Chemistry of Natural Compounds, Vol. 49, No. 3, July, 2013 [Russian original No. 3, May–June, 2013]

CHEMICAL PROPERTIES OF THE OIL FROM *Pistacia khinjuk* FRUITS GROWING WILD IN IRAN

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UDC 547.913

Pistacia is a genus of the family Anacardiaceae, consisting of 11 or more species, which are shrubs or trees. Three *Pistacia* species occur naturally in Iran, including *P. vera* Linnaeus, *P. khinjuk* Stocks, and *P. atlantica* Desf. *P. khinjuk* [1].

The study of the chemical properties of edible fats and oils and their relation to oxidative stability, which in turn affects the oil quality, is of scientific interest. The oils from *P. khinjuk* fruits are considered important among the newer sources of edible oils, but little has been published on their physicochemical characteristics.The fatty acid composition of the oil from *P. khinjuk* fruits showed that the amounts of oleic, palmitic, linoleic, palmitooleic, stearic, and linolenic acids were 52.12, 17.82, 17.44, 5.73, 2.31, and 1.5%, respectively [2]. A study of the chemical properties of the oil from the *P. khinjuk* fruits has not been done previously. Therefore, this study was undertaken to investigate the chemical properties of the oil from *P. khinjuk* fruits (PKF) grown in Iran and to compare its properties with those of *P. vera* L. cv. Ohadi kernel (PVOK) oil (the most plentiful cultivar of *P. vera* in Iran) used as the control.

The chemical composition and characteristics of the PKF and PVOK oils are shown in Table 1. The percentage of saturated fatty acids (SFA) of the PKF oil (20.76%) was significantly higher than that of the PVOK (9.81%) oil. Among the monounsaturated fatty acid (MUFA), the percentage of palmitoleic acid (C16:1) in the PKF oil (5.94%) was about 6.6 times higher than that of the PVOK (0.98%) oil. There was no statistically signicant difference between the percentages of oleic acid of the two oils studied (51.91–52.05). The polyunsaturated fatty acids (PUFA) content of the PVOK oil (37.06%) was significantly greater than that of the PKF oil (19.52%). From the information stated above, the PKF oil showed a ratio of unsaturated and saturated fatty acids (USFA/SFA) (3.81) and a Cox value (2.66) that were lower than those of PVOK (9.18 and 4.46, respectively) oil, which makes it particularly resistant to oxidation.

The PV of the PKF oil (1.99 meq per kg oil) was significantly greater than that of the PVOK oil (0.25 meq per kg oil) (Table 1). This indicates that the PKF oils were oxidized more than the PVOK oil. This might be due to its improper storage and handling conditions as compared with the PVOK oil.

The IV, which is considered as a measure of oil unsaturation for the PKF and PVOK oils, was 85.15 and 110.78, respectively. The difference in IVs among the PKF and PVOK oils was due to their different fatty acid composition. The PKF oil has a greater SFA content (C16:0 and C18:0) and a lower PUFA content (mainly, C18:2) than those of PVOK oil.

As shown in Table 1, the SN of the PKF oil (79.46) was significantly lower than that of the PVOK oil (125.71) and also much lower than that of common vegetable oils with an average SN range of 175–250. Because there is an inverse relationship between SN and weight of fatty acids in the oil, it can be inferred that the PKF oil contains a great number of fatty acids of high molecular weight [3]. However, as can be seen in Table 1, the fatty acid composition of the oils studied cannot be the only reason for the considerable differences in their average molecular weight. In fact, their different wax and USM contents constitute the main contribution of these differences [4].

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Published in *Khimiya Prirodnykh Soedinenii*, No. 3, May–June, 2013, pp. 465–468. Original article submitted January 16, 2012.

0009-3130/13/4903-0546

TABLE 1. The Chemical Characteristics of the PKF and PVOK Oils

Fatty acid (%)	PKF oil	PVOK oil	Parameter	PKF oil	PVOK oil
16:0	18.01 ± 0.17^a	8.4 ± 0.45^b	MUFA	59.66 ± 0.51^a	53.03 ± 0.48^b
16:1	5.94 ± 0.12^a	0.98 ± 0.4^b	PUFA	19.52 ± 0.58^b	37.06 ± 0.69^a
17:0	0.5 ± 0.06	–	USFA/SFA	3.81 ± 0.12^b	9.18 ± 0.37^a
17:1	0.43 ± 0.02	–	Oxidizability (Cox) value	2.66 ± 0.02^b	4.46 ± 0.05^a
18:0	2.25 ± 0.13^a	1.41 ± 0.45^b	PV (meq O2 per kg oil)	1.99 ± 0.08^a	0.25 ± 0.04^b
18:1	51.91 ± 0.26^a	52.05 ± 0.57^a	IV (g of I_2 per 100 g oil)	85.15 ± 0.75^b	110.78 ± 1.44^a
18:2	18.32 ± 0.85^b	36.04 ± 0.73^a	SN (mg KOH per g oil)	79.46 ± 1.28^b	125.71 ± 1.816^b
18:3	1.2 ± 0.37^a	1.02 ± 0.50^a	USM content (% of oil)	5.24 ± 1.02^a	5.61 ± 0.42^a
20:1	0.66 ± 0.17	–	TT content (mg α-tocopherol per kg oil)	981.14 ± 13.63^a	832.13 ± 15.85^b
22:1	0.72 ± 0.14	–	TP content (mg gallic acid per kg oil)	120.64 ± 1.21^a	65.78 ± 1.2^b
SFA	20.76 ± 0.19^a	9.81 ± 0.38^b	TS content (% of oil)	2.63 ± 0.5^b	5.61 ± 0.22^a
			Wax content (% of oil)	15.45 ± 0.15^a	5.87 ± 0.34^b

Mean ± SD within a row with the same lowercase letters are not significantly different at $p < 0.05$. PKF, *Pistacia khinjuk* fruits; PVOK, *P. vera* L.cv.*Ohadi* kernels., SFA, saturated fatty acids, MUFA, monounsaturated fatty acids, PUFA, polyunsaturated fatty acids, USFA, unsaturated fatty acids, Cox, calculated oxidizability, PV, peroxide value, IV, iodine value, SN, saponification number, USM, unsaponifiable matter, TT, total tocopherols, TP, total phenolics, TS, total sterols.

The wax content of the PKF oil (15.45%) was significantly greater than that of the PVOK (5.87%) oil (Table 1). It should be mentioned that the wax content of the PKF oil was greater than that of rice bran oils, which are characterized by their high wax contents (3–6%) among vegetable oils [5]. Waxes are a group of insoluble high-melting-point compounds that occur naturally in crude vegetable oils. These compounds can be disadvantageous since they are primarily responsible for the dark color (turbidity) of refined oil [6]. On the other hand, these compounds have potential applications in the cosmetic, lubricant, plasticizer, pharmaceutical, food, polymer, and leather industries [7].

The USM contents of the PKF and PVOK oils were statistically in the same range of 5.24–5.61 (Table 1). The USM fraction of vegetable oils naturally contains hydrocarbons, terpene alcohols, sterols, tocopherols, and other phenolic compounds that usually make up 0.5 to 2.5% and sometimes 5 to 6% of vegetable oils [8]. The USM content is extensively used as an index of the quality of refined fat or oil or as a control index of the refining process. For example, according to the Japanese Agricultural Standards, the USM content of edible safflower oils, edible soybean oils, and edible palm oils should not exceed 1.0%. The effectiveness of USMs in retarding oil deterioration has been demonstrated by many investigators.

Plant sterols, called phytosterols, are important minor components that are present in almost all vegetable oils [9]. Oil samples can contain a complex composition of sterols with an average total sterol content of 0.3–2% in the oil, but the sterol content can reach more than 10% in some plant oils [10]. As shown in Table 1, the USM fractions were composed mainly of sterols, and their amounts were 2.63 and 5.61% for the PKF and PVOK oils, respectively. Phytosterols have attracted the interest of food chemists because they are of great importance for food labeling and nutritional purposes. They are also one of the indexes of the authenticity of vegetable oils [11]. Clinical studies have demonstrated that the dietary intake of phytosterols may decrease blood cholesterol levels, resulting in a significant reduction in the risk of heart disease [12].

Tocopherols together with phenolic compounds are particularly important functional constituents of a minor fraction of vegetable oils [13]. Tocopherols have antioxidant properties and they are active as vitamin E, which makes them particularly important for human health. Interest in phenolic compounds is related primarily to their antioxidant activity; nevertheless, they also show important biological activity *in vivo* and may be beneficial in combating diseases related to excessive oxygen radical formation that exceeds the antioxidant defense capacity of the human body. The TT content of the PKF oil (981.14 mg per kg oil) was significantly higher than that of the PVOK oil (832.13 mg per kg oil) (Table 1). The PKF oil is considered to be rich source of tocopherols since their tocopherol concentration is greater than that reported for some common vegetable oils, e.g., canola, sunflower, cottonseed, and corn oils with TT contents of about 695, 640, 630, and 605 mg/kg, respectively [14]. The TP content of the PKF oil (120.64 mg per kg oil) was significantly higher than that of the PVOK oil (65.78 mg per kg oil) (Table 1).

The oxidative stability of the PKF and PVOK oils was studied by following the formation of primary and secondary lipid oxidation products in these oil during 8 h of heating at 170°C. The CDV and CV are good classical indices of primary and secondary oxidative changes in lipids [15].

TABLE 2. The Conjugated Diene Value (CDV) of the PKF and PVOK Oils during Heating at 170°C

Heating time, h	CDV, mmol L^{-1}		Heating time, h	CDV, mmol L^{-1}	
	PKF oil	PVOK oil		PKF oil	PVOK oil
0	19.69 ± 0.56[a]	6.43 ± 0.26[b]	5	21.74 ± 0.72[a]	13.92 ± 0.80[b]
1	20.16 ± 0.09[a]	9.22 ± 0.54[b]	6	21.79 ± 1.66[a]	13.91 ± 0.32[b]
2	22.12 ± 0.03[a]	10.06 ± 0.88[b]	7	23.86 ± 1.91[a]	16.98 ± 0.52[b]
3	19.75 ± 1.70[a]	9.78 ± 0.70[b]	8	25.42 ± 1.13[a]	19.35 ± 0.19[b]
4	18.96 ± 0.24[a]	10.75 ± 0.74[b]			

Mean ± SD within a row with the same lowercase letters are not significantly different at $p < 0.05$. PKF, *Pistacia khinjuk* fruits; PVOK, *P. vera* L. cv. *Ohadi* kernels.

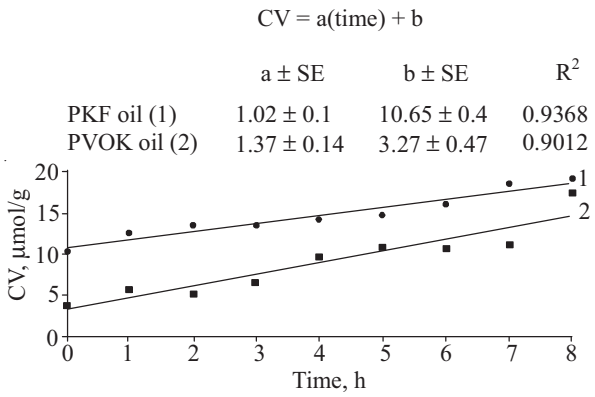

Fig. 1. The carbonyl value (CV) (μmol·g^{-1}) of the PKF (1) and PVOK (2) oils during heating at 170°C. PKF – *Pistacia khinjuk* fruits; PVOK – *P. vera* L.cv. Ohadi kernels.

Table 2 shows the changes in the CDV of the PKF and PVOK oils during 8 h of heating at 170°C. Measurement of the CDV provides a good indication of the oxidative stability of edible fats and oils. During the oxidation of PUFAs containing methylene-interrupted dienes and polyenes, there is a shift in the double bond positions due to isomerization and conjugation. This is accompanied by an increase in UV absorption at 234 nm due to dienes. The increase in absorbance at 234 nm is an indicator of oxidation and is due to the uptake of oxygen and formation of peroxides during the early stages of oxidation [16]. The initial CDV of the PKF oil (19.69 mmol/L) was significantly higher than that of the PVOK oil (6.43 mmol/L). This autoxidation indicator increased parallel to the increase in heating time, with a lower rate for the PKF oil; thus, after 8 h of heating, PKF oil showed an increase of 29% (25.42 mmol/L), whereas this amount for the PVOK oil was 201% (19.35 mmol/L). This indicates that the resistance of the PKF oil to the production of conjugated diene hydroperoxides during heating was about 6.9 that of the PVOK oil.

The CV of these oils during 8 h of heating at 170°C is shown in Fig. 1. In the CV test, we measured the secondary decomposition products of lipid oxidation such as aldehydes and ketones. Peroxides are transformed into secondary products that contain carbonyl groups. These compounds are more stable than peroxides, and the CV was a good index of the oxidative changes that occurred in the oils during heating. The determination of carbonyl compounds in heated and frying oils is very important for evaluating their quality because these compounds often contribute to rancid and unpleasant flavors and can reduce the nutritional value of fried foods [17]. As can be seen in Fig. 1, the CV of the PVOK oil ($a = 1.37$) increased linearly at a greater rate than that of the PKF oil ($a = 1.02$). This indicates that the PKF oil is more resistant to oxidation and deterioration than the PVOK oil.

In general, based on the formation of primary and secondary lipid oxidation products, the oxidative stability of the PKF oil is obviously higher than that of the PVOK oil. With regard to the very high oxidative stability of the PKF oil and also its high TT and TP contents, it was expected that the addition of the PKF oil to other common vegetable oils can improve their oxidative stability. Therefore, different percentages (up to 15%) of the PKF oil were added to olive oil, and using the Rancimat test, its antioxidant activity was compared with that of a synthetic antioxidant of wide commercial use such as

tert-butylhydroquinone (TBHQ), which has very strong antioxidant activity, especially at high temperatures. A power equation can describe well the antioxidant behavior of the PKF oil with high determination coefficient ($R^2 = 0.9953$); that is, the OSI (Oxidative Stability Index) of olive oil initially rose steeply from 2.86 (0% PKF oil) to 5.29 h (2% PKF oil), but then increased less rapidly at higher percentages of PKF oil (2–15%).

The OSI of olive oil increased linearly with the TBHQ concentration up to 200 ppm ($R^2 = 0.9987$). With regard to the common levels of 50, 100, 150, and 200 ppm for the TBHQ in edible oils, their corresponding OSI were calculated to be 3.29, 3.72, 4.19, and 4.66 h, respectively. Substituting these quantities into the equation, we calculated the corresponding percentages of the PKF oil in the olive oil to be 0.012, 0.1, 0.38, and 0.95%, respectively. It was very intresting to find that the PKF oil at low concentrations (lower than 1%) had the same effect of different levels of TBHQ (50 to 200 ppm) in the olive oil.

Our results in the present study showed that the PKF oil was more resistant to lipid oxidation than the PVOK oil, which is considered to be very stable oil among the common edible oils. This can be attributed to the very high levels of the TT and TP contents and very low amounts of the USFA/SFA ratio and Cox values in the PKF oil as compared with the PVOK oil, although the PKF oil had an initial quality no better than that of the PVOK oil (Table 1). Therefore, the PKF oil can be considered as a new source of safe and effective natural antioxidants and should be used in common oils that are prone to oxidation in order to increase their storage time.

Plant Material. The fruit samples of *P. khinjuk* and *P. vera* L. cv. Ohadi were collected from fields in Meimand in Fars Province and in Rafsanjan in Kerman Province, respectively and were stored at –18°C until analysis. Virgin olive oil without antioxidants in glass bottles was purchased from local shops in Tehran City and was stored at –18°C until analysis.

Chemical Material. Fatty acid methyl ester (FAME) standards and all chemicals and solvents used in this study were of analytical reagent grade and were supplied by Merck (Darmstadt, Germany) and Sigma Chemical Companies.

Oil Extraction. After drying in the shade, the whole fruits of *P. khinjuk* (consisting of outer and inner skins and kernel) and *P. vera* L. cv. Ohadi were ground to powdered in a grinder (Moulinex Coffee Mill Model 980, Paris, France). The powders were extracted with *n*-hexane (1:4, wt/vol) by agitation in the dark at ambient temperatures for 48 h. The solvent was evaporated *in vacuo* at 40°C for drying [2].

Chemical Composition. The fatty acid composition of the vegetable oils was determined by gas-liquid chromatography and reported in relative area percentages. Fatty acids were transesterified into their corresponding FAMEs by vigorous shaking a solution of the oil in hexane (0.3 g in 7 mL) with 2 mL of 7 M methanolic potassium hydroxide at 50°C for 10 min. The FAMEs were identified using an HP-5890 chromatograph (Agilent, Palo Alto, CA) equipped with a CP-FIL 88 (Supelco Inc., Bellefonte, PA) capillary-fused silica column, 60 m × 0.22 mm I.D., 0.2 mm film thickness, and a flame ionization detector. Nitrogen was used as a carrier gas with a flow rate of 0.75 mL/min. The oven temperature was maintained at 198°C, and that of the injector and the detector at 250°C. The oxidizability (Cox) value of the oils was calculated from the percentage of unsaturated C18 fatty acids using the formula proposed by Fatemi and Hammond [18]:

$$\text{Cox value} = (1\,[18{:}1\%] + 10.3\,[18{:}2\%] + 21.6\,[18{:}3\%])/100.$$

The iodine value (IV) was determined according to the AOAC Official Method 920.158 (Hanus method) [19]. The unsaponifiable matter (USM) content was determined by the method described by [20]. The total tocopherol (TT) content was determined according to the colorimetric method described by [21]. The total phenolics (TP) content was determined spectrophotometrically using Folin–Ciocalteau′s reagent according to the method described by [22]. A calibration curve of gallic acid in methanol was performed in the concentration range 0.04–0.40 mg/mL. The total sterol (TS) content was determined according to the Lieberman–Burchard color reaction [23]. Lieberman–Burchard reagent (sulfuric acid and acetic anhydride) reacts with sterols to produce a characteristic green color whose absorbance is determined by spectrophotometry at 640 nm. The saponification number (SN) was determined according to the AOAC Official Method 920.160 [19]. The spectrophotometric method of the International Dairy Federation as described by Shantha and Decker [24] was used to determine the peroxide value (PV) (thiocyanate method).

Oxidative Stability. The oil samples extracted (200 g) were placed in a fryer (Kenwood DF280, Havant, Hampshire, UK) and maintained at 170°C for 8 h with no stirring. The surface-to-volume ratio of the oil samples in the fryer was around 0.7 cm^{-1}. After intervals of 60 min of heating, the samples were removed and analyzed [25]. The conjugated diene value (CDV) was measured spectrophotometrically at 234 nm and read against HPLC grade hexane as a blank. The oil samples were diluted 1:600 with hexane. An extinction coefficient of 29.000 mol/L was utilized to quantify the concentration of conjugated dienes formed during oxidation [26]. The carbonyl value (CV) of the oils was measured according to the method developed by [17]. A Metrohm Rancimat model 743 (Herisau, Switzerland) was used for the oil/oxidative stability Index (OSI) analysis. The tests were done with 3 g oil samples at temperatures of 120°C at an airflow rate of 15 L/h.

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