The Effects of Pistachio Oil on Oxidative Stability of Sunflower Oil under Accelerated Oxidation Conditions

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Abstract

Introduction: Nowadays, identifying the natural ways of preventing food deterioration due to the deleterious effects of synthetic additives has gained high significance. In this study, the effects of pistachio oil (PO), as a natural antioxidant, on the oxidative stability of sunflower oil (SFO) have been evaluated under accelerated oxidation conditions.

Materials and Methods: The antioxidant properties of unrefined SFO and PO were evaluated using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method. The peroxide value (PV), thiobarbituric acid-reactive substances (TBARS), and the TOTOX value of SFO supplemented with the concentrations of 0.1, 0.2, and 0.4% of PO (T3, T4, and T5 treatments, respectively) were assessed as the oxidation indices, over 30 days. In addition, SFO alone and SFO in combination with butylated hydroxytoluene (BHT) were considered as negative and positive controls (T1 and T2 treatments), respectively. The statistical analysis of the indices was carried out using the one-within one-between ANOVA.

Results: Treatments with 0.2 and 0.4% of PO delayed SFO oxidation for 20 days through their antioxidant contents, but the treatment with 0.1% of PO increased SFO oxidation after 30 days. The increase by the later might be due to the pro-oxidant nature of PO. It is worth noting that the high concentrations of PO (0.2 and 0.4%), a generally recognized as a safe (GRAS) additive, showed stronger oxidation-inhibitory effects than the authorized concentration of BHT (0.02%) ($P \leq 0.05$).

Conclusion: In conclusion, it was identified that the use of PO, as a natural antioxidant, was effective in the prevention of SFO oxidation.

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1. Introduction

Edible oils, as highly energetic nutrients, are used extensively in everyday life [1]. The fatty acids' profile of edible oils affects their susceptibility to oxidation. Rancid physical changes and the lowered quality and safety of nutritional oil are from among the effects of oxidation [2, 3].

Sunflower oil (SFO) is used as cooking oil in many countries [4]. Oleic and linoleic fatty acids form about 90% of SFO fatty acids, yet their amount can be affected by the plant and its environmental properties [5]. In SFO, the oleic: linoleic ratio ranges from 1:1 to 1:6 [6]. Despite the known beneficial edible and cooking properties of SFO, it has some unpleasant properties. Crude SFO oxidation and particularly polymerization during the food frying process lead to the gumminess of the frying vessel walls, with this being one of the unpleasant properties [7, 8]. Studies have shown that frying SFO is toxic [9]. Nowadays, the edible oil industry uses generally synthetic antioxidants to retard the oxidative deterioration of oils. Relevant synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), can easily induce carcinogenesis, especially at high levels and after long-term applications [10-12]. Hence, there has always been a tendency to use natural antioxidants of plant origins to replace such synthetic antioxidants [13]. Since the last two decades, plants have been considered as the richest source of natural antioxidants [14, 15]. The major identified antioxidants of plant origins include tocopherols, carotenoids, phenolics, and vitamin C [16]. For many reasons, natural herbal antioxidants have been utilized in the food-related oil industry [17].

The composition of raw pistachio nuts consists in approximately 45 to 72 % of fats, depending on the variety and stage of the harvest [12]. Pistachio kernel oil is rich in unsaturated fatty acids, with its predominant fatty acid being oleic acid (56- 64%), followed by linoleic acid (23- 31%) [18]. Pistachio nuts have a great deal of antioxidant materials, including polyphenols, tocopherols, and carotenoids, with these being the cause of their high antioxidant activity [19- 23]. Considering the high amount of oil in pistachio kernels and its safe nature, it is suggested to be utilized as a safe and economic additive in the food industry at low concentrations.

The current study has been conducted to investigate SFO supplementation with pistachio oil (PO) as a natural antioxidant, under accelerated oxidation conditions. Despite the lack of broad consensus, the primary oxidative products of SFO can be evaluated by the PV assay, while the secondary ones, such as aldehydes can be measured by the thiobarbituric acid test [8]. In this study, the oxidative stability of SFO supplemented with PO and also synthetic antioxidants was monitored by measuring peroxides and TBARS. In addition, its hydrolytic rancidity was assessed by evaluating the acid value, during the accelerated oxidation storage [24].

2. Materials and Methods

2.1. Materials

In order to prepare PO, pistachio kernels (Kalle-Ghuchi cultivar, Kerman, Iran) were directly entered into a screw press (Iran Cold Press®, Model 85 mm express, Iran). The oil

extraction process was enhanced by the pre-heating of the tip of the apparatus at temperatures below 45°C. Besides, the unrefined SFO was provided from a traditional refinery by the cold-pressing process. The SFO contained no synthetic antioxidants. The oils were stored at a refrigerator at 4°C before use.

2.2. The DPPH radical scavenging assay

The antioxidant activities of PO and SFO were determined via the assessment of their DPPH-radical scavenging ability, according to the method introduced by Gulluce et al., with some modifications. An amount equaling 2.5 ml of the 0.004% MeOH solution of DPPH (D9132; Sigma Aldrich, Germany) was added to 0.1 ml of oil samples at different concentrations. The mixtures were kept in the dark at room temperature. After 30 minutes, the absorbance of the solution was measured at 517 nm [25]. The synthetic antioxidant, i.e. BHT, was used as the positive control. The DPPH radical-scavenging activity (RSA, %) was calculated by equation1 as follows:

Equation1: $RSA(\%) = \frac{A\,blank - A\,sample}{A\,blank} \times 100$

Where,"A blank" is the absorbance of the control reaction, and "A sample" is the absorbance of the test compound. The oil concentration that provided the 50% inhibition (IC50) was calculated using the RSV/oil concentration graph. All tests were done at least in triplicate.

2.3. Sample preparation for accelerated oxidation conditions

For this research, five treatments (T1-T5) were considered. The T1 sample contained unrefined SFO as the negative control. The T2 sample, as the positive control, consisted of the unrefined SFO that received the synthetic antioxidant and BHT within the legal limit (0.02%; w/w) [26]. T3-T5 treatments had been supplemented with 0.1%, 0.2%, and 0.4% (w/w) of PO, respectively. Three replications were provided for every sample. All samples were placed in dark brown amber bottles. The bottles were capped tightly and stored in an oven under accelerated oxidation conditions (60°C). The oxidative and hydrolytic rancidity indices of SFO were assessed at 10-day intervals for 30 days.

2.4 The peroxide value (PV)

In order to evaluate the PV of the treatments, different SFO treatments (2 g) were dissolved in chloroform: acetic acid mixture (3:2, v/v; 30 ml). Next, the saturated potassium iodide solution (1 ml) was added. The mixture was shaken heavily for one minute and then kept in the dark for five minutes. After adding distilled water (75 ml) to it, the mixture was titrated against sodium thiosulfate (0.002 M) with high agitation to make the yellow color disappear. Afterwards, the starch indicator solution (1%) was added. Titration was continued again until the blue color disappeared. "The blank" was analyzed under similar conditions [13]. The PV (meq/kg) was calculated using Equation2 as follows:

Equation 2: $PV = \frac{C \times (VS - VB) \times 12.69 \times 78.8}{m}$

Where, C represents the sodium thiosulfate concentration (M), VS and VB represent the volume of sodium thiosulfate for the sample and the blank (ml), and m is the mass of SFO (g).

2.5. The thiobarbituric acid-reactive substances (TBARS) assay

To measure TBARS, the oil samples (0.05 ml) were combined with distilled water (0.95 ml) and 2.0ml of the TBA reagent in test tubes. The mixture was stirred well and heated for 15 minutes using a boiling water bath. The TBA reagent consisted of trichloroacetic acid (15 g), thiobarbituric acid (0.375 g), hydrochloric acid (2 ml), and distilled water (82.9 ml). The tubes were cooled for 10 minutes at room temperature, and then the mixture was centrifuged (1000g; 15 min). The absorbance of the pinkish supernatant was measured at 532 nm. The TBARS concentrations of the oils were determined from the standard curve plotted using 1,1,3,3-tetraethoxypropane (Sigma, T9889) [27].

2.6. The total oxidation (TOTOX) value

Total oxidation (TOTOX) values of the sunflower oil samples were assessed based on PV and TBARS values [28]. For this purpose, TBARS values were used instead of p-anisidine values to measure the total oxidation. Total oxidation values were obtained using Equation 3 as follows:

Equation 3: $$TOTOX\,(TBA) = 2PV + TBARS$$

2.7. Acid values

The acid values of the oil samples were determined using a titration method introduced by Rao et al. (2009), with some modifications. Two grams of the oil samples were mixed thoroughly in a mixture of ether: ethanol (2:1, v/v; 50 ml). The mixture was cooled down to room temperature and titrated against KOH using phenolphthalein (1%) as the indicator [29]. The acid value was calculated using Equation 4 as follows:

Equation 4: $$\text{Acid value} = \frac{V \times C \times 56.11}{m}$$

Where, V represents the volume of potassium hydroxide used by the samples (ml), C shows the KOH concentration, and (m)stands for the mass of SFO (g).

2.8. The statistical analysis

The experiments were replicated three times on three different occasions. All analyses were reported as mean± SEM. The statistical data analysis was performed for each feature of SFO on different accelerated oxidation days, using one-within one-between ANOVA (via SPSS 11.5 statistical software; SPSS Inc., Chicago, IL). Significant differences among the means of the parameters were determined using the Duncan's multiple range test ($P \leq 0.05$).

3. Results

In this study, the antioxidant activities of PO and SFO were assessed through the DPPH spectrophotometric method. Based on the results, the DPPH radical scavenging activities of PO and SFO increased at higher oil concentrations (Fig.1). The synthetic antioxidant BHT was used as the positive control. The results also showed that the anti-radical activity of the PO was more than that of the SFO (Table 1).

Table 1. 50% DPPH radical inhibitory activity concentration of PO, SFO and, BHT

Compounds	IC50 values (means)	SEM
Pistachio oil	14.79 mg/ml	0.14
Sunflower oil	20.89 mg/ml	0.28
BHT (positive control)	19.10 µg/ml	0.49

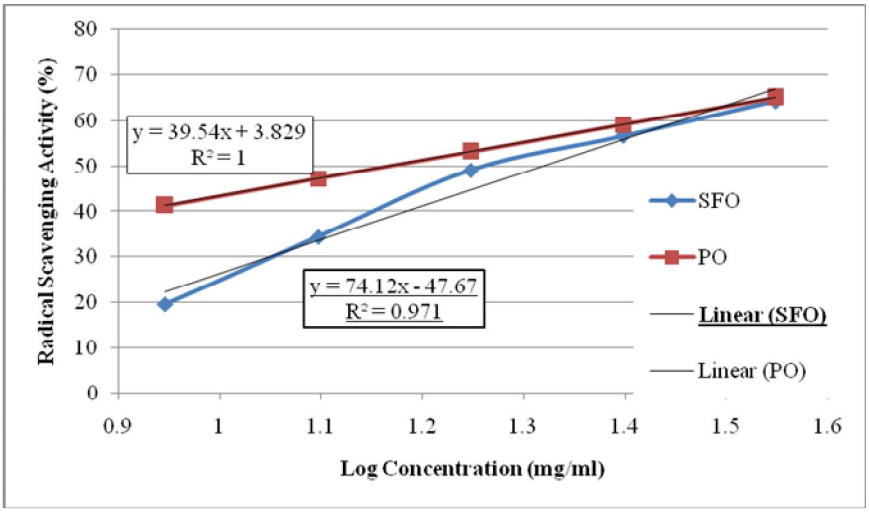

Fig. 1: DPPH radical-scavenging activity of different concentrations of PO and SFO

The initial PV of the SFO was 5.49±0.49 (mean± SEM) on day 1. Peroxide formation increased with an increase in the temperature. The PVs of all SFO treatments were less than that of the negative control group (P≤0.05), after 10 days under intensified oxidation conditions. The amount of the peroxide in treatment T5 was notably low after this period (Table 2). By the extension of the time to 20days, the PVs of the BHT group and the T3 treatment were similar to that of the negative control, while the PVs of treatments T4 and T5 decreased compared to that of the negative control group (P≤0.05). At the end of day 30 of SFO storage under accelerated oxidation conditions, although the PV decreased apparently, the PV of SFO supplemented with PO did not differ significantly from that of the control group (P≤0.05).

Table 2. Effects of different SFO treatments on its peroxide value (mean ± SEM) (meq/kg)

Treatments	Time (Day)		
	10	20	30
T1	57.490± 2.49^{a}	67.5± 2.49^{ab}	77.49± 2.49^{bc}
T2	46.99± 0.00^{b}	62.49± 2.49^{b}	84.99± 4.94^{b}
T3	44.49± 2.49^{bc}	72.99± 0.99^{a}	97.49± 2.49^{a}
T4	49.49± 0.49^{b}	53.99± 0.99^{c}	73.49± 1.49^{c}
T5	39.99± 0.00^{c}	52.99± 0.99^{c}	67.49± 2.49^{c}

T1: negative control (SFO); T2: positive control (SFO+ 0.02% BHT); T3: SFO+ 0.1% PO; T4: SFO+ 0.2% PO; T5: SFO+ 0.4% PO (w/w). a-c: The different letters in each column indicate a statistically significant difference (P≤0.05)

The TBARS index indicates the amount of malondialdehyde (MDA) in milligrams, present in one kilogram of a sample. In this study, the initial TBARS value of SFO was 11.75±0.04 ppm (mean ±SEM) on day 1. TBARS values of the SFO samples were measured at different concentrations of PO and BHT, as the positive control, and the SFO of the negative control was measured for 30 days at 60°C (Table 3).

Table 3. Effects of different SFO treatments on its TBARS (ppm) (mean ± SEM)

Treatments	Time (Day)		
	10	20	30
T1	14.8± 0.89[a]	15.85± 0.54[a]	12.75± 0.54
T2	12.35± 0.44[b]	11.8± 0.39[b]	12.4± 0.49
T3	12.35± 0.04[b]	14.9± 1.99[ab]	11.8± 0.79
T4	11.75± 0.04[b]	14.6± 0.49[ab]	13.1± 0.19
T5	12.5± 0.59[b]	12.4± 0.89[ab]	13.3± 0.49

T1: negative control (SFO); T2: positive control (SFO+ 0.02% BHT); T3: SFO+ 0.1% PO; T4: SFO+ 0.2% PO; T5: SFO+ 0.4% PO (w/w). a-b: The different letters in each column indicate a statistically significant difference (P≤0.05)

The TBARS values of all SFO treatments were less than that of the control group (P≤0.05), after 10 days of the accelerated oxidation. After 20 days, despite a decrease in the TBARS value of SFO treatments containing PO, only the reduction of the TBARS value in the BHT treatment (T2) was statistically significant (P≤0.05), compared to the negative control group. Therefore, a significant decrease in the TBARS value in the intensified oxidation conditions occurred only within the first 10 days of SFO storage supplemented with PO.

The overall oxidation status of the SFO containing PO and BHT was determined for 30 days at 60°C by obtaining the TOTOX value (Table 4). The TOTOX value of the SFO was 22.79±0.57 (mean± SEM), immediately after the oil extraction task. In the first 10 days of SFO storage under accelerated oxidation conditions, all treatments containing PO showed a lower TOTOX value than that of the negative control. During the mentioned period, the TOTOX value of the synthetic antioxidant, i.e. BHT, was also lower than that of the negative control (P≤0.05). By the progressive prolongation of the storage time to 20days, the TOTOX value of the SFO decreased in treatments T4 and T5 (containing PO) as well as the treatment T2, compared to the negative control group (P≤0.05). After 30 days, the TOTOX value of the group T5 was less than that of the negative control (P≤0.05).

The acid value was measured as an indicator of the SFO samples hydrolysis in the presence of different concentrations of PO for 30 days at accelerated oxidation conditions (Table 5). At first, the SFO had an acid value of 6.66±0.34 (mean± SEM). The reduction in acid value was observed only in the T5 group compared to the control group after 20 days. This decrease was not observed at day 30 of PO supplemented SFO evaluation (P≤0.05).

Table 4. Effects of different SFO treatments on its Totox value (mean± SEM).

Treatments	Time (Day)		
	10	20	30
T1	129.85± 2.92^{a}	150.85± 5.78^{b}	167.80± 2.9^{c}
T2	106.41± 0.26^{bc}	136.86± 2.89^{c}	182.41± 5.78^{b}
T3	101.38± 2.88^{c}	160.92± 1.62^{a}	206.86± 2.92^{a}
T4	110.79± 0.57^{b}	122.64± 1.19^{d}	160.14± 1.73^{c}
T5	92.55± 0.34^{d}	118.45± 1.26^{d}	148.35± 2.9^{d}

T1: negative control (SFO); T2: positive control (SFO+ 0.02% BHT); T3: SFO+ 0.1% PO; T4: SFO+ 0.2% PO; T5: SFO+ 0.4% PO (w/w). a-d: The different letters in each column indicate a statistically significant difference (P≤0.05).

Table 5. Effects of different SFO treatments on its acid value (mean± SEM)

Treatments	Time (Day)		
	10	20	30
T1	7.71± 0.7	10.51± 0.7^{a}	14.02± 0.00^{b}
T2	8.41± 0.00	9.81± 0.00^{a}	9.11± 0.7^{c}
T3	8.41± 0.00	9.11± 0.7^{a}	16.83± 0.00^{a}
T4	7.71± 0.7	8.41± 0.00^{a}	14.02± 0.00^{b}
T5	8.41± 0.00	6.30± 2.10^{b}	13.32± 0.7^{b}

T1: negative control (SFO); T2: positive control (SFO+ 0.02% BHT); T3: SFO+ 0.1% PO; T4: SFO+ 0.2% PO; T5: SFO+ 0.4% PO (w/w). a-c: The different letters in each column indicate a statistically significant difference (P≤0.05)

4- Discussion

SFO, like all other vegetable oils, contains antioxidant compounds, such as tocopherols and phospholipids [8, 30]. However, oxidation is one of the major reasons for the decrease in the SFO quality during storage [8].

PO has a high antioxidant capacity, so it is one of the 50 food products with the highest antioxidant potential [20, 31]. The presence of a wide range of antioxidant compounds in pistachio kernels results in the anti-radical activity of PO [32].

In this study, PO caused the in vitro 50% DPPH radical inhibition at a concentration of 14.79 mg/ml. In addition, the anti-radical effect of PO was higher than that of SFO. PO is regarded as one of the most stable nut oils for its antioxidant compounds [21, 31, 33-35].

The PO obtained using the screw press method, which is part of cold-pressing, has a higher content of polyphenols than the one obtained by other extraction methods [36].

In this study, the results demonstrated that the prevention of SFO oxidation using PO depended on some factors, such as the PO concentration and the duration of exposure to accelerated oxidation conditions. The oxidation stability of oils is usually evaluated under accelerated oxidation conditions, by considering the primary and secondary oxidation products [8]. Using the oil PV, the primary oxidation, and using the TBARS value, the secondary oxidation can be assessed [27, 37]. In advanced oxidation cases, high TBARS values are associated with the high content of fatty acids with three or more double bonds [38]. The TBARS value of the SFO does not increase highly during oxidation, due to the low content of these fatty acids. However, a little amount of SFO's linolenic acids leads to the formation of these compounds, hence the TBARS test has been used in some studies as a reliable method for evaluating SFO oxidation [27, 37, 39, 40]. Many studies have so far been done to delay the oxidation of edible oils, such as SFO by natural additives [13, 27, 37, 40, 41]. In this study, the application of PO as a natural antioxidant agent was investigated during the storage of SFO due to the lower risks associated with it than synthetic antioxidants.

It was demonstrated that the production of SFO peroxides increased after 9 days of accelerated storage [13]. In the evaluation of the first 10 days of SFO oxidation in the presence of all PO amounts, all oxidation monitoring assays showed a significant decrease in the oxidation rate. The presence of some compounds such as tocopherols (mainly γ-tocopherols and α- tocopherols) and phytosterols such as β-sitosterol in PO contributed to its antioxidant property [20, 42, 43].

The presence of benzoic acid derivatives, regarded as the main component of polyphenols, including gallic acid, protocatechuic acid, and 4-hydroxybenzoic acid also plays an important role in the antioxidant properties of PO [44, 45]. During the first 10 days, the reduction of peroxides in the mixture of SFO and PO (T5) was even higher than that of the treatment BHT as a synthetic antioxidant. The reductions in peroxide formation were detected at 0.2 and 0.4%concentrations of PO in SFO, even after 20 days. The decrease was not only observed in the PV, but also in the TOTOX value. The decreased amount of SFO peroxides by PO after 20 days, in contrast to the BHT positive control group, at the legal concentration (T2), will be promising if it is applied in controlling the accelerated oxidation. Besides, the TOTOX value of SFO reduced at the 0.4% concentration of PO after 30 days, yet it was not associated with the reduction of the peroxides. The results of the present study indicated the effect of PO in at least 20 days on inhibiting the oxidation of SFO. The increase in the oxidation rate in T3 (containing the lowest concentration of PO) after 30 days might have been due to the pro-oxidant properties of bioactive compounds in PO, including polyphenols (tables 2 and 4). It has been shown that at low concentrations, some of reducing antioxidants, such as polyphenols increase the oxidation rate by generating radicals through the Fenton reaction [46].

SFO has triglycerides containing high levels of polyunsaturated fatty acids. Pistachios, like many other plants, contain a series of diverse bioactive compounds, with

some of which inducing oxidation and others preventing it. Oxygenase and lipase are pistachio enzymes that intensify oxidative and hydrolytic deterioration [47]. In addition to the antioxidant and anti-inflammatory properties of PO, this oil can also contribute to the prevention of lipid hydrolysis, perhaps via enzymatic inhibition [48-50]. In this study, the highest concentration of PO (0.4%) reduced the acid value in SFO samples after 20 days. The reduction of the SFO's acid value by PO might have resulted from the SFO's hydrolytic rancidity suppression, with this having affected its oxidative deterioration. In contrast, the presence of active compounds such as lipase in pistachios and the role of heat in accelerated conditions are likely to have affected the hydrolysis of SFO triglycerides.

The increase in the acid value in SFO at the lowest concentration of PO (0.1%) might have occurred due to the effects of the mentioned factors, after 30 days.

5- Conclusion

In general, the results indicated that PO, as a natural antioxidant, inhibited the accelerated oxidation of SFO for 10 days, at all concentrations of 0.1, 0.2 and 0.4%. This potent natural antioxidant demonstrates a higher antioxidant activity at a concentration of 0.4% than the legal concentration of BHT as a synthetic antioxidant, after 10days. After 20 days, the 0.2 and 0.4%concentrations of PO reduced the accelerated oxidation of SFO. These findings imply that the use of PO can efficiently prevent the accelerated oxidation of SFO for at least 20 days. The results also showed that the 0.4%concentration of PO could inhibit and delay the hydrolytic rancidity of SFO for 20 days.

Conflict of interest

The authors declare no conflict of interest.

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