Effect of unsaponifiable matter extracted from *Pistacia khinjuk* fruit oil on the oxidative stability of olive oil

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Abstract The present study was carried out to investigate the improvement of oxidative stability of refined olive oil using various concentrations of unsaponifiable matters extracted from *Pistacia khinjuk* fruit oil (UFO). For further elucidation of UFO antioxidative power, tertbutylhydroquinone (TBHQ) was used in an olive oil sample, too. Oxidative stability of olive oil samples without and with different levels of UFO (50, 100, 250, 500, 750 and 1000 ppm) and TBHQ (100 ppm) were studied via evaluation of conjugated diene value, carbonyl value, oil/oxidative stability index, acid value and total tocopherol (TT) contents through 8 h thermal process at 170 °C. Results obtained by oxidative stability assays revealed that the highest antioxidative activity of olive oil was obtained by 100 ppm of UFO, followed using 100, 250, 500, 750, and 1000 ppm of UFO and 100 ppm TBHQ, respectively. Evaluation of the relationship between oxidative stability indexes and TT changes indicated a strong correlation ($R^2 = 0.9718$) between mean relative resistance to oxidation and relative resistance to TT reduction during thermal process. By promotion of relative resistance to TT reduction, olive oil samples' relative resistance to oxidation was enhanced exponentially; implying importance of TT in promotion of oxidative stability of edible oils. The results obtained in this study showed that UFO has higher antioxidative activity compared to TBHQ; thus UFO can be considered as a natural antioxidant with ideal antioxidative activity.

Keywords *Pistacia khinjuk* fruit oil · Unsaponifiable matter · Oxidative stability · Total tocopherol · Olive oil

Introduction

Oxidation is major factor in quality loss of edible oils. Antioxidants are used to prevent negative consequences of this reaction (Frankel 1998). While harmful effects of synthetic antioxidants on health are well documented; the benefits of natural antioxidants on human health have been confirmed by scientific bodies (Huang et al. 2013; Sosa et al. 2013). As a result, natural antioxidants have gained widespread application and there are many investigations conducted on identification of natural antioxidants (Abdelazim et al. 2013; Siddeeg and Xia 2015; Yang et al. 2010). However, natural antioxidants suffer from lack of sufficient and available supply and instability in food at different thermal and temporal conditions. There have been many experiments conducted on antioxidative activities of natural antioxidants in Iran (Bamoniri et al. 2010; Farhoosh et al. 2011; Tavakoli et al. 2013; Asnaaashari et al. 2015; Tavakoli et al. 2016). Zagros forest represents a natural resource in Iran stretching from west to southwest harboring various tree species such as wild pistachio, wild almond and oak. *Pistacia khinjuk* (called Kolkhoung in Persian) is a type of wild pistachio trees covering an area of 700,000 hectares in Zagros forest. Its annual productivity is 20–30 kg and its fruit oil content is nearly 39% (Browicz 1988; Saffarzadeh et al. 1999). In a previous research, it has been observed that *P. khinjuk* fruit oil has a high oxidative stability at 170 °C (Tavakoli and Khodaparast 2013). This stability is attributed to presence of antioxidants such as tocopherols, polyphenols, sterols, etc.; all belonging to unsaponifiable matters of *P. khinjuk* fruit oil

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(Shahidi 2005). Regarding availability and abundance of *P. khinjuk* trees in Iran and excellent oxidative stability of its oil, the present study was carried out to investigate improvement of oxidative stability of refined olive oil using various concentrations of unsaponifiable matters extracted from *P. khinjuk* fruit oil (UFO), with refined olive oil without antioxidant being used as control. For further elucidation of UFO antioxidative power, tert-butylhydroquinone (TBHQ) (the strongest and the most popular synthetic antioxidant in food industry) was used in an olive oil sample, too.

Materials and methods

Materials

Pistacia khinjuk fruits (three replications) were collected from Meimand forest, Fars province (summer of 2015). Moreover, refined olive oil without antioxidant was purchased from Shiraz province, Fars (three replications). The samples were stored at -18°C until experiment. All standards, chemicals and solvents were purchased from Sigma Aldrich and Merck Companies.

Oil extraction

For extraction of unsaponifiable matters, the *P. khinjuk* fruit oil was extracted at first. For this purpose, whole fruit of *P. khinjuk* (consisting of a kernel, thick woody shell and a soft external hull) were shade-dried and powdered in mill. The prepared powder was mixed with normal hexane in 1:4 ratio and placed in shaker for 48 h at darkness. The mixture was then placed under vacuum condition at 40°C for 6–12 h to remove the solvent (Tavakoli et al. 2015). Oil yields of *P. khinjuk* fruits was 40.2%.

Preparation of various samples of olive oil

To perform thermal process, different refined olive oil samples containing 50, 100, 250, 500, 750 and 1000 ppm UFO and 100 ppm of the synthetic antioxidant TBHQ were prepared for comparison with refined olive oil without antioxidant as control (olive oil containing 50 ppm of UFO, olive oil containing 100 ppm of UFO, olive oil containing 250 ppm of UFO, olive oil containing 500 ppm of UFO, olive oil containing 750 ppm of UFO, olive oil containing 1000 ppm of UFO, olive oil containing 100 ppm of TBHQ).

Extraction of unsaponifiable matters (USM)

To saponify *P. khinjuk* fruit oil, 5 g of this oil was mixed with 50 ml 1 N ethanolic KOH and heated at 95°C for 1 h.

The mixture was cooled and then 100 ml distilled water was added and mixed well. The resulting solution was extracted twice using 100 ml diethyl ether in decanter funnel. In every extraction step, the upper organic layer was collected and washed twice with 75 ml distilled water and once with 100 ml 0.5 N ethanolic KOH and finally neutralized with 100 ml distilled water. Organic layers were then separated and dried by Na_2SO_4 . The solution was filtered and evaporated for drying in vacuum oven at 45°C . For further purification of USM, the compounds were dissolved in chloroform, filtered and finally evaporated at 45°C under vacuum condition (Lozano et al. 1993).

Chemical composition

Gas chromatography (GC) method using Betulin as standard was applied to quantify total sterol (TS) compounds of *P. khinjuk* fruit oil and refined olive oil without antioxidant. The compounds were separated on a SE 54 CB (Macherey-Nagel, Duren, Germany; 50 m long, 0.25 mm ID, 0.25 μm film thickness). Other parameters included hydrogen as carrier gas, split ratio 1:20, injection and detection temperature adjusted to 320°C , temperature program, $240\text{--}255^{\circ}\text{C}$ at $4^{\circ}\text{C}/\text{min}$ (ISO12228 1999). Total tocopherol (TT) compounds in the oils was determined using a high-performance liquid chromatograph (HPLC) (WATERS, Alliance system, USA) equipped with a Spherisorb column (25 cm $\times$ 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. Hexane/isopropanol (98.5:0.5 v/v) at a flow rate of 1 ml/min was used as mobile phase. Tocopherol content was measured by comparison of retention times with those of reference standards (ISO9936 1997). Total Phenolic (TP) content was determined by the method developed by Capannesi et al. (2000); and results were reported in terms of mg gallic acid per kilogram of oil. Folin–Ciocalteu reagent was used in this spectroscopic method.

Oxidative stability

Oil samples (refined olive oil without antioxidant as control, olive oil containing 50 ppm of UFO, olive oil containing 100 ppm of UFO, olive oil containing 250 ppm of UFO, olive oil containing 500 ppm of UFO, olive oil containing 750 ppm of UFO, olive oil containing 1000 ppm of UFO, olive oil containing 100 ppm of TBHQ) (250 g for each replication) were poured in Erlenmeyer flask and placed without any shaking in hot paraffin bath in an oven for 8 h. Temperature of the samples was carefully kept at 170°C . At 60-min intervals,

samples (20 g) was taken and stored under -18°C for further analysis (Farhoosh and Tavakoli 2008; Farhoosh et al. 2008; Tavakoli et al. 2016). Conjugated diene value (CDV) was measured using the procedure described by Saguy et al. (1996). In this method, sample oil was mixed with HPLC grade hexane in 1:600 ratio, and the absorption was measured spectroscopically at 234 nm. Carbonyl Value (CV) of the oils was measured according to the method developed by Endo et al. (2001). Oil/oxidative stability index (OSI) was measured using Rancimat device model 743. For each assay, 3 g of oil was used and temperature and airflow velocity of the device was set at 120°C and 15 l/h, respectively. Acid value (AV) was determined according to the AOCS Official Method Cd 3d-63 (1993).

Estimating relative resistance of olive oil samples against variation of various oxidative quantities during thermal process

The following formula was used to estimate resistance of various olive oil samples against increase of CDV, CV and AV or reduction of OSI and TT during thermal process:

$$\text{Resistance} = \frac{100}{X}$$

X = Percentage of increase of CDV, CV and AV or reduction of OSI and TT of various olive oil samples during thermal process compared to t_0 .

Relative resistance of various oil samples was calculated using this formula:

$$\text{Relative resistance} = \frac{\text{Resistance of various olive oil samples}}{\text{Resistance of refined olive oil without antioxidant as control}}$$

Statistical analysis

At first, chemical properties of studied oils in the present research were analyzed in triplicate based on randomized complete (RCD). Then, thermal processes of the oils were planned in the context of factorial approach based on

randomized complete design (RCD) with three replications (the variable in this experiments included oil samples and time of heating process). All data were analyzed by analysis of variance (ANOVA). MStatC and SlideWrite software were used for ANOVA and regression analysis, respectively. Mean comparison was performed by Duncan multi-step test to determine significance of the differences ($p < 0.05$).

Results and discussion

Chemical properties

Some of chemical properties of *P. khinjuk* fruit and olive oils are presented in Table 1. USM includes tocopherol, polyphenol, sterol, hydrocarbon and carotenoid compounds possessing food value and antioxidative properties considered as index of edible oils quality. USM content in edible oils is typically in the range of 0.5–2.5%; occasionally reaching 5–6% (Shahidi 2005). USM content of *P. khinjuk* fruit and olive oils were determined 1.5 and 1.2%; respectively (Table 1). The content of this compounds in rice bran, corn, sesame, raw soybean, canola, extra virgin olive and cotton seed oils are 4.2%, at most 2.8%, 0.9–2.3%, 1.6%, 0.2–1.5%, and 0.5–0.7; respectively (Gunstone et al. 1994). Thus USM content of *P. khinjuk* fruit is close to that of sesame and soybean oils.

Tocopherol or vitamin E is naturally found in the forms of alpha, beta, gamma, and delta in animal and plant foods. Tocopherols have vitamin and antioxidative properties that provide them with food importance. Application of natural antioxidants such as tocopherols has been increasingly growing due to their ability in neutralizing free radicals and carcinogenic effects of synthetic antioxidants (Gunstone et al. 1994). TT content of *P. khinjuk* fruit and olive oils was determined 639.83 and 350 mg/kg; respectively (Table 1). TT contents in walnut, cotton seed, canola, olive, palm, peanut, soybean, and sunflower oils are 1500, 830–900, 690–695, 30–300, 360–560, 330–450, 900–1400, and 630–700 mg/kg (Shahidi 2005). TT content of *P. khinjuk* fruit oil was close to that of canola and sunflower oils.

Table 1 Some of chemical composition of *Pistacia khinjuk* fruit and refined olive oil without antioxidant

Parameter	<i>Pistacia khinjuk</i> fruit oil	Refined olive oil without antioxidant
USM content (% of oil)	1.5 ± 0.2^a	1.2 ± 0.3^a
TT content (mg/kg oil)	639.83 ± 14.3^a	350 ± 1.2^b
TP content (mg gallic acid/kg oil)	85.23 ± 2.4^a	26.5 ± 4.5^b
TS content (mg/kg oil)	2167.5 ± 23.2^a	1076.3 ± 17.4^b

Mean $\pm$ SD (standard deviation) within a row with the same lowercase letters are not significantly different at $p < 0.05$

USM unsaponifiable matter, TT total tocopherol, TP total phenolic, TS total sterols

Table 2 Changes in conjugated diene value (CDV, mmol/L) and carbonyl value (CV, $\mu\text{mol/g}$) of olive oil as affected by unsaponifiable matters of *P. khinjuk* fruit oil (UFO) and TBHQ during heating process at 170 °C

Time (hour)	Refined olive oil without antioxidant	Olive oil samples containing						
		UFO						TBHQ
		50 ppm	100 ppm	250 ppm	500 ppm	750 ppm	1000 ppm	100 ppm
<i>CDV</i>								
0	11.5 ± 0.04 ^a	11.5 ± 0.04 ^a	11.51 ± 0.05 ^a	11.52 ± 0.04 ^a	11.55 ± 0.04 ^a	11.56 ± 0.04 ^a	11.56 ± 0.03 ^a	11.5 ± 0.03 ^a
2	19.23 ± 0.06 ^c	14.92 ± 0.05 ^g	21.4 ± 0.03 ^b	21.65 ± 0.03 ^a	16.26 ± 0.02 ^e	15.34 ± 0.05 ^f	17.84 ± 0.08 ^d	14.51 ± 0.02 ^h
4	20.34 ± 0.08 ^c	20.55 ± 0.06 ^b	18.25 ± 0.02 ^e	21.89 ± 0.06 ^a	16.77 ± 0.02 ^g	17.8 ± 0.04 ^f	19.17 ± 0.03 ^d	14.54 ± 0.04 ^h
6	31.34 ± 0.07 ^a	18.45 ± 0.14 ^e	15.65 ± 0.03 ^g	22.08 ± 0.04 ^b	17.22 ± 0.03 ^f	18.58 ± 0.03 ^d	19.72 ± 0.03 ^c	16.5 ± 0.04 ^h
8	29.74 ± 0.05 ^a	28.48 ± 1.36 ^b	19.97 ± 0.03 ^e	26.11 ± 0.08 ^c	18.67 ± 0.03 ^f	18.69 ± 0.03 ^f	21.74 ± 0.09 ^d	16.96 ± 0.02 ^g
<i>CV</i>								
0	7.22 ± 0.32 ^a	7.24 ± 0.32 ^a	7.22 ± 0.32 ^a	7.22 ± 0.32 ^a	7.22 ± 0.33 ^a	7.22 ± 0.32 ^a	7.22 ± 0.32 ^a	7.22 ± 0.48 ^a
2	8.02 ± 0.22 ^d	7.95 ± 0.35 ^d	9.08 ± 0.04 ^c	9.87 ± 0.41 ^b	10.1 ± 0.4 ^b	9.98 ± 0.4 ^b	13.1 ± 0.4 ^a	9.11 ± 0.21 ^c
4	8.56 ± 0.46 ^d	8.56 ± 0.36 ^d	10.22 ± 0.51 ^c	12.85 ± 0.36 ^b	12.39 ± 0.45 ^b	10.81 ± 0.46 ^c	16.27 ± 0.5 ^a	10.91 ± 0.62 ^c
6	9.22 ± 0.45 ^e	9.22 ± 0.25 ^e	10.83 ± 0.47 ^d	10.28 ± 0.58 ^d	12.83 ± 0.5 ^b	11.22 ± 0.48 ^c	18.81 ± 0.52 ^a	12.13 ± 0.38 ^b
8	17.07 ± 0.51 ^b	10.03 ± 0.26 ^e	10.92 ± 0.41 ^d	10.58 ± 1.60 ^{de}	17.47 ± 1.77 ^{ab}	16.57 ± 1.08 ^b	19.43 ± 1.11 ^a	12.82 ± 0.27 ^c

Mean $\pm$ SD (standard deviation) within a row with the same lowercase letters are not significantly different at $p < 0.05$

TP content was determined 85.2 and 26.5 mg/kg in *P. khinjuk* fruit and olive oils; respectively. We had previously reported that TP content in *P. khinjuk* fruit oil was 81.12 mg/kg (Tavakoli and Khodaparast 2013). Although polyphenols are interesting substances due to their antioxidative properties, they have biological activities in organisms and play critical role in preventing diseases resulting from extra free radicals exceeding of human body (Robards et al. 1999; Ryan and Robards 1998).

Plant sterols called phytosterol play important role in decreasing blood cholesterol and thereby reduction of heart disease risk. They are important factors in food grading and used as indices for originality of edible oils. Most of plant oils contain 1000–5000 ppm of sterol compounds (Crane et al. 2005; Lagarda et al. 2006). TS content of *P. khinjuk* fruit and olive oils were calculated 2167.5 and 1076.3 ppm; respectively.

Oxidative stability tests

To assess positive effect of UFO on oxidative stability of olive oil, various samples of this oil (containing different concentrations of UFO and 100 ppm TBHQ) were exposed to at 170 °C for 8 h and the changes in CDV, CV, AV, OSI and TT were monitored.

Conjugated diene value (CDV)

Measurement of CDV is a good indication of primary oxidative of oil samples. CDV test is suitable for edible oil containing high linoleic acid. To assay the primary

oxidation, hydroperoxides content should be measured but these compounds are decomposed in high temperature (170 °C); whereas, conjugated diene hydroperoxides are stable under this condition (Frankel 1998). Thus CDV changes of olive oil samples were evaluated to monitor primary oxidation. Olive oil has a low amount of linoleic acid. Oxidative stability of linoleic acid is lower than that of other fatty acids contained in olive oil. Thus, CDV changes can be used as a relative index of primary oxidation of olive oil in high temperature. CDV variation in different olive oil samples (refined olive oil without antioxidant as control, olive oil containing 50 ppm of UFO, olive oil containing 100 ppm of UFO, olive oil containing 250 ppm of UFO, olive oil containing 500 ppm of UFO, olive oil containing 750 ppm of UFO, olive oil containing 1000 ppm of UFO, olive oil containing 100 ppm of TBHQ) during 8 h of thermal process at 170 °C are presented in Table 2. As can be seen, there is no significant difference among the samples at t_0 regarding CDV. During thermal process, CDVs of olive oils show increasing trend. Table 5 shows relative resistance of olive oils samples to increase in CDV during thermal process. Resistance of olive oils containing 50, 100, 250, 500, 750 and 1000 ppm of UFO and 100 ppm of TBHQ were 1.07, 2.16, 1.25, 2.57, 2.59, 1.81, and 3.34 times as much as that of refined olive oil without antioxidant, respectively. It was observed that addition of different concentrations of UFO increased olive oil resistance to formation of CDV compared to refined olive oil without antioxidant. The best sample of olive oil was that containing 100 ppm TBHQ; followed by those

containing 500, 750, 100, 1000, 250 and 50 ppm of UFO, respectively. Therefore, relative resistance of olive oils with 100, 500 and 750 ppm UFO to primary oxidation was 64.7, 79.94 and 77.54% of that of TBHQ. It should be mentioned that in contrast to TBHQ, UFO is not a pure compound. If all TT, TP and TS content of *P. khinjuk* fruit oil have antioxidative power (these compounds constitute 0.29% of PKF oil), then about one-fifth of UFO constituents had antioxidative properties.

It was also found that CDV variation follows an irregular pattern in different samples of UFO-containing olive oil. UFO, compared to TBHQ, is not pure and hence, the interactions among different fractions of UFO may explain irregular changes of CDV.

Carbonyl value (CV)

Carbonyl compounds such as aldehydes and ketones are the products of secondary oxidation of lipids resulting from hydroperoxides degradation representing the main cause of unfavorable taste, deterioration and reduced value of fried foods (Antolovich et al. 2002). These compounds are more resilient than peroxides in thermal process and thus, CV is used as an appropriate index of oxidative changes in oils during thermal process (Farhoosh et al. 2008). CV variation of different olive oil samples (refined olive oil without antioxidant as control, olive oil containing 50 ppm of UFO, olive oil containing 100 ppm of UFO, olive oil containing 250 ppm of UFO, olive oil containing 500 ppm of UFO, olive oil containing 750 ppm of UFO, olive oil containing 1000 ppm of UFO, olive oil containing 100 ppm of TBHQ) during 8 h of thermal process at 170 °C are presented in Table 2. As can be seen, there is no significant difference among the samples at t_0 regarding CV. During thermal process, CVs of olive oils show increasing trend. Table 5 shows relative resistance of olive oils samples to increase in CV during thermal process. Resistance of olive oils containing 50, 100, 250, 500, 750 and 1000 ppm of UFO and 100 ppm of TBHQ were 3.53, 2.66, 2.93, 0.96, 1.05, 0.81 and 1.76 times as much as that of refined oil without antioxidant, respectively. It was observed that addition of different concentrations of UFO had different effects on prevention of CV increase. The best olive oil sample in CV test was the sample containing 50 ppm UFO, followed by oil samples containing 250 and 100 ppm UFO, 100 ppm TBHQ and 500, 750, and 1000 ppm UFO. Relative resistance of olive oils with 50, 100 and 250 ppm UFO to secondary oxidation was 2, 1.5 and 1.66 times as much as that of TBHQ indicating high antioxidative power of UFO in olive oil especially in the concentrations lower than 250 ppm. Reduced antioxidative power of olive oils containing UFO concentrations above

250 ppm indicates formation of peroxidation condition in the olive oil. In some cases, by increase in antioxidant concentration, instead of antioxidative activity, peroxidant property occurs which enhances oxidation process (Frankel 1998). Comparison of CDV and CV test results showed that there was no correlation between them. Power of Antioxidant compounds against primary oxidation (CDV test), secondary oxidation (CV test) and the hydrolysis of triglycerides (AV test) are different. An antioxidant may strongly prevent from primary oxidation, but its ability to prevent from secondary oxidation is poor. So there is not permanently a correlation between the power of an antioxidant in various stages of oxidation.

Acid value (AV)

Free fatty acids concentration is increased during thermal process and frying. This increase is attributed to either degradation of triglycerides or presence of carboxyl groups in the corresponding oxidative or polymeric product. By degradation of triglycerides and consequent increase of free fatty acids, oxidation process in edible oils is promoted and hence, their shelf life diminishes (Frankel 1998). AV variation in different olive oil samples (refined olive oil without antioxidant as control, olive oil containing 50 ppm of UFO, olive oil containing 100 ppm of UFO, olive oil containing 250 ppm of UFO, olive oil containing 500 ppm of UFO, olive oil containing 750 ppm of UFO, olive oil containing 1000 ppm of UFO, olive oil containing 100 ppm of TBHQ) during 8 h of thermal process at 170 °C are presented in Table 3. As can be seen, there is no significant difference among the samples at t_0 regarding AV. During thermal process, AVs of olive oils follow an increasing trend. As can be seen from Table 5, the most resistant sample to increase of free fatty acids was olive oils containing 50 ppm of UFO (4.25), followed by olive oils containing 250 ppm of UFO (3.4), olive oils containing 500 ppm of UFO (2.83), olive oils containing 750 ppm and 1000 ppm of UFO (both 2.43), olive oils containing 100 ppm of TBHQ (1.99) and olive oils containing 100 ppm UFO (1.89). Regarding AV test, UFO had higher antioxidative power than TBHQ. This means that UFO caused lower hydrolysis of the triglycerides than TBHQ in olive oil and the possibility of oxidation reaction reduced in this oil. In this test, as well as CV and CDV tests, was not observe systematic changes, too. Interactions between different fractions of UFO can be a reason of irregular changes of AV.

Oil/oxidative stability index (OSI)

To create oxidation in Rancimat test, oil samples are simultaneously exposed to high temperature (usually

Table 3 Changes in acid value (AV) and oil/oxidative stability index (OSI, h) of olive oil as affected by unsaponifiable matters of *P. khinjuk* fruit oil (UFO) and TBHQ during heating process at 170 °C

Time (hour)	Refined olive oil without antioxidant	Olive oil samples containing						
		UFO						TBHQ
		50 ppm	100 ppm	250 ppm	500 ppm	750 ppm	1000 ppm	100 ppm
<i>AV</i>								
0	0.24 ± 0.02 ^a	0.24 ± 0.02 ^a	0.24 ± 0.03 ^a	0.24 ± 0.03 ^a	0.24 ± 0.04 ^a	0.24 ± 0.03 ^a	0.21 ± 0.02 ^a	0.24 ± 0.03 ^a
2	0.26 ± 0.01 ^a	0.29 ± 0.02 ^a	0.28 ± 0.02 ^a	0.27 ± 0.02 ^a	0.26 ± 0.01 ^a	0.29 ± 0.02 ^a	0.26 ± 0.03 ^a	0.27 ± 0.02 ^a
4	0.31 ± 0.03 ^{ab}	0.31 ± 0.03 ^{ab}	0.29 ± 0.02 ^{ab}	0.29 ± 0.02 ^{ab}	0.27 ± 0.02 ^b	0.31 ± 0.01 ^a	0.27 ± 0.02 ^b	0.31 ± 0.03 ^{ab}
6	0.37 ± 0.02 ^a	0.29 ± 0.02 ^{bc}	0.26 ± 0.02 ^c	0.31 ± 0.02 ^b	0.30 ± 0.03 ^{bc}	0.31 ± 0.03 ^{bc}	0.30 ± 0.03 ^{bc}	0.31 ± 0.02 ^b
8	0.41 ± 0.02 ^a	0.28 ± 0.03 ^c	0.33 ± 0.03 ^{bc}	0.29 ± 0.02 ^c	0.30 ± 0.02 ^{bc}	0.31 ± 0.02 ^{bc}	0.31 ± 0.02 ^{bc}	0.33 ± 0.02 ^b
<i>OSI</i>								
0	3.35 ± 0.26 ^c	4.25 ± 0.36 ^b	4.31 ± 0.42 ^b	3.43 ± 0.28 ^c	4 ± 0.32 ^{bc}	2.73 ± 0.41 ^d	2.5 ± 0.29 ^d	9.5 ± 0.45 ^a
2	2.5 ± 0.25 ^e	4 ± 0.21 ^b	4.21 ± 0.27 ^{bc}	3.03 ± 0.24 ^d	3.75 ± 0.15 ^c	2.43 ± 0.25 ^e	2.45 ± 0.24 ^e	7.2 ± 0.51 ^a
4	1.9 ± 0.24 ^e	3.8 ± 0.18 ^c	4.25 ± 0.28 ^b	2.2 ± 0.21 ^d	3.44 ± 0.28 ^c	2.23 ± 0.26 ^d	2.31 ± 0.26 ^d	5.8 ± 0.44 ^a
6	1.02 ± 0.15 ^d	3.6 ± 0.24 ^b	4.11 ± 0.31 ^a	1.4 ± 0.22 ^d	3.15 ± 0.29 ^b	1.98 ± 0.26 ^c	2.1 ± 0.22 ^c	3.1 ± 0.34 ^b
8	0.55 ± 0.55 ^e	3.32 ± 0.28 ^b	4.06 ± 0.32 ^a	0.96 ± 0.33 ^d	2.92 ± 0.44 ^c	1.79 ± 0.43 ^d	1.53 ± 0.39 ^d	1.57 ± 0.2 ^d

Mean ± SD (standard deviation) within a row with the same lowercase letters are not significantly different at $p < 0.05$

between 100 and 130 °C) and air flow. Oxidation progress in this test is followed by measuring electric conductance changes. The change in electric conductance in Rancimat test is due to formation of volatile acids during thermal oxidation. This test is typically conducted along with other tests such as CDV and CV (Gertz et al. 2000). OSI changes of oil samples (refined olive oil without antioxidant as control, olive oil containing 50 ppm of UFO, olive oil containing 100 ppm of UFO, olive oil containing 250 ppm of UFO, olive oil containing 500 ppm of UFO, olive oil containing 750 ppm of UFO, olive oil containing 1000 ppm of UFO, olive oil containing 100 ppm of TBHQ) during 8 h of thermal process at 170 °C are presented in Table 3. Addition of various concentrations of UFO to unheated olive oil results in different outputs. Among different unheated olive oil samples (t_0), oil containing 100 ppm TBHQ had the highest OSI (9.5 h). Among different levels of UFO, only 50 and 100 ppm concentrations increased OSI (4.25 and 4.31 h, respectively) of refined olive oil without antioxidant at t_0 ; while UFO at concentration of 250 ppm (3.43 h) was not able to create significant difference in refined olive oil without antioxidant (3.35 h) at t_0 . Moreover, addition of UFO at concentration of 750 and 1000 ppm (2.73 and 2.5 h, respectively) reduced OSI of refined olive oil without antioxidant. In the other words, by increase in UFO concentration and antioxidant compounds in the olive oil, peroxidative properties were observed in the oil. Moreover, OSI variation of oil samples during thermal process showed a descending trend so that the highest decrease was observed in samples containing TBHQ. Relative resistance of oil samples against reduction of OSI is presented in Table 5.

Relative resistance of olive oils containing 50, 100, 250, 500, 750 and 1000 ppm of UFO and 100 ppm of TBHQ were 4.55, 14.99, 1.18, 3.27, 2.53, 4.35 and 1.03 times as much as that of refined olive oil without antioxidant, respectively. It was observed that addition of different concentrations of UFO had different effects on prevention of OSI reduction. The most resistant sample was that containing 100 ppm UFO, followed by oil samples containing 50, 1000, 500, 750 and 250 ppm UFO and 100 ppm TBHQ. Interestingly, the weakest resistance to OSI reduction was observed for olive oil containing 100 ppm TBHQ whose resistance was tightly close to that of refined olive oil without antioxidant. Therefore, it can be concluded that under thermal condition, in contrast to unheated olive oil, UFO has higher thermal resistance and antioxidative power than TBHQ.

Total tocopherol (TT) content

Primary TT content of edible oils affects their antioxidative power; however, thermal resistance of TT during thermal process is of higher importance. TT changes of olive oil samples (refined olive oil without antioxidant as control, olive oil containing 50 ppm of UFO, olive oil containing 100 ppm of UFO, olive oil containing 250 ppm of UFO, olive oil containing 500 ppm of UFO, olive oil containing 750 ppm of UFO, olive oil containing 1000 ppm of UFO, olive oil containing 100 ppm of TBHQ) during 8 h of thermal process at 170 °C are presented in Table 4. TT content of oil samples at t_0 was increased by addition of UFO. This can be attributed to accumulation of TT of *P. khinjuk* fruit oil in UFO which finally increases TT content

in the oil. TT changes during thermal process in olive oil samples showed a descending trend. Relative resistance of oil samples to decrease of TT during thermal process is presented in Table 5. Resistance of olive oils containing 50, 100, 250, 500, 750 and 1000 ppm of UFO and 100 ppm of TBHQ were 1.96, 2.18, 1.59, 1.7, 1.16, 1.5 and 0.96 times as much as that of refined olive oil without antioxidant, respectively. It was observed that addition of different concentrations of UFO had positive effect on prevention of TT reduction. The most resistant sample to TT reduction during 8 h of thermal process was olive oil sample containing 100 ppm UFO, followed by samples containing 50, 500, 250, 1000 and 750 ppm UFO and 100 ppm TBHQ. The remarkable note was higher reduction of TT in samples containing TBHQ compared to refined olive oil without antioxidant, confirming harmfulness of synthetic antioxidants because apart from their own harmful effects, these antioxidants degrade their natural counterparts such as tocopherols. Regarding critical role of tocopherols in

retention of oxidative stability of edible oils and their high food value in human body, minimum decrease of these compounds in edible oils is sought by researchers.

Relation between total tocopherol (TT) content changes and resistance to oxidation

For better interpretation of oxidative stability tests' results, relative resistance of various oil samples in CDV, CV, AV and OSI assays were summed and the mean values were estimated (Table 5). Mean relative resistance of olive oils containing 50, 100, 250, 500, 750 and 1000 ppm of UFO and 100 ppm of TBHQ was 2.68, 4.34, 1.75, 1.93, 1.72, 1.88 and 1.62 times as much as that of refined olive oil without antioxidant, respectively. In this regard, the best sample was that containing 100 ppm of UFO, followed by samples containing 50, 500, 1000, 250, and 750 ppm of UFO and 100 ppm of TBHQ. The relation between oil samples' resistance to TT reduction and

Table 4 Changes in total tocopherol (TT, mg/kg oil) content of olive oil as affected by unsaponifiable matters of *P. khinjuk* fruit oil (UFO) and TBHQ during heating process at 170 °C

Time (hour)	Refined olive oil without antioxidant	Olive oil samples containing						
		UFO						TBHQ
		50 ppm	100 ppm	250 ppm	500 ppm	750 ppm	1000 ppm	100 ppm
0	350 ± 12.1 ^c	356.1 ± 13.43 ^{de}	360.5 ± 14.9 ^{de}	374.4 ± 6.6 ^d	401.4 ± 7.7 ^c	438.7 ± 4.8 ^b	477.8 ± 6.3 ^a	350 ± 8.9 ^e
2	301.2 ± 7.9 ^d	331.2 ± 10.3 ^c	329.8 ± 10.7 ^c	302.8 ± 4.5 ^d	350.2 ± 7.5 ^b	310.2 ± 9.2 ^d	401.3 ± 11.1 ^a	250.1 ± 7.6 ^e
4	225.6 ± 8.9 ^e	276.8 ± 14.5 ^b	284.4 ± 7.2 ^b	241.5 ± 12.3 ^c	280.3 ± 4.3 ^b	234.5 ± 8.8 ^c	320.2 ± 10.3 ^a	124.5 ± 8.8 ^d
6	97.2 ± 7.6 ^e	229.1 ± 13.7 ^b	251.4 ± 6.9 ^a	200.1 ± 10.7 ^c	235.2 ± 9.9 ^b	145.5 ± 7.6 ^d	251.9 ± 11.8 ^a	62.5 ± 7.7 ^f
8	30 ± 3.03 ^e	189.7 ± 11.2 ^b	209.5 ± 5.9 ^a	159.3 ± 3.9 ^e	186 ± 5.6 ^b	92.7 ± 6.3 ^d	186 ± 9.2 ^b	16.9 ± 2.3 ^f

Mean ± SD (standard deviation) within a row with the same lowercase letters are not significantly different at $p < 0.05$

Table 5 Relative resistance of olive oil samples against changes in different oxidation factors and TT compounds during heating process at 170 °C

Parameter	Olive oil samples containing						
	UFO						TBHQ
	50 ppm	100 ppm	250 ppm	500 ppm	750 ppm	1000 ppm	100 ppm
Resistance against CDV increase	1.07 ± 0.05 ^f	2.16 ± 0.07 ^c	1.25 ± 0.09 ^e	2.57 ± 0.1 ^b	2.59 ± 0.05 ^b	1.81 ± 0.07 ^d	3.34 ± 0.08 ^e
Resistance against CV increase	3.53 ± 0.2 ^a	2.66 ± 0.15 ^c	2.93 ± 0.1 ^b	0.96 ± 0.07 ^c	1.05 ± 0.08 ^c	0.81 ± 0.07 ^f	1.76 ± 0.1 ^d
Resistance against AV increase	4.25 ± 0.1 ^a	1.89 ± 0.06 ^e	3.4 ± 0.09 ^b	2.83 ± 0.1 ^c	2.43 ± 0.1 ^d	2.43 ± 0.08 ^d	1.99 ± 0.07 ^e
Resistance against OSI reduction	4.55 ± 0.2 ^b	14.99 ± 0.25 ^a	1.18 ± 0.1 ^e	3.27 ± 0.15 ^c	2.53 ± 0.13 ^d	4.35 ± 0.13 ^b	1.03 ± 0.05 ^e
Mean resistance against oxidation	2.68 ± 0.13 ^b	4.34 ± 0.22 ^a	1.75 ± 0.06 ^d	1.93 ± 0.05 ^c	1.72 ± 0.08 ^{de}	1.88 ± 0.05 ^c	1.62 ± .04 ^e
Resistance against TT reduction	1.96 ± 0.04 ^b	2.18 ± 0.09 ^a	1.59 ± 0.08 ^{cd}	1.70 ± 0.03 ^c	1.16 ± 0.04 ^e	1.5 ± 0.03 ^d	0.96 ± 0.04 ^f

Mean ± SD (standard deviation) within a row with the same lowercase letters are not significantly different at $p < 0.05$

UFO unsaponifiable matters of *P. khinjuk* fruit oil, CDV conjugated diene value, CV carbonyl value, AV acid value, OSI oil/oxidative stability index, TT total tocopherols

Fig. 1 Correlation between olive oil samples' relative resistance against TT reduction and their mean relative resistance against oxidation during 8 h of thermal process at 170 °C. TT total tocopherols

their mean relative resistance to oxidation is shown in Table 5 and Fig. 1. As can be inferred, samples with higher resistance to TT reduction had higher mean relative resistance to oxidation (in sample containing TBHQ, a part of resistance is due to presence of this synthetic antioxidant) (Table 5). Figure 1 shows the relation between resistance to TT reduction and mean resistance to oxidation. As can be seen from the figure, by increase in relative resistance to TT reduction, mean relative resistance to oxidation is enhanced and a strong correlation ($r^2 = 0.9718$) is observed between them. By increase in relative resistance to TT reduction, mean relative resistance to oxidation was exponentially enhanced showing importance of TT in oxidative stability of edible oils during thermal process.

Conclusion

Results of oxidative stability tests showed that UFO (although not pure), especially at 50 and 100 ppm concentrations, had higher antioxidative power than TBHQ which is regarded as the strongest and most popular antioxidant. Therefore, UFO can be introduced as a natural antioxidant with high antioxidative power and thermal stability which can be an alternative to synthetic antioxidants such as TBHQ whose harmful effects on health is well documented. Moreover, the reason of irregular changes in oxidative stability tests of olive oil samples in the presence of UFO could be attributed to its impure nature. UFO is composed of different factions that during the heating process can be created interactions between them. A strong correlation between oxidative stability of olive oil samples and their TT content confirms the critical role of tocopherol compounds as natural

antioxidants in the oxidative stability of edible oils. The remarkable note of present study was higher reduction of TT in oil samples containing TBHQ compared to other olive oil samples, confirming harmfulness of synthetic antioxidants, too.

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