

INTRODUCING *Pistacia khinjuk* (KOLKHOUNG) FRUIT HULL OIL AS A VEGETABLE OIL WITH SPECIAL CHEMICAL COMPOSITION AND UNIQUE OXIDATIVE STABILITY

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UDC 547.915

The chemical and qualitative characteristics of *P. khinjuk* hull oil (PKHO), *P. atlantica* hull oil (PAHO), and *Sesamum indicum* L. cv. Dezful seed oil (SISO) were compared. The high values of wax and tocopherol compounds and low values of the saponification number of PKHO were interesting. PKHO was also unique due to its high content of tocotrienols (92.6% of total tocol compounds). Oxidation stability data showed that PKHO was the most stable oil against lipid oxidation, followed by PAHO and SISO. The conjugated diene hydroperoxides increase during heat treatment (8 h) at 170°C for PKHO, PAHO, and SISO and were 18.41, 25.28, and 300.66%, respectively. Moreover, the carbonyl value was significantly increased at the lowest speed for PKHO, followed by PAHO and SISO. During heat treatment, there was no difference between PKHO and PAHO concerning total polar compounds, whereas the parameter showed a linear increase for SISO. The antioxidative activity of PKHO at low concentration (0.0099%) in olive oil was also almost equal to that of TBHQ (100 ppm).

Keywords: *P. khinjuk* hull oil, oxidative stability, chemical composition, *P. atlantica* hull oil, *Sesamum indicum* L. cv Dezful seed oil.

Lipid oxidation, including unfavorable reactions in edible oils, is considered the main cause of reduced shelf life of food products [1]. Synthetic antioxidants are usually used to combat the negative effects of these reactions. Many scientists are investigating the identification and extraction of natural antioxidants. A large number of investigations are devoted to studying the oxidation stability and antioxidative properties of different species of *Pistacia* oil [2].

Out of eleven known species of *Pistacia*, three species, namely *P. vera* Linnaeus, *P. khinjuk* Stocks, and *P. atlantica* Desf exist in Iran. *P. khinjuk* and *P. atlantica* grow as wild populations and are called in the Persian language as Kolkhoung and Bene, respectively. Kolkhoung and Bene are similar to each other in shape and morphology. Kolkhoung fruit, like that of Bene, is composed of kernel, wooden rough shell, and soft outermost hull, which constitute 37%, 26%, and 37% of the fruit, respectively. The fatty acid composition of oil from the kernel of Kolkhoung 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 [3].

The goal of the present study was to investigate the chemical composition and oxidation stability of *P. khinjuk* (Kolkhoung) hull oil (PKHO) accompanied by *P. atlantica* (Bene) hull oil (PAHO, the most similar species to *P. khinjuk* with a highly stable oil) and *Sesamum indicum* L. cv Dezful seed oil (SISO, as one of the most stable oil) as control. Sesame variety Dezful, which is very common in Iran, was selected for the investigation. Furthermore, the antioxidative power of PKHO was compared to that of PAHO and TBHQ; the latter is a powerful synthetic antioxidant.

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TABLE 1. Chemical Characteristics of *P. khinjuk* Hull Oil, *P. atlantica* Hull Oil, and *Sesamum indicum* L. cv Dezful Seed Oil

Parameter	<i>P. khinjuk</i>	<i>P. atlantica</i>	<i>Sesamum indicum</i>
Fatty acid, %			
14:0	—	—	0.08 ± 0.13
16:0	23.4 ± 0.24 a	22.55 ± 0.07 a	10.2 ± 0.32 b
16:1	7.74 ± 0.07 b	14.05 ± 0.22 a	0.28 ± 0.1 c
17:0	0.05 ± 0.08 b	0.4 ± 0.03 a	—
17:1	0.2 ± 0.16 a	0.24 ± 0.13 a	—
18:0	2.39 ± 0.05 c	3 ± 0.14 b	5.71 ± 0.22 a
18:1	52.03 ± 0.16 a	52.12 ± 0.17 a	30.01 ± 0.42 b
18:2	12.02 ± 0.2 b	5.35 ± 1.02 c	47.1 ± 0.69 a
18:3	1.5 ± 0.19 b	1.16 ± 0.12 b	5.01 ± 0.45 a
20:1	0.25 ± 0.05 b	0.76 ± 0.06 a	0.44 ± 0.11 b
22:0	—	—	0.59 ± 0.22
SFA	25.84 ± 0.21 a	25.95 ± 0.9 a	16.87 ± 0.51 b
MUFA	60.22 ± 0.3 b	67.17 ± 0.7 a	30.73 ± 0.58 c
PUFA	13.52 ± 19 b	6.51 ± 0.11 c	52.11 ± 0.52 a
PUFA/SFA	0.52 ± 0.03 b	0.26 ± 0.02 c	3.06 ± 0.05 a
Oxidizability (Cox) value	2.14 ± 0.1 b	1.35 ± 0.1 c	6.24 ± 0.07 a
PV (mequiv O ₂ /kg oil)	1.84 ± 0.07a	0.84 ± 0.09 b	1.8 ± 0.04 a
AV (mg KOH/g oil)	6.86 ± 0.05 a	6.58 ± 0.04 b	0.44 ± 0.07 c
IV (g of I ₂ /100 g oil)	77.01 ± 0.56 b	69.92 ± 0.79 c	120.76 ± 0.39 a
SN (mg KOH /g oil)	64.46 ± 1.28 c	77.71 ± 1.81 b	179.72 ± 1.07 a
USM content (% of oil)	2.58 ± 0.33 a	2.98 ± 0.2 a	2.37 ± 0.43 a
TT content (mg /kg oil)	2043.4 ± 18.9 a	649.91 ± 12.2 c	993.69 ± 16.5 b
TP content (mg gallic acid /kg oil)	99.67 ± 7.07 b	81.73 ± 9.85 c	645.73 ± 13.9 a
TS content (mg /kg oil)	1600 ± 13.1 c	1640 ± 12.2 b	5400 ± 15.5 a
Wax content (% of oil)	16.87 ± 2.8 a	13.14 ± 1.34 b	5.49 ± 0.77 c

Mean ± SD within a row with the same lowercase letters is not significantly different at $p < 0.05$. SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, Cox: calculated oxidizability, PV: peroxide value, AV: acid value, IV: iodine value, SN: saponification number, USM: unsaponifiable matter, TT: total tocopherols, TP: total phenolics, TS: total sterols.

The chemical properties and structure of PKHO, PAHO, and SISO are presented in Table 1. The fatty acid composition of an oil is an important determinant of its oxidation stability and food value [4]. Oleic acid was the dominant fatty acid in PKHO and PAHO (52.03–52.12), whereas its value in SISO was 30.01%. The amount of palmitoleic acid in PKHO, PAHO, and SISO was 7.74%, 14.05%, and 0.28%, respectively, indicating a significant difference. The value of palmitoleic acid in PKHO and PAHO was higher than that reported for edible oils [5]. Negligible amounts of margaroleic acid and gadoleic acid were also found in the experimented oils. The values of saturated fatty acids in PKHO and PAHO (25.84 and 25.95%, respectively), which were statistically equal to each other, were higher than that of SISO (16.87%). The dominant saturated fatty acid in all the three oils was palmitic acid, followed by stearic acid. The highest value of unsaturated fatty acids (mainly linoleic acid) was found in SISO (52.11%), followed by PKHO (13.52%) and PAHO (6.51%). Based on a composition of the fatty acids, SISO had the highest food value, followed by PKHO and PAHO. It was also found that the ratio between polyunsaturated to saturated fatty acids (PUFA/SFA) (0.52) and calculated oxidizability (Cox) value (2.14) of PKHO were higher than those of PAHO (0.26 and 1.35, respectively) and lower than those of SISO (3.06 and 6.24, respectively). Therefore, it can be expectable that PKHO has suitable oxidative stability.

The peroxide value (PV) and acid value (AV) of edible oils indicate their initial quality [4]. The PV of PKHO, PAHO, and SISO was 0.84, 1.84, and 1.8 meq/kg, respectively. Furthermore, the highest AV was observed in PKHO (6.86) followed by PAHO (6.58) and SISO (0.44) (Table 1). The initial PV and AV of the three oils indicated the highest oxidation in PKHO, followed by PAHO and SISO. This can be due to unsuitable harvesting, transfer, storage, and processing of PKHO compared to PAHO and SISO.

The iodine value (IV) of PKHO, PAHO, and SISO was 77.01, 69.92, and 120.76, respectively (Table 1). Compared to SISO, PKHO and PAHO had higher SFA and lower PUFA.

TABLE 2. Sterol Composition of *P. khinjuk* Hull Oil, *P. atlantica* Hull Oil, and *Sesamum indicum* L. cv Dezful Seed Oil, mg/kg oil

Compound	<i>P. khinjuk</i>	<i>P. atlantica</i>	<i>Sesamum indicum</i>
Sitosterol	1139.7 ± 9.4 b	1024.1 ± 10.2 c	3387.4 ± 15.2 a
Δ ⁷ -Stigmasterol	202.4 ± 5.3 c	367.2 ± 8.7 a	326.2 ± 7.7 b
Campesterol	59.7 ± 4.1 b	64.5 ± 3.8 b	1134 ± 9.4 a
Δ ⁷ -Avenasterol	33.3 ± 2.5 b	27.4 ± 2.1 c	56.7 ± 4.6 a
Sitostanol	32.64 ± 2.4 b	29.2 ± 1.4 c	230.4 ± 8.1 a
Δ ⁵ -Avenasterol	29 ± 2.1 b	18.1 ± 1.2 c	78.8 ± 6.6 a
Stigmasterol	28.5 ± 1.9 b	8.1 ± 1.3 c	102.6 ± 5.3 a
Clerosterol	27.9 ± 2.1 a	17.9 ± 2.7 b	17.8 ± 2.1 b
Cholesterol	20.5 ± 1.4 b	8.3 ± 0.9 c	27 ± 2.6 a
Δ ^{5,24} -Stigmastadienol	17.1 ± 1.5 c	66.4 ± 0.6 a	27.6 ± 1.9 b
Brassicasterol	8.96 ± 1.2 ab	8.6 ± 0.9 b	11.8 ± 2 a
Total sterols	1600 ± 13.1 c	1640 ± 12.2 b	5400 ± 15.5 a

Mean ± SD within a row with the same lowercase letters is not significantly different at $p < 0.05$.

TABLE 3. Tocopherol Composition of *P. khinjuk* Hull Oil, *P. atlantica* Hull Oil, and *Sesamum indicum* L. cv Dezful Seed Oil, mg/kg oil

Compound	<i>P. khinjuk</i>	<i>P. atlantica</i>	<i>Sesamum indicum</i>
α-Tocopherol	36.17 ± 2.5 b	105.58 ± 7.2 a	3.9 ± 0.5 c
β-Tocopherol	54.96 ± 3.8 b	471.87 ± 10.1 a	15.31 ± 2.3c
δ-Tocopherol	3.07 ± 1.2 c	6.35 ± 0.9 b	935.13 ± 12.2 a
Δ-Tocopherol	55.17 ± 0.8 a	7.93 ± 1 c	18.96 ± 1.4 b
α-Tocotrienol	1076.87 ± 20.1 a	53.17 ± 2.4 b	5.59 ± 0.8 c
Δ-Tocotrienol	815.12 ± 13.1 a	5.01 ± 4.1b	14.79 ± 1.9 c
Total	2043.4 ± 18.9 a	649.91 ± 12.2 c	993.69 ± 16.5 b

Mean ± SD within a row with the same lowercase letters is not significantly different at $p < 0.05$.

As can be seen in Table 1, the saponification number (SN) of PKHO, PAHO, and SISO was 64.46, 77.71, and 179.72, respectively. Considering the mean range of SN in different vegetative oils (175–250), the SN of PKHO was far lower than the common value. Since this parameter is inversely proportional to the molecular weight of fats [6], PKHO must contain a large number of fatty acids of high molecular weight, which was not observed in the analysis of the fatty acid composition. Another factor, such as a high amount of wax, therefore, is responsible for the low SN of PKHO [2].

The amount of minor compounds such as wax is an important determinant of quality and stability of edible oils. When an edible oil is stored in the cold, the waxes undergo crystallization, creating turbidity in the oil [7]. The highest wax value was observed in PKHO (16.87%), followed by PAHO (13.14%) and SISO (5.49%) (Table 1). It should be mentioned that the wax value of PKHO was higher than that found in rice bran oil, a product characterized for its high wax value (3–6%) among edible oils [8].

The unsaponifiable matter (USM) values for PKHO, PAHO, and SISO were 2.58, 2.98, and 2.37, respectively (Table 1), showing no significant difference. USM values vary in different oils. The USM value is usually 0.5–2.5% in edible oils, reaching up to 5–6% in some cases [9, 10]. The amount of these compounds is therefore widely used as a quality index of refined oils and fats [5].

Phytosterols play an important role in food classification and are indices for fakeness determination [11]. Phytosterols are found in different kinds. Sitosterol is the dominant phytosterol (50–80%) in vegetative oils, accompanied by campesterol, stigmasterol, and Δ⁵-avenasterol. Brassicasterol is absent in most plant oils (except for rapeseed oil). Cholesterol is also found in very minor amount (20–50 ppm) compared to animal oils (up to 1000 ppm) [12]. As can be seen from Table 1, the total sterol (TS) content in PKHO, PAHO, and SISO was 1600, 1640, and 5400 mg/kg, respectively. The kinds of sterol compounds in the three oils are presented in Table 2. The main sterol in PKHO, PAHO, and SISO was sitosterol. Δ⁷-Stigmasterol and campesterol are also important sterols of PKHO, PAHO, and SISO. Δ⁷-Avenasterol, stigmasterol, clerosterol, cholesterol, Δ^{5,24}-stigmastadienol, and brassicasterol are other sterol compounds in PKHO, PAHO, and SISO.

TABLE 4. Conjugated Diene Value of *P. khinjuk* Hull Oil, *P. atlantica* Hull Oil, and *Sesamum indicum* L. cv. Dezful Seed Oil during Heat Treatment at 170°C

Heating time, h	Conjugated diene value, mmol L ⁻¹		
	<i>P. khinjuk</i>	<i>P. atlantica</i>	<i>Sesamum indicum</i>
0	14.94 ± 0.4 Ca	12.5 ± 0.87 Cb	12.01 ± 0.92 Hb
1	17.11 ± 0.36 ABa	13.95 ± 0.34 Bb	13.12 ± 0.52 Hb
2	16.56 ± 0.22 Ba	13.43 ± 0.35 Bb	17.57 ± 0.71 Ga
3	16.38 ± 0.6 Bb	13.8 ± 0.24 Bc	20.39 ± 0.88 Fa
4	16.62 ± 0.22 Bb	13.8 ± 0.34 Bc	23.60 ± 0.23 Ea
5	16.85 ± 0.18 Bb	13.8 ± 0.53 Bc	27.55 ± 0.39 Da
6	17.53 ± 0.31 Ab	13.38 ± 0.17 Bc	35.74 ± 0.99 Ca
7	18.22 ± 0.4 Ab	14.06 ± 0.4 Bc	40.35 ± 1.36 Ba
8	17.69 ± 0.39 Ab	15.66 ± 0.35 Ac	48.12 ± 1.16 Aa

Means ± SD within a row with the same lowercase letters are not significantly different at $p < 0.05$.

Means ± SD within a column with the same uppercase letters are not significantly different at $p < 0.05$.

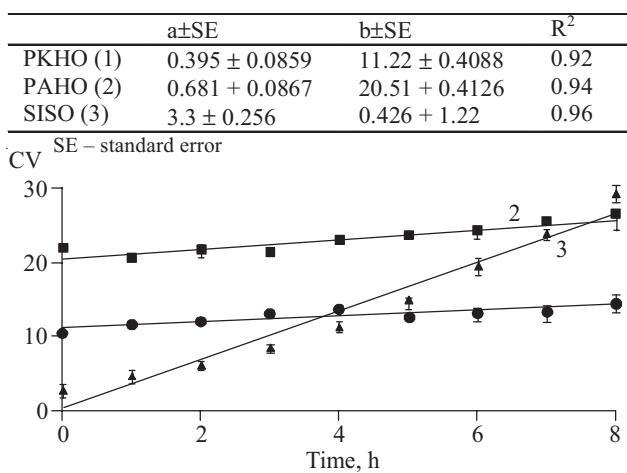


Fig. 1. Carbonyl value (CV) ($\mu\text{mol}\cdot\text{g}^{-1}$) of PKHO (1), PAHO (2), and SISO (3) during heat treatment process at 170 °C.

Phenolic compounds are natural antioxidants and important factors for evaluation of edible oil quality. There is a direct relation between phenolic compound content and oxidation stability and organoleptic characteristics of edible oils [13]. The total phenolics (TP) contents of the three oils were significantly different. SISO had the highest content of phenolic compounds (645.73 mg/kg), followed by PKHO (99.67 mg/kg) and PAHO (81.73 mg/kg) (Table 1).

Tocols (tocopherol and tocotrienol) are oil-soluble compounds that act as vitamin and antioxidant agents. The highest vitamin activity of the compounds belongs to the α kind (1.0), followed by β (0.5), γ (0.1), and δ (0.03). Palm oil is the only oil containing a high content of tocotrienols together with tocopherols [14]. The total tocol (TT) content of PKHO (2043.4 mg/kg) was significantly higher than those of the other investigated oils, followed by SISO and PAHO (993.69 and 649.91 mg/kg, respectively) (Table 3). PKHO is considered a valuable source of tocols because the tocol content reported for this oil is much higher than that of common oils such as sunflower, cottonseed, soybean, canola, and palm oil. There was also a significant difference between the PKHO tocol composition and that of SISO, PAHO, and common edible oils. PKHO contains a high level of tocotrienols. The tocol composition of PKHO is completely unique among common edible oils. In contrast to palm oil, in which γ -tocotrienols are the dominant tocol (43.6% in average), α - and γ -tocotrienols are the main components in PKHO (52.7 and 39.9%, respectively).

Table 4 shows the changes in conjugated diene value (CDV) of PKHO, PAHO, and SISO during 8 h heat treatment at 170°C. The initial CDV of PKHO was significantly higher than those of PAHO and SISO, which showed no significant difference from each other. This autoxidation index increased in parallel with heat treatment duration, and its rate increase was faster for PKHO than for PAHO and SISO, so that after 8 h of heat treatment, the increase in CDV of PKHO, PAHO, and SISO was 18.41, 25.28, and 300.66%, respectively. This fact shows the higher resistance of PKHO to the formation of diene hydroperoxides during heat treatment compared to PAHO and SISO.

TABLE 5. Total Polar Compound Content of *P. khinjuk* Hull Oil, *P. atlantica* Hull Oil, and *Sesamum indicum* L. cv. Dezful Seed Oil during Heat Treatment at 170°C

Heating time, h	Total polar compounds, %		
	<i>P. khinjuk</i>	<i>P. atlantica</i>	<i>Sesamum indicum</i>
0	12.02 ± 0.95 Aa	9.6 ± 0.58 Ab	5.19 ± 0.56 Fc
1	11.14 ± 1.11 Aa	10.06 ± 0.38 Aa	6.57 ± 0.27 Eb
2	11.97 ± 0.99 Aa	9.82 ± 0.89 Aa	7.96 ± 0.66 Dc
3	10.72 ± 0.95 Aa	9.34 ± 1.12 Aa	7.94 ± 0.41 Db
4	10.53 ± 0.82 Aa	9.75 ± 0.84 Aa	7.91 ± 0.39 Db
5	10.88 ± 1.58 Aa	9.55 ± 1.05 Aa	8.41 ± 0.22 Db
6	10.87 ± 0.82 Aa	9.2 ± 0.78 Ab	8.9 ± 0.11 Cb
7	11.71 ± 0.74 Aa	9.95 ± 0.36 Ab	11.40 ± 0.28 Ba
8	12.38 ± 0.98 Aa	9.03 ± 0.88 Ab	13.89 ± 0.49 Aa

Means ± SD within a row with the same lowercase letters are not significantly different at $p < 0.05$.

Means ± SD within a column with the same uppercase letters are not significantly different at $p < 0.05$.

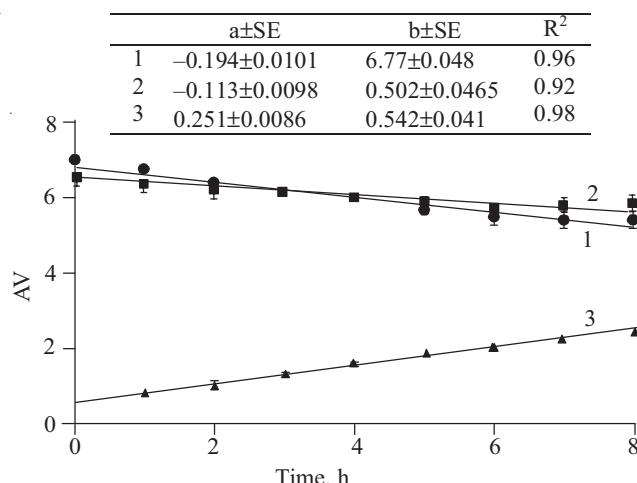


Fig. 2

Fig. 2. Acid value (AV) (mg KOH/g oil) of PKHO (1), PAHO (2), and SISO (3) during heat treatment at 170°C. SE – standard error.

Fig. 3. Effect of different percentages of PKHO (A) and PAHO (B) on the oxidative stability index (OSI) of olive oil.

The carbonyl value (CV) of the oils during heat treatment is presented in Fig. 1. Carbonyl compounds such as aldehydes and ketones are products of the secondary oxidation of lipids generated by hydroperoxide degradation and are the main cause of the unfavorable taste, degeneration, and reduced food value of fried foods [15]. As can be seen in Fig. 1, the CV increased more rapidly for SISO, followed by PAHO and PKHO. This suggests that PKHO is the most stable oil against oxidation and degradation in quality, followed by PKHO and SISO.

Figure 2 shows the changes in AV of the three oils during heat treatment. As seen in Fig. 2, the AV followed a linear increase for SISO and decreased in PKHO and PAHO. The authors propose that the reduced AV of PKHO and PAHO can be due to oxidation of free fatty acids and generation of a complex with other oil components or due to evaporation of free fatty acids during heat treatment.

Total polar compounds (TPC) is a proper criterion for comparing the quality of heated and fried oils and fats [16]. In many European countries it is recommended that edible oils containing 24–27% of TPC be banned [17]. Changes in TPC of PKHO, PAHO, and SISO during 8 h of heat treatment at 170°C are shown in Table 5. Heat treatment brought about no significant change in the initial value of TPC in PKHO and PAHO, whereas TPC of SISO underwent a linear increase. In other words, PKHO and PAHO were very resistant to TPC generation during heat treatment.

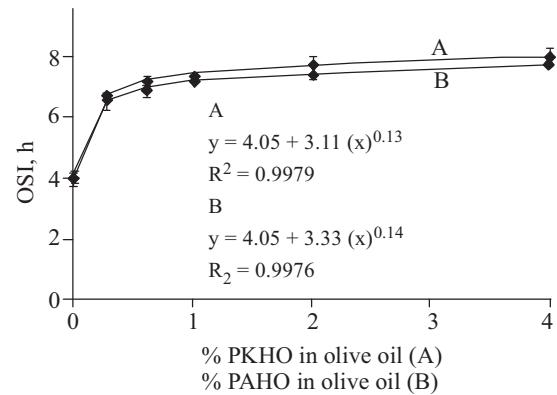


Fig. 3

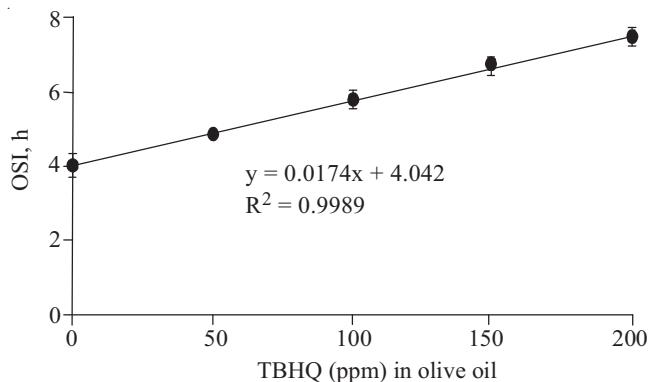


Fig. 4. Effect of different concentrations of TBHQ on the oxidative stability index (OSI) of olive oil.

In general, based on primary and secondary oxidation compound generation, the oxidation stability of PKHO was better than that of SISO and PAHO. One way of evaluating the antioxidant properties of edible oils is to add oils of lower oxidation stability and investigate their effects [2]. Considering the high oxidation stability of PKHO, adding this oil to common edible oils can enhance their oxidation stability. So, different concentrations of PKHO (up to 4%) were added to olive oil, and its antioxidant activity was compared to those of PAHO (as a very stable oil) and TBHQ (a synthetic antioxidant of high antioxidative activity especially at high temperatures) using the Rancimat test. Based on Fig. 3, the antioxidant activity of PKHO and PAHO can be explained by means of an exponential equation. The addition of different concentrations of PKHO and PAHO caused a slope increase in the oxidative stability index (OSI) of olive oil, but the highest OSI was achieved by adding 0.3% of PKHO and PAHO. The OSI was increased from 4.05 to 6.81 h (oil containing 0.3% PKHO) and 6.66 h (olive oil containing 0.3% PAHO); however, incorporating an additional amount of PKHO and PAHO (0.3 to 4%) to olive oil caused a slower increase in the OSI.

As can be seen from Fig. 4, as the TBHQ concentration is increased (up to 200 ppm), the OSI of olive oil is enhanced. The usual content of TBHQ in edible oils is 100 ppm, so determining the amounts of PKHO and PAHO to bring the olive oil OSI to this value is interesting. Based on Fig. 4, the OSI for 100 ppm of TBHQ was determined as 5.77 h by incorporating the value in Fig. 3, and PKHO and PAHO percentage was determined as 0.0099% and 0.0121%, respectively. An interesting point was the fact that the PKHO antioxidant activity was similar to that of TBHQ at low concentrations. The PAHO percentage in olive oil was near the value reported by Farhoosh et al.

Our results showed that despite its lower initial quality, PKHO was the most stable oil against oxidation, followed by PAHO and SISO, oils that are considered as very stable oils among common edible oils. The high oxidation stability of PKHO can be explained by its high antioxidant, especially tocol compounds, content. Based on our results, PKHO can be considered as a new resource of natural and effective antioxidants, and by addition of PKHO to oxidation-sensitive common oils, their oxidation characteristics can be improved.

EXPERIMENTAL

Plant Material. Fruit samples of Kolkhoung (*P. khinjuk*) and Bene (*P. atlantica*) were gathered from forest areas of Meimand and Marvdasht (autumn of 2011), respectively. Sesame seeds (*Sesamum indicum* L. cv Dezful) were gathered from a farmland in Dezul, Khoozestan Province (summer of 2011). Virgin olive oil without antioxidant was purchased from local markets of Mashhad. All the samples were stored at -18°C until initiation of the experiments.

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

Oil Extraction. The soft green hull of Kolkhoung and Bene fruits was removed using a dehuller device. The hulls of the two pistachio species, together with sesame seed, were powdered by a mill. The prepared powder was mixed with hexane solvent in 1:4 ratio and put in a shaker incubator in the dark at room temperature for 48 h. To remove the solvent, the solution was then incubated under vacuum at 40°C for 6–12 h [18].

Oil Oxidation. The extracted oil samples (250 g) were put into glass vials and, without agitation, into a hot paraffin bath for 8 h. The device was set to provide the oil samples with a temperature of 170°C. Twenty grams of the oil was sampled after 60 min and stored at –18°C for further analyses [19].

Fatty Acid 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 of 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.

Oxidizability Value. The oxidizability (Cox) value of the samples was calculated according to the formula of Fatemi and Hammond [18]: Cox value = (1[18:1%] + 10.3[18:2%] + 21.6[18:3%])/100 [20].

Iodine Value. The iodine value (IV) was determined according to the AOAC Official Method 920.158 (Hanus method) [21].

Unsaponifiable Matter Content. Unsaponifiable matter (USM) content was determined by the method described in [22].

HPLC Analysis for Tocopherols. The content of tocopherols in the oils was determined using a high-performance liquid chromatograph (Waters, Alliance system, USA) with a Spherisorb column (25 cm × 4 mm i.d., Waters, USA) packed with silica (5 µm particle size) and a fluorescence detector operating at an excitation wavelength of 290 nm and an emission wavelength of 330 nm [23]. The mobile phase was hexane-isopropanol (98.5:0.5, v/v) at a flow rate of 1 mL/min. The tocopherols in the test samples were verified by comparison of their retention times with those of reference standards.

GC Analysis for Sterols. The composition of the sterol fraction was determined by gas chromatography (GC) using betulin as internal standard [24]. The compounds were separated on an SE 54 CB chromatograph (Macherey–Nagel, Duren, Germany; 50 m long, 0.25 mm i.d., 0.25 µm film thickness). Other parameters were as follows: hydrogen carrier gas, split ratio 1:20, injection and detection temperature adjusted to 320°C, temperature program 240–255°C at 4°C/min.

Total Phenolics Content. The total phenolics (TP) content was determined based on the method developed by Capannesi et al. [25]. Folin–Ciocalteau reagent was used.

Saponification Number. The saponification number (SN) was determined according to the AOAC Official Method 920.160 [21].

Peroxide Value. The peroxide value (PV) was determined using the International Dairy Federation spectroscopic method described by Decker and Shantha [26].

Acid Value. The acid value (AV) was determined according to the AOCS Official Method Cd 3d-63 [21].

Conjugated Diene Value. The conjugated diene value (CDV) was measured by the method developed by Saguy et al. [27]. Based on this method, the oil was mixed with hexane of HPLC grade in 1:600 ratio, and the absorbance of the mixture was read at 234 nm.

Carbonyl Value. The carbonyl value (CV) of the oils was measured according to the method developed by Endo et al. [28].

Total Polar Compounds Content. Total polar compounds (TPC) content was determined according to the economical micromethod developed by Schulte [29].

Oxidative Stability Index. The oxidative stability Index (OSI) was determined using a Metrohm Rancimat model 743. Three grams of oil was used for each test. Temperature and air flow rate were set at 120°C and 151 L/h, respectively [19].

Wax Value. To determine the wax value, 10 g of the sample was mixed with 50°C of acetone and incubated at 4°C for 24 h to initiate crystallization. The precipitated solid fractions were then filtered through Whatman filter paper No.1 with predetermined weight and put in a vacuum oven at 45°C for drying. The weight of the solid fraction left on the filter paper is the weight of the wax contained in the oil [8].

Statistical Analysis. All the experiments were replicated three times and the data were analyzed by analysis of variance (ANOVA). MStatC and Slide Write software were used for ANOVA and regression analyses, respectively. Mean comparison was performed using the Multi step Duncan test ($p < 0.05$).

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