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ORIGINAL ARTICLE

Effects of pistachio green hull crude extract and its polyphenol fraction on oxidative stability of sunflower oil during accelerated storage

Farideh Zandi-Darehgharibi[1] · Hedayat Haddadi[1] · Mahmoud Rafieian-Kopaei[2] · Aziz A. Fallah[3]

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Abstract

Pistachio green hull (PGH) contains considerable amount of polyphenolic compounds, which are the most important natural antioxidants. In this research, the antioxidative activity of PGH crude extract and polyphenolic fraction, as the alternatives to synthetic antioxidants, were evaluated. Polyphenol fraction was isolated with Amberlite XAD16 resin from the crude extract. Effects of the crude extract, polyphenol fraction, and synthetic antioxidant (BHT) to inhibit the sunflower oil oxidation were studied under the heat accelerated storage (60 °C) for 16 days. Thermal stability was performed with Rancimat at 110 °C. The results showed that polyphenol fraction of PGH at 200 and 600 ppm had the same effects to retard oxidative processes in sunflower oil and even more potent than BHT and crude extract.

Keywords Oxidation · Antioxidant · Polyphenol fraction · Extract · Pistachio green hull

Abbreviations

AnV	Anisidine value
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
Control	Sunflower oil without antioxidant
DPPH	2, 2-Diphenyl-1-picrylhydrazyl
E200	Sunflower oil containing 200 ppm of PGH crude extract
E600	Sunflower oil containing 600 ppm of PGH crude extract
FFA	Free fatty acids
GAE/DW	Gallic acid equivalent per dry wheat
GRAS	Generally recognized as safe
IP	Induction period
MDA	Malondialdehyde
PF200	Sunflower oil containing 200 ppm of PGH polyphenol fraction
PF600	Sunflower oil containing 600 ppm of PGH polyphenol fraction
PGH	Pistachio green hull
PV	Peroxide value
TBA	2-Thiobarbituric acid
TBARS	Thiobarbituric acid reactive substances assay
TBHQ	Ter-butyl hydroquinone
TCA	Trichloroacetic acid
TPC	Total phenolic content

1 Introduction

Lipid peroxidation results from the reactivity of oxygen molecules with unsaturated fatty acids, which causes many problems in industrial food products and edible oils. The initial oxidation products (hydroxides), which are unstable compounds, convert to secondary oxidation products such as aldehydes, alkanes, acids, and alcohols. Many of these secondary oxidation products tend to react with themselves and may start the oxidative chain process. Consequently, oxidation processes reduce the quality and shelf-life of oils and fatty foods [1, 2].

Antioxidants can retard the oxidative processes and increase the shelf-life of foodstuffs [3]. Synthetic

✉ Mahmoud Rafieian-Kopaei
rafieian@Yahoo.com

[1] Department of Chemistry, Faculty of Basic Sciences, Shahrekord University, Shahrekord, Iran

[2] Medical Plants Research Center, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Shahrekord, Iran

[3] Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran

antioxidants such as TBHQ, BHA, and BHT are widely used to protect foodstuffs against oxidation but have several adverse side effects, including carcinogenicity [1, 4–9]. The TBHQ, as the most potent synthetic antioxidant, is not authorized for use in foodstuffs in several countries such as Japan, Canada, and Europe; moreover, BHA has been removed from the official list of GRAS [5]. Therefore, the need is evident to identify natural and safe antioxidants to protect foodstuffs from oxidation processes.

Recently, the use of natural antioxidants derived from plants, such as tocopherols, flavonoids, and phenolic acids to enhance the shelf-life of foodstuffs, has been increased. Natural antioxidants can react with free radicals and chelate the catalytic metals; therefore, they have a prominent role in prevention of the oxidation processes [3]. Recently, there has been a great desire to add polyphenols to foodstuffs and biological systems due to their well-known abilities in scavenging free radicals [3]. In recent years, several studies have been reported the application of plant crude extracts to control oxidative degradation and increase the shelf-life of edible oils [5–7, 9].

Iran is a leading pistachio producer and the world's largest pistachio exporter [10]. The green hull of pistachios has been proven to have high levels of polyphenolic compounds [3]. Pistachio production is associated with the production of lusts that is mainly pistachio green hull; therefore, tens of thousands of tons of pistachio seeds annually are created in the country as agricultural waste, which can be a valuable source for the preparation of polyphenolic compounds as a natural antioxidant [11]. This study aimed to isolate the polyphenolic fraction of pistachio green hull and compare its antioxidative activity with the crude extract and synthetic antioxidant (BHT) in sunflower oil.

2 Materials and methods

2.1 Plant and materials

PGH was obtained from the village of Nouq, north of Rafsanjan, Iran. The green hull of the plant was dried at room temperature without light and moisture and then powdered by the mill. The particle size was 0.595 mm. A portion of PGH powder (100 g) was mixed with distilled water (800 ml) inside a glass jar, left for 72 h at room temperature, and then flattened with a cloth. The mixture was stirred regularly by hand every day [11].

2.2 Chemicals and reagents

Folin-Ciocalteu's phenol reagent, 2, 2-diphenyl-1-picrylhydrazyl (DPPH˙), gallic acid (97.5–102.5% (titration)), and Iron (III) chloride (≥ 98%) were obtained from Merck Chemicals. Amberlite XAD16 resin was obtained from Sigma, and other chemicals and solvents were purchased from Lobachemi (Tehran, Iran).

2.3 Identification of phenolic compounds

A portion of the crude extract or polyphenol fraction (2 ml) was placed in the test tube, and $FeCl_3$ (3%, w/v) was added drop by drop. The development of bluish-black precipitate demonstrates the presence of polyphenolic compounds [12].

2.4 Isolation of polyphenol fraction with polymeric resin XAD16

To isolate polyphenol fraction, the polymeric resin XAD16 was used. The resin bed's surface, where the absorption of molecules occurs, was activated by methanol. The resin was placed in water overnight, and then 250 g of resin was packed in a glass column (50 cm length × 5 cm ID) [8]. A 15 ml of pistachio crude extract was injected into the column in separate cycles. Waste compounds, including pectins, salts, and sugars, do not absorb the column. The polyphenol fraction, through the interactions of the π-π groups of phenol, is absorbed by the aromatic rings of the stationary phase. Pectins, salts, and sugars were removed with 500 ml of distilled water, and then the polyphenolic compounds were eluted with 500 ml of ethanol. Then, polyphenol fraction was concentrated and dried under reduced pressure at 40 °C.

2.5 Determination of TPC

The Folin-Ciocalteu method was used to determine the total phenol content of crude extract and polyphenol fraction of PGH [13]. A portion of dried crude extract or polyphenol fraction (0.01 g) was dissolved in 60% methanol and then brought to 10 ml. Afterwards, 2 ml of above solution (10 mg/ml) was added to 0.5 ml of 10% Folin-Ciocalteu reagent, kept for 3–8 min at room temperature, and then mixed with 0.4 ml of sodium carbonate solution (7.5%, w/v). The samples were left in the darkness for 30 min, and the absorbance was determined by UV–Vis spectrophotometer at 765 nm. Gallic acid was used as the standard, and the data were calculated as mg GAE/g DW.

2.6 Determination of DPPH radical scavenging activity

The antioxidant power of PGH crude extract and polyphenol fraction was determined by the method described by Moein et al. [13] with some modifications. Two milliliters of a fresh solution of DPPH free radical (0.1 mM in methanol) and 2 ml of crude extract or polyphenol fraction were mixed and

placed for 15 min at ambient temperature. The DPPH radical scavenging activity was determined at 517 nm by a UV–Vis spectrophotometer. The antioxidant activity was calculated by the following formula:

$$100 - [\frac{(A)sample}{(A)control} \times 100]$$

where A is the absorbance of sample and control (DPPH).

2.7 Preparation of sunflower oil samples

The concentrations of 200 and 600 ppm of PGH aqueous extract or isolated polyphenol fraction were dissolved in 200 µl distilled water and added to virgin sunflower oil. Synthetic antioxidant (BHT) was added to virgin sunflower oil at the maximum permissible level (200 ppm). A portion of distilled water (200 µl) was added to virgin sunflower oil as a control sample. All sunflower oil samples were placed in dark brown bottles and incubated in an oven at 60 °C to accelerate the oxidation process. Progression of sunflower oil oxidation was studied using PV, FFA, TBARS, and AnV, on days 0, 4, 8, 12, and 16 of storage. The Rancimat test was performed at 110 °C after the samples' preparation.

2.7.1 Analyses of PV

The peroxide value of the sunflower oil samples was determined according to GB/T 5009.37–2003 with slight modifications [9]. A portion of sample (2 g) was added to 30 ml of chloroform-glacial acetic acid (3:2, v/v) and completely dissolved. Then, 1 ml of potassium iodide saturation solution (KI) was added; the mixture was shaken for 1 min, and kept in darkness for 5 min. Afterwards, 75 ml of distilled water and 0.5 ml of 1% starch solution (as an indicator) were added. The mixture was stirred and titrated with thiosulfate sodium 0.002 M. A blank was also prepared and analyzed under the same condition. The PV (meq O_2/kg oil) was calculated with the following formula:

$$PV = (C \times (V - V_k) \times 12.69 \times 78.8)/m$$

where, C: thiosulfate sodium concentration (M), V: volume of thiosulfate sodium for sample (mL), V_k : volume of sodium thiosulfate for blank(mL) and, m: the mass of the sample (g).

2.7.2 TBARS assay

The TBARS assay, which determines the secondary lipid oxidation products, was performed according to the method of Okuda et al. [14] with a slight modification. Oil sample (1 ml) was mixed with 2 ml of TCA-TBA aqueous solution (15% w/v TCA and 20 mM TBA). The above mixture was placed in a water bath at 96 °C for 30 min. Afterwards, it was placed in a bath containing cold water for 30 min and centrifuged at 4000 g for 20 min. The control sample was prepared with 1 ml of distilled water under the same condition. The absorbance of the sample was read at 532 nm by a spectrophotometer against the control. TBARS value was expressed as mg MDA equivalent per kg oil (mg MDA/kg oil).

2.7.3 Measurement of FFA

Free fatty acids were measured as the percentage of oleic acid in the oil samples by the alkaline titration according to GB/T500937–2003 method [9]. A portion of sunflower oil (2 g) and a mixture of 50 ml neutral ether:ethanol (2:1, v/v) were appropriately stirred on a magnetic stirrer, and then few drops of phenolphthalein solution (10 g/l) were added as an indicator. Afterwards, the mixed solution was titrated with potassium hydroxide (0.005 M) until the appearance of purple color. The amount of free fatty acids (mg/g) was calculated by the following formula:

$$FFA = (V \times C \times 56.11)/m$$

where, V: hydroxide potassium volume (mL) for samples, C: potassium hydroxide concentration (M) and, m: sunflower oil sample mass (g).

2.7.4 Measurement of AnV

The AnV was measured according to method 2.504 of IUPAC [9]. A portion of sunflower oil (2 g) was dissolved in 25 ml of isooctane, and the absorbance of this solution was determined at 350 nm by UV–Vis spectrophotometer. The mixture (5 mL) was added to 1 mL of 0.25% para-anisidine in acetic acid (w/v), kept in a dark place for 10 min, and the absorbance was measured at 350 nm. The anisidine value was calculated by the following formula:

$$AnV = (25 \times (1.2A_s - A_b))/m$$

where,

A_s: sample solution absorbance after reacted with the reagent of p-anisidine.

A_b: sample solution absorbance.

m: sunflower oil mass (g).

2.7.5 Determination of IP

The thermal stability of oil samples containing synthetic and natural antioxidants was measured with the 743 Metrohm Rancimat [15]. Oxidation acceleration conditions in oil samples (5 g) were carried out using airflow velocity at 20 l/h at 110 °C. Conduction of water-filled cells was continuously controlled to detect conduction changes. The IP in terms of

Table 1 Total phenol content of the crude extract and polyphenol fraction of PGH in milligram of gallic acid per gram dry weight (mg GAE/g DW)

Treatment	Total phenol content
PGH crude extract	$109.00 \pm 0.45^{a*}$
PGH polyphenol fraction	213.00 ± 1.50^{b}

*Non-similar letters in the column indicate a significant difference between the treatments ($p \leq 0.05$). The data represent the mean ± standard deviation ($n = 3$)

Table 2 The antioxidant activity of the crude extract and the polyphenol fraction of PGH in micrograms per milliliter (μg/mL)

Treatment	IC_{50}
BHT	$189.81 \pm 12.92^{a*}$
PGH crude extract	25.30 ± 2.50^{b}
PGH polyphenol fraction	10.20 ± 0.40^{b}

*Non-similar letters in the column indicate a significant difference between the treatments ($p \leq 0.05$). The data represent the mean ± standard deviation ($n = 3$)

hours for oil samples was automatically recorded as a suitable endpoint for the oxidative stability estimation.

2.8 Statistical analyzes

SPSS software was used for data analysis. The data were reported as mean ± standard deviation (SD) of 3 replicates. One-way ANOVA and Tukey's complement tests were used to compare the means. The $p \leq 0.05$ was considered statistically significant. The Pearson's linear correlation coefficient was employed to assess the relationship between oxidative indexes.

3 Results and discussion

3.1 TPC and antioxidant activity of crude extract and polyphenol fraction of PGH

Phenolic compounds are the most important components of the plants with the ability to quench free radicals by their hydroxyl groups. Therefore, the plant's antioxidative activity can be related to their polyphenolic compounds [9]. The total phenol content of crude extract and polyphenol fraction of PGH is shown in Table 1. The total phenol content was significantly higher in polyphenol fraction of PHG than its crude extract. Several compounds such as pectin, polyphenols, tocopherols, dietary fibers, essential oils, and unsaturated fatty acids are present in the crude extract of PGH [16]. In this research, the polyphenolic compounds were isolated from the other components; therefore, this process concentrates the polyphenols in the fraction and increases its total phenol content.

The DPPH test is the most commonly used method to evaluate the antioxidative activity and determines the compound's ability to transfer hydrogen atoms to the radicals. This transfer leads to a white band that has a maximum absorption around 515–528 nm. The amount of antioxidants required to reduce 50% of the initial concentration of DPPH radicals is determined and indicated by IC_{50} [17]. The antioxidative power of the crude extract and the polyphenol fraction of PGH and BHT were 25.30, 10.20, and 189.81 μg/ml, respectively (Table 2). There was no significant difference between IC_{50} of the crude extract and polyphenol fraction of PGH, but the IC_{50} of them was significantly lower than IC_{50} of synthetic antioxidant (BHT). In other words, a smaller amount of polyphenol fraction can inhibit 50% of the DPPH radicals compared to the BHT and PGH crude extract. Phenolic compounds are considered strong antioxidants due to their radical scavenging activity. This activity is attributed to their ability to donate hydrogen [16]. A negative significant correlation ($r = -1.000$, $p < 0.01$) was found between the TPC of the PGH crude extract and its IC_{50} and TPC of polyphenol fraction and its IC_{50}.

In other studies, different amounts of total phenol content and IC_{50} have been reported for pistachio green hull extract. Roostaee et al. [18] reported that total phenol content and IC_{50} for pistachio green hull extract were 614.91 ± 0.45 mg GAE/g and 10 ± 0.05 μg/mL. In another study, total phenol content and IC_{50} for the green hull extract were 49.32 mg GAE/g and 2.53 μg/mL, respectively [19]. The variations depend on the species, extraction method (the type of solvent, time, and temperature), geographical location, and agriculture condition [18, 20].

3.2 Effect on PV

In the PV test, the amount of the initial products of lipid oxidation was measured [9, 21]. The oxidation rate of sunflower oil was evaluated by determining the peroxide value in the presence and absence of natural and synthetic antioxidants at 60 °C for 16 days (Fig. 1a). The results indicated that the PV increased with the storage period in all groups and accelerated from the fourth day. On the 16th day of storage, the control sample reached the highest amount of PV.

According to Fig. 1a, PV in all treated groups was significantly lower than the control group from the 4^{th} to 16^{th} days. On the 16^{th} day, the PV of PF200 was lower compared to the PV of E200 and BHT, but the difference was not statistically significant. The PV of E600 was significantly lower than the PV of BHT on the 8^{th}, 12^{th}, and 16^{th} days; and also, the peroxide value of PF600 was significantly lower than the PV of the other groups on these days.

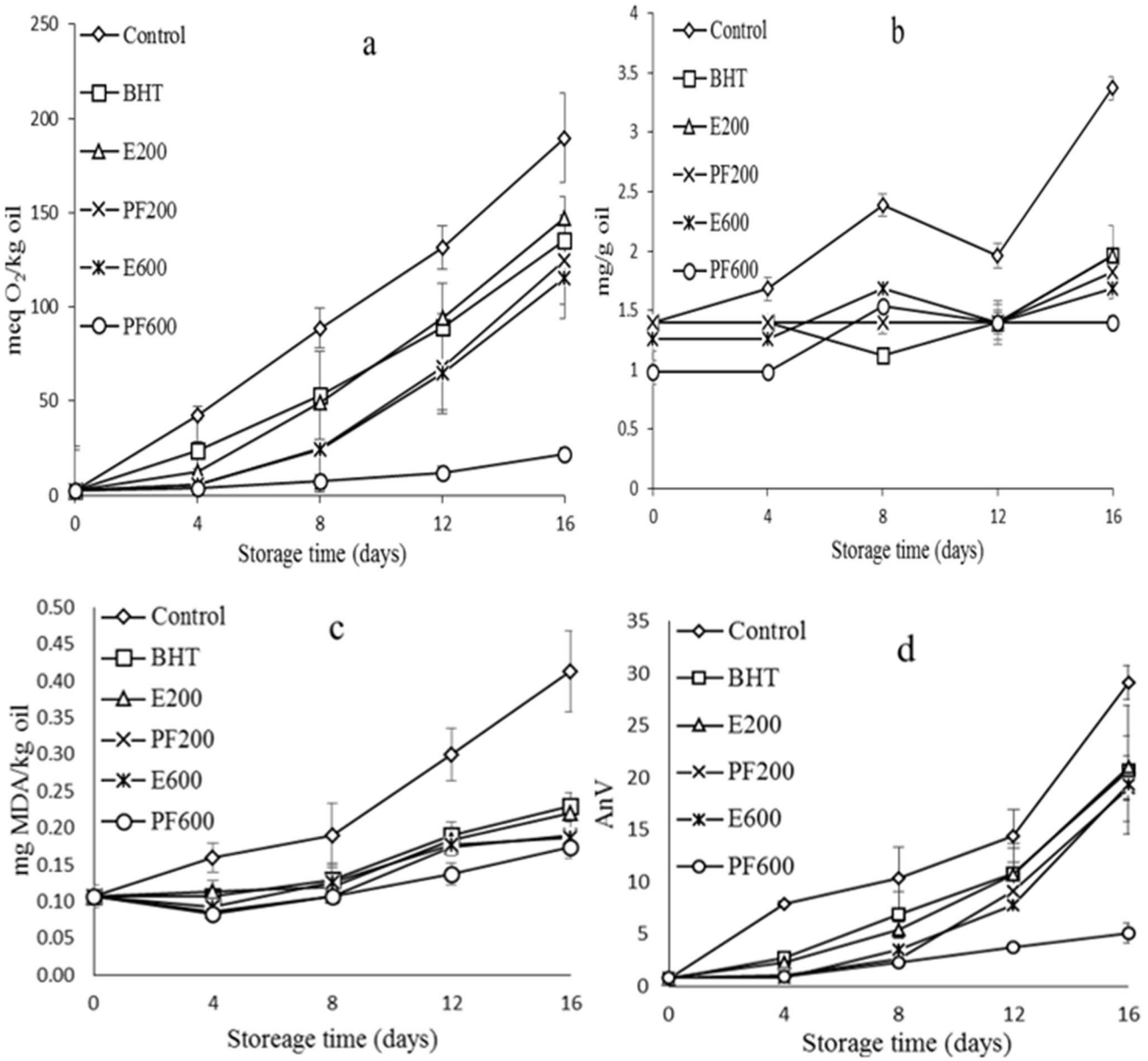

Fig. 1 Influence of adding antioxidants on (**a:** PV, **b:** FFA, **c:** TBARS, **d:** AnV) of sunflower oil under accelerated storage at 60 °C for 16 days

The result of PGH crude extract is in accordance with the findings of Goli et al. [3]. They found that the peroxide value of the soybean oil containing BHT (200 ppm) was comparable to the oil sample containing pistachio green hull extract (200 ppm) on the 15th day (Last day of storage). Also, the finding of the PGH crude extract of this research is in accordance with the findings of Roostaei et al. [18]. Their results showed that 500 ppm of pistachio green hull extract was more effective than BHT to delay the oxidative process of soybean oil. Significant positive correlation was found between PV and FFA, TBARS, and AnV ($p < 0.01$); and the Pearson's linear correlation coefficients were $r = 0.758$, $r = 0.901$, and $r = 0.975$, respectively. Significant negative correlation was found between PV and IP ($r = -0.432$; $p < 0.05$). The significant positive correlations exist between PV and TBARS, and between PV and AnV may be due to the instability of hydroperoxides and rapid conversion of these oxidation products to secondary oxidation products [22].

These findings show that the process of oxidation is slowed down by increasing the concentration of the crude extract and polyphenol fraction. Also, these findings showed that the polyphenol fraction is more effective in delaying the oxidative process than the same concentration of the crude extract. Several compounds such as pectin, polyphenols, tocopherols, dietary fibers, essential oils, and

unsaturated fatty acids are present in the crude extract of PGH [16]. In this research, the polyphenolic compounds were isolated from the other components; therefore, this process concentrates the polyphenols in the fraction. Polyphenolic compounds have strong antioxidative activity; therefore, they are more effective than the crude extract to retard the oxidation processes in the oil.

3.3 Effect on FFA

The FFA presence may be the main criterion for fats rancidity. FFA is a product of the hydrolyzes of the triglycerides and increases with moisture [9]. The oil samples FFA were measured in the presence and absence of natural and synthetic antioxidants at 60 °C for 16 days (Fig. 1b). These findings indicate that addition of crude extract and polyphenol fraction of PGH leads to a decrease in the FFA of oil samples. The content of FFA was increased from day zero to 16th day in all groups, and the control group reached the highest level. The FFA content of treated groups was significantly lower than the control group during the storage period. Moreover, the FFA content in PF200, E200, and BHT did not show significant difference during the storage, but the FFA content of E600 and PF600 groups was significantly lower than the aforementioned groups on the last day of storage. However, on 16th day the FFA of PF600 was lower than the FFA of E600 but, no significant difference was observed between them. These results indicate that by increasing the concentration of polyphenol fraction and crude extract of PGH, the process of free fatty acids production was decreased. The PGH polyphenol fraction was more effective than the crude extract and BHT to prevent the hydrolyzes of triglycerides and the formation of the FFA. There was a significant positive correlation between FFA and PV, TBARS, and AnV ($r = 0.758$, $r = 0.855$, and $r = 0.778$; respectively; $p < 0.01$). A significant negative correlation was found between FFA and IP (r =—0.556; $p < 0.01$).

3.4 Effect on TBARS

The primary oxidation products (hydroperoxides) are unstable and convert to secondary oxidation products, which may cause the bad smell of oxidized oils [23, 24]. The effect of crude extracts and polyphenol fractions of PGH and synthetic antioxidants during the storage period at 60 °C on the TBARS level of sunflower oil are shown in Fig. 1c. On day 0, no significant difference was found among the TBARS levels of all groups. The TBARS index in all groups increased linearly from day the 4th to 16th days, and the control group reached the highest amount of TBARS. TBARS level of treated groups was significantly lower than the control group from the 4th to 16th days. However, no significant difference was observed between samples of sunflower oil containing crude extract, polyphenol fraction, and BHT during the storage time. The effect of crude extract is in accordance with the previous study that found no significant difference in the TBARS levels among the various concentrations (200–600 ppm) of PGH extract and BHT (200 ppm) in soybean oil [3].

According to Fig. 1c, with an increase in the concentration of PGH polyphenol fraction, the amount of malondialdehyde formation decreased. Therefore, phenolic compounds can be a good alternative to synthetic antioxidants such as BHT. A significant positive correlation was found between TBARS index and PV, FFA, and AnV ($r = 0.901$, $r = 0.855$, and $r = 0.889$, respectively), while a negative significant correlation was found between TBARS and IP (r =—0.473; $p < 0.01$).

3.5 Effect on AnV

AnV expresses the secondary oxidation products such as aldehydes and ketones. This test's basis is the reactivity of the carbonyl aldehyde transplantation in the amine group of p-anisidine, which leads to the production of a Schiff base [9].

$$R = CH = O + H2\ N - \Phi - OMe \rightarrow R - CH = N - \Phi - OMe + H2O$$

The results for the AnV of sunflower oil in the presence and absence of natural and synthetic antioxidants during storage at 60 °C demonstrate in Fig. 1d. There was no significant difference between the initial AnV of all groups. The AnV of all groups increased during storage time and the control group had the highest AnV on the 16th day.

From the 4th to 16th days, the AnV of all treated groups was significantly lower than the control group. During the storage time, the AnV of PF200, E200, E600, and BHT did not show significant differences. However, the AnV of PF600 was significantly lower than the AnV of the other groups on the 8th, 12th, and 16th days. These results indicate that the higher concentration of PGH polyphenol fraction is more effective to slow down the increasing rate of AnV of sunflower oil. Significant positive correlation was found between AnV and PV, FFA, and TBARS ($r = 0.975$, $r = 0.778$, and $r = 0.889$, respectively; $p < 0.01$). However, no significant correlation was found between AnV and IP (r = -0.359; $p < 0.05$).

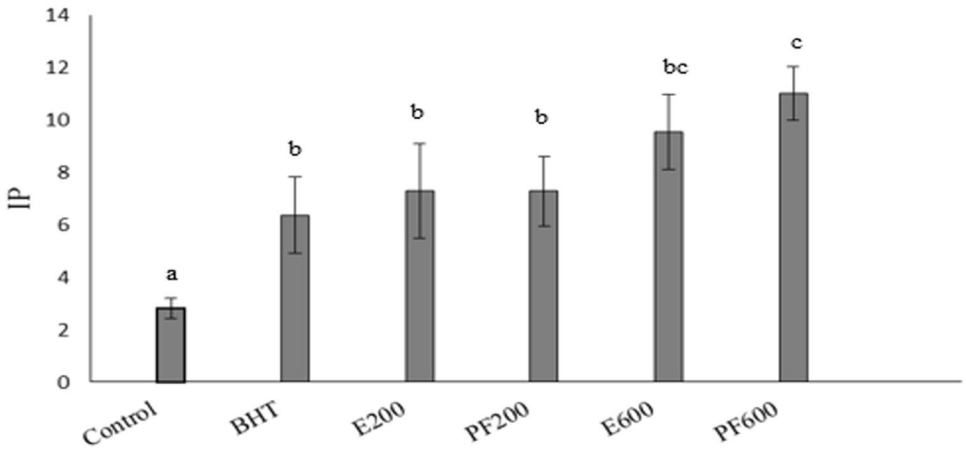

Fig. 2 Influence of adding antioxidants on induction period (IP) sunflower oil at 110 °C. The dissimilar letters (**a**), (**b**), and (**c**) indicate a significant difference between different samples ($p < 0.05$)

3.6 Effect on IP

The antioxidative power of the control and treated groups containing natural (aqueous extract and polyphenol fraction of PGH) and synthetic (BHT) antioxidants was measured at 110 °C with the Rancimat method (Fig. 2). The time required for the formation of volatile acids, which causes a sudden increase in conductivity, is called the induction period or thermal stability of the oil. Addition of antioxidants to virgin oil increases the induction period [25]. The stability time of all treated groups containing antioxidants was significantly higher than the control group (2.82 h). In other words, synthetic antioxidants, crude extract, and polyphenol fraction of PGH delayed the rancidity of sunflower oil.

The stability time of E200, E600, and PF200 was more than BHT, but no significant difference was found among them. Although the IP of PF600 (11 h) was higher than the IP of E600 (9.53 h), no significant difference was observed between them. However, the IP of PF600 was significantly higher than the stability time of the other groups. The result indicates a positive effect of increasing concentration of PGH polyphenol fraction to prevent the rancidity of sunflower oil. Moreover, the polyphenol fraction of PGH is more effective than PGH crude extract and BHT to prevent the rancidity of sunflower oil.

4 Conclusion

In this study, polyphenolic fraction was purified from crude aqueous extract of pistachio green hull using XAD_{16}. The PGH polyphenolic fraction had a higher total phenol content and better antioxidant properties than the PGH crude extract. Both PGH crude extract and polyphenolic fraction of PGH slowed down the lipid oxidation process in sunflower oil during accelerated storage condition. PGH polyphenolic fraction showed better performance in slowing down the oxidation process than crude extract and BHT. Therefore, the polyphenolic fraction of PGH can be used as a natural antioxidant replacement for synthetic antioxidants in edible oils.

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Author contribution Farideh Zandi-Darehgharibi performed experiments and collected laboratory data; prepared the original draft; and reviewed, edited, and revised the paper. Hedayat Haddadi provided project idea, project guidance, and laboratory equipment. Mahmoud Rafieian-Kopaei provided project guidance, laboratory materials, and edited manuscript. Aziz A. Fallah consulted on the project and revised the paper.

Declarations

Conflict of interest The authors declare no competing interests.

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