlorophyllic derivatives was treated with a 2% Na_2SO_4 solution at 0°C and transferred to 100 mL of a mixture of hexane/ethyl ether (1 : 1; v/v). The aqueous phase was eliminated polyphenols and other water soluble compounds. The ether was evaporated in a rotary evaporator at reduced pressure at 30°C. The dry residue was dissolved in methanol, and analyzed by HPLC. Separation was performed using C18 Column (25 cm × 4.6 mm i.d., 5 μm particle size, Tracer Excel 120 ODSA) and elution was performed at a flow rate of 1.0 mL/min at room temperature. The mobile phase was a mixture of methanol and water (8 : 2, v/v) containing 0.025% ammonium acetate and 0.05% triethylamine as phase A and methanol and acetone (1 : 1, v/v) as phase B. The pigments were eluted according to solvent gradient as follow: 0–10 min, 75% A, 25% B, 10–14 min, 50% A, 50% B, 14–21 min 20% A, 80% B, 21–40 min, 0% A, 100% B, 40–50 min 75% A, 25% B. Identification of compounds was achieved by comparing with standards.

3. Results and Discussion

3.1. Fatty Acid Composition. Table 1 shows the fatty acid composition of *Pistacia atlantica* seed oil. The most predominant fatty acid (FA) was oleic acid, with a mean value of ~51%. In addition to oleic acid, seed oil of *Pistacia atlantica* contained high amount of linoleic acid (~30%). The seed oil also contains saturated fatty acid especially palmitic and stearic acids. The level of palmitic acid was ~13% and higher than the amount of stearic acid 3%. The polyunsaturated (PU) fatty acids of the oil amounted to ~30% of the total fatty acid, while the monounsaturated (MU) and saturated (SA) fatty acids amounted to 53% and 17%, respectively. The ratio of unsaturated/saturated fatty acid of *Pistacia atlantica* seed oil was 5.1.

Table 1: Fatty acid composition of *Pistacia atlantica* seed oil (%).

Fatty acid	Value
Myristic $C_{14:0}$	0.15 ± 0.12
Palmitic $C_{16:0}$	13.12 ± 0.21
Palmitoleic $C_{16:1}$	2.04 ± 0.10
Margaric $C_{17:0}$	0.07 ± 0.02
Heptadecenoic $C_{17:1}$	0.09 ± 0.02
Stearic $C_{18:0}$	2.78 ± 0.17
Oleic $C_{18:1}$	50.65 ± 0.82
Linoelaidic $C_{18:2}$	0.04 ± 0.15
Linoleic $C_{18:2}$	29.76 ± 0.30
α-Linolenic $C_{18:3}$	0.59 ± 0.008
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
MUFA	53.10
PUFA	30.39

Oleic acid has an important role in food industry. Foods prepared with oleic acid will remain safe to eat for longer periods, even without refrigeration. Oleic acid is also used as a cleaning agent in the manufacturing of soaps and detergents and as an emollient or softening agent, in creams, lotions, lipsticks, and skin products [16]. It has been found to be fungistatic against a wide spectrum of saprophytic moulds and yeasts.

According to the results of this study, *Pistacia atlantica* seed oil is regarded as oleiclinoleic oil because oleic acid is most abundant, followed by linoleic acid and it may be used as edible cooking or salad oils or for margarine manufacture [17].

3.2. Sterol Composition. Table 2 shows sterol composition of seed oil of *Pistacia atlantica*. Results from the quantitative analysis of sterols from *Pistacia atlantica* (expressed in mg/100 g of oil) showed that the major sterol of the oil is betasitosterol (189.9 mg/100 g oil), which amounted to ~87% of the total amount of sterols. Campesterol (9.4 mg/100 g oil), and Δ^5-avenasterol (4.9 mg/100 g oil) were presented, with about 4 and 2% of the total sterols, respectively.

Each of the $\Delta^{5,23}$ stigmastadienol (2.6 mg/100 g oil), Δ^7-avenasterol (2.3 mg/100 g oil), and stigmasterol (2.1 mg/100 g oil) amounted to about 1% of the total amount of sterols. Among the minor sterols, cholesterol (0.9 mg/100 g oil) was 0.4%.

Phytosterol has good effectiveness in decreasing serum low-density lipoprotein (LDL) cholesterol levels that could be effective in protecting against cardiovascular diseases, thus it can be used to improve the functional foods.

Table 2: Sterol composition of *Pistacia atlantica* seed oil (%).

Constituent	Value
Cholesterol	0.44 ± 0.02
Campesterol	4.35 ± 0.04
Stigmasterol	0.98 ± 0.02
Betasitosterol	87.73 ± 0.16
Delta-5-avenasterol	2.28 ± 0.04
Delta-7-avenasterol	1.04 ± 0.06
Delta-5,23-stigmastadienol	1.19 ± 0.02
Others	1.59

In nuts (walnuts, almonds, peanuts, hazelnuts, and the macadamia nuts), beta-sitosterol is the most abundant sterol, the total sterol contents ranging from 99.12 to 207.17 mg/100 g oil [18]. *Pistacia atlantica* has the high level of beta-sitosterol (189.9 mg/100 g oil).

3.3. TGA Composition. The fatty acid composition can be used to evaluate the stability and nutritional quality of fats and oils, but not always their functional properties. The type and the amounts of the various TAG species in the oil are too important. It is the TAG compositions that determine the final physical and functional properties of the oils. Table 3 shows the TAG composition of *Pistacia atlantica*. The most predominant TGA species are SLL + PLO (21.82%) and then SOL + POO (16.56%), OOLn + PLL (15.68%), OOO (14.07), and SOO (13.72). Other TGAs are minor (OLL + PoOL, PLnP, POS, SLS, LLL, PPP, OLLn, SOS). Where P, palmitic; S, stearic; O, oleic; L, linoleic; Ln, linolenic; Po, palmitoleic. TGAs in *Pistacia atlantica* with ECN of 48 were dominant (30.63%), followed by triacylglycerols with ECN of 46 (27.65%).

3.4. Tocopherol Composition. Because of the critical role of the tocopherols in nutrition and their relative instability, qualitative and quantitative analyses are very important. To the authors' knowledge it is the first time that tocopherol composition of cold press *Pistacia atlantica* fruit oil has been evaluated. Table 4 shows the tocopherol content of *Pistacia atlantica* seed oil. High level of tocopherols in *Pistacia atlantica* cold press oil (409.97 mg/kg oil) was determined. α-tocopherol was in highest concentration. It was 379.68 mg/kg. (γ + β)-tocopherol and δ-tocopherol were 20.70 and 9.59 mg/kg oil, respectively.

Tocopherol content in nuts (almonds, Brazil nuts, hazelnuts, pecans, pine nuts, pistachios, and walnuts), which oil extracted with different solvent, were obtained from 106.8 to 321.9 mg/kg oil [19].

Radical-chain breaking antioxidant in membranes, lipoproteins and foods is the main function of α-tocopherol [20]. α-tocopherol ability to act as an antioxidant and various functions at the molecular level reduce the risk of cancer and cardiovascular diseases [6, 7].

3.5. Phenol Content. This research examined the phenolic fraction of *Pistacia atlantica* seed oil. As Table 4 shows, caffeic acid was the predominant phenolic compounds. It was 1.96 mg/kg oil, followed by cinnamic acid (0.67 mg/kg oil) and pinoresinol (0.64 mg/kg oil). Vanillin (0.28 mg/kg oil), *p*-coumaric acid (0.36 mg/kg oil), ferulic acid (0.16 mg/kg oil), and *o*-coumaric acid (0.19 mg/kg oil) were determined. Total phenol was 57.57 mg/kg oil.

A direct relationship has been found between the content of total phenolics and antioxidant capacity of plants [8]. Phenolic acids have been widely investigated as potential models for the development of new primary antioxidants, which can prevent or delay in vitro and/or in vivo oxidation processes [21] (see Table 5).

3.6. Pigment Content. This work contains the first qualitative quantitative investigation of the chlorophyll and carotenoid pigments composition of *Pistacia atlantica* kernel oil. As Table 6 shows, pheophytin a was the major component (12.02 mg/kg), followed by luteoxanthin (10.41 mg/kg).

Table 3: Triacylglycerol composition of *Pistacia atlantica* seed oil.

Triacylglycerol species	ECN[a]	TAG content (%)
LLL	42	0.55
OLLn	42	0.10
OLL + PoOL	44	7.04
OOLn + PLL	44	15.68
PLnP	46	5.82
SLL + PLO	46	21.83
OOO	48	14.07
SOL + POO	48	16.56
SOO	50	13.72
POS	50	2.41
SLS	50	1.90
SOS	52	0.09
PPP	54	0.23

[a]: ECN, equivalent carbon number (carbon number −2 × number of double bonds).

Table 4: Tocopherol composition of *Pistacia atlantica* seed oil.

Tocopherol	Content (mg/kg oil)
α-tocopherol	379.68 ± 0.92
(γ + β)-tocopherol	20.70 ± 0.04
δ-tocopherol	9.59 ± 0.02
Total	409.97

Table 5: Phenol composition of *Pistacia atlantica* seed oil.

Constituent	Value (mg/kg oil)
Caffeic acid	1.96 ± 0.04
Vanillin	0.28 ± 0.01
p-coumaric acid	0.36 ± 0.01
Ferulic acid	0.16 ± 0.02
o-coumaric acid	0.19 ± 0.02
Pinoresinol	0.64 ± 0.01
Cinnamic acid	0.67 ± 0.02

Table 6: Levels of *Pistacia atlantica* pigment (mg/kg).

Constituent	Value
Neoxanthin	0.15 ± 0.02
Violaxanthin	0.23 ± 0.03
Luteoxanthin	10.41 ± 0.04
Lutein	5.20 ± 0.07
Lutein isomers	1.2 ± 0.02
Chlorophyll a	1.19 ± 0.06
Chlorophyll a′	0.92 ± 0.01
Pheophytin a	12.02 ± 0.03
Pheophytin a′	1.46 ± 0.02
Total chlorophylls	17.21
Total carotenoids	14.80

Neoxanthin (0.15 mg/kg), violaxanthin (0.23 mg/kg), lutein (5.2 mg/kg), lutein isomers (1.2 mg/kg), chlorophyll a (1.19 mg/kg), chlorophyll a′ (0.92 mg/kg), and pheophytin a′ (1.46 mg/kg) were also presented.

Chlorophyll and carotenoid play key roles in photosynthesis. Animals cannot synthesize chlorophylls and carotenoids, thus they must obtain them from foods. Several reports have demonstrated that plant pigments play important roles in health [22, 23]. Carotenoids have antioxidant activity, which protects cells and tissues from free radicals and singlet oxygen. Lutein have a fundamental role in the protection of the macula region of the retina and in the prevention of the cataracts; other beneficial actions of carotenoids include enhancement of the immune response, protection against solar radiation, inhibition of some cancers and prevention of degenerative and cardiovascular diseases [24, 25].

4. Conclusion

The cold-pressing procedure involves neither heat nor chemical treatments, and it is becoming an interesting substitute for conventional practices because of consumers' desire for natural and safe food products. The consumption of new and improved products such as cold pressed oils may improve human health and may prevent certain diseases. Improved knowledge on the composition of *Pistacia atlantica* seed oil would assist in efforts to achieve industrial application of this plant. Data about cold pressed *Pistacia atlantica* seed oil are very few. In concluding this investigation, it is clear that the performed chromatographic techniques constituted a flexible analytical system, which gave valuable information about the structure of the seed oil. *Pistacia atlantica* seeds give a considerable yield of oil and the cold press oil seems to be a good source of fatty acids and lipid-soluble bioactives. The high linoleic and oleic acid content makes the oil nutritionally valuable. Tocopherols and sterols, at the level estimated, may be of nutritional importance in the application of the seed oil.

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References

[1] H. R. Karimi, Z. Zamani, A. Ebadi, and M. R. Fatahi, "Morphological diversity of *Pistacia* species in Iran," *Genetic Resources and Crop Evolution*, vol. 56, no. 4, pp. 561–571, 2009.

[2] M. Pourreza, J. D. Shaw, and H. Zangeneh, "Sustainability of wild pistachio (*Pistacia atlantica* Desf.) in Zagros forests, Iran," *Forest Ecology and Management*, vol. 255, no. 11, pp. 3667–3671, 2008.

[3] F. B. Hu and M. J. Stampfer, "Nut consumption and risk of coronary heart disease: a review of Epidemiologic Evidence," *Current Atherosclerosis Reports*, vol. 1, no. 3, pp. 204–209, 1999.

[4] K. Warner and E. N. Frankel, "Effects of β-carotene on light stability of soybean oil," *Journal of the American Oil Chemists' Society*, vol. 64, no. 2, pp. 213–218, 1987.

[5] E. N. Frankel, "Review. Recent advances in lipid oxidation," *Journal of the Science of Food and Agriculture*, vol. 54, no. 4, pp. 495–511, 1991.

[6] G. W. Burton, "Vitamun E: molecular and biological function," *Proceedings of the Nutrition Society*, vol. 53, no. 2, pp. 251–262, 1994.

[7] G. W. Burton and M. G. Traber, "Vitamin E: antioxidant activity, biokinetics, and bioavailability," *Annual Review of Nutrition*, vol. 10, pp. 357–382, 1990.

[8] I. C. F. R. Ferreira, L. Barros, M. E. Soares, M. L. Bastos, and J. A. Pereira, "Antioxidant activity and phenolic contents of *Olea europaea* L. leaves sprayed with different copper formulations," *Food Chemistry*, vol. 103, no. 1, pp. 188–195, 2007.

[9] D. Kritchevsky and S. C. Chen, "Phytosterols-health benefits and potential concerns: a review," *Nutrition Research*, vol. 25, no. 5, pp. 413–428, 2005.

[10] J. M. Del Valle and J. M. Aguilera, "High pressure CO_2 extraction. Fundamentals and applications in the food industry," *Food Science and Technology International*, vol. 5, pp. 1–24, 1999.

[11] M. Yousfi, B. Nedjmi, R. Bellal, D. Ben Bertal, and G. Palla, "Fatty acids and sterols of *Pistacia atlantica* fruit oil," *Journal of the American Oil Chemists' Society*, vol. 79, no. 10, pp. 1049–1050, 2002.

[12] M. Yousfi, B. Nadjemi, R. Belal, I. Bombarda, and E. M. Gaydou, "Triacylglycerol composition of oil from *Pistacia atlantica* fruit growing in algeria," *Journal of the American Oil Chemists' Society*, vol. 82, no. 2, pp. 93–96, 2005.

[13] H. Benhassaini, M. Bendahmane, and N. Benchalgo, "The chemical composition of fruits of *Pistacia atlantica* desf. subsp. atlantica from Algeria," *Chemistry of Natural Compounds*, vol. 43, no. 2, pp. 121–124, 2007.

[14] R. Farhoosh, J. Tavakoli, and M. H. H. Khodaparast, "Chemical composition and oxidative stability of kernel oils from two current subspecies of *Pistacia atlantica* in Iran," *Journal of the American Oil Chemists' Society*, vol. 85, no. 8, pp. 723–729, 2008.

[15] M. H. Givianrad, S. Saffarpour, K. Larijani, and P. Beheshti, "Investigation of some compositional properties of *Capparis spinosa* seed oil growing wild in Iran from commercial utilization approach," *Chemistry of Natural Compounds*, vol. 47, no. 3, pp. 428–430, 2011.

[16] http://www.livestrong.com/article/438717-what-is-oleic-acid/ .

[17] M. H. Givianrad, S. Saffarpour, and P. Beheshti, "Fatty acid and triacylglycerol compositions of *Capparis spinosa* seed oil," *Chemistry of Natural Compounds*, vol. 47, no. 5, pp. 798–799, 2011.

[18] M. J. Lagarda, G. García-Llatas, and R. Farré, "Analysis of phytosterols in foods," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 41, no. 5, pp. 1486–1496, 2006.

[19] H. Miraliakbari and F. Shahidi, "Antioxidant activity of minor components of tree nut oils," *Food Chemistry*, vol. 111, no. 2, pp. 421–427, 2008.

[20] A. Kamal-Eldin and L. Å. Appelqvist, "The chemistry and antioxidant properties of tocopherols and tocotrienols," *Lipids*, vol. 31, no. 7, pp. 671–701, 1996.

[21] C. Siquet, F. Paiva-Martins, J. L. F. C. Lima, S. Reis, and F. Borges, "Antioxidant profile of dihydroxy- and trihydroxyphenolic acids-A structure-activity relationship study," *Free Radical Research*, vol. 40, no. 4, pp. 433–442, 2006.

[22] S. B. Kritchevsky, "β-Carotene, carotenoids and the prevention of coronary heart disease," *Journal of Nutrition*, vol. 129, no. 1, pp. 5–8, 1999.

[23] G. Van Poppel and R. A. Goldbohm, "Epidemiological evidence for β-carotene and cancer prevention," *The American Journal of Clinical Nutrition*, vol. 62, pp. 1493–1503, 1995.

[24] N. I. Krinsky, "The biological properties of carotenoids," *Pure and Applied Chemistry*, vol. 66, pp. 1003–1010, 1994.

[25] H. Van den Berg, R. Faulks, H. F. Granado et al., "The potential for the improvement of carotenoid levels in foods and the likely systemic effects," *Journal of the Science of Food and Agriculture*, vol. 80, no. 7, pp. 880–912, 2000.