CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: **http://chimie-biologie.ubm.ro/carpathian_journal/index.html**

EFFECTS OF POMEGRANATE (*PUNICA GRANATUM L.*) FRUIT AND RIND EXTRACTS ON PHYSICO-CHEMICAL, COLOUR, AND OXIDATIVE STABILITY OF RAINBOW TROUT FILLET

Ali Salehi[1], Gholamreza Jahed Khaniki[1]✉, Nabi Shariatifar[1], Parisa Sadighara[1], Mahmood Alimohammadi[1], Arash Akbarzadeh[2]

[1] *Division of Food Safety and Hygiene, Department of Environmental Health Engineering, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*
[2]*Department of Biostatistics and Epidemiology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*
✉*ghjahed@sina.tums.ac.ir*

https://doi.org/10.34302/crpjfst/2022.14.2.5

Article history:
Received:
18 July 2021
Accepted:
10 April 2022

Keywords:
Pomegranate extracts;
Fish;
Shelf life;
Chemical quality;
Oxidative Stability.

ABSTRACT

Colour changes, oxidation of fat and physicochemical status of rainbow trout fillet were examined after adding water extracts of pomegranate rind (WEPR), ethanolic extracts of pomegranate rind (EEPR), water extracts of pomegranate fruit (WEPF), and ethanolic extracts of pomegranate fruit (EEPF) during four days of refrigerated aerobic storage. These extracts were added in a concentration of 0.01%. Results unveiled that the WEPR group had the highest total phenolic compounds amount and anti-radical activity. However, pH values for the extract treatments did not show a meaningful difference. Analysis of variance of colors showed a remarkable difference ($p<0.05$) about the effects of extracts and storage time. The values of Lightness for both control and EEPF sample at day 0 higher than the other samples. At the end of storage time, total volatile base nitrogen (TVB-N), peroxide value (PV) and thiobarbituric acid reactive substances (TBARS) values of the control sample were significantly ($p<0.05$) higher than those of the treated fillets with pomegranate extracts. Overall acceptability scores of water extracts of pomegranate fruit and rind treated fillets were higher than those of ethanolic extracts of pomegranate samples. The results indicated that pomegranate extracts can retard fish spoilage and they may be beneficial as natural antioxidant sources in minimizing the physicochemical changes of fish products during cold storage.

1. Introduction

Fish preservation is a key factor for increasing the shelf life and conserving nutritional value, texture, and flavor which prevents spoilage without affecting the quality (Lakshmanan et al., 2003). Spoilage is the post-harvest change and spoiled fish is typically observed as the change in physical features such as colour, odour, texture, eyes, gills and softness of muscle (Barbosa-Pereira et al., 2013). The process of spoilage of fish shows high complexity in which enzymes, bacteria and chemical components are involved and begins rapidly after the death of fish (Ghaly et al., 2010). The maintenance of freshness and quality of fish fillet as a safe food is important.

Chemical changes can occur in fish fillets during storage. Chemical changes such as lipid oxidation and auto-oxidation are leading culprits for deterioration of the quality of seafood and can reduce its shelf life (Secci et al., 2016). Lipid oxidation may lead to changes in seafood quality-related factors such as color, off-flavor, rancidity, odor, texture, and also nutritional quality (Annamalai et al., 2015). Fresh fish and its products undergoing oxidative changes are tremendously vulnerable products owing to their biological components (Yilmaz et al., 2009). Synthetic antioxidants like butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and tertiary butylhydroquinone (TBHQ) have been successfully utilized to prevent lipid oxidation in fish products (Gai et al., 2014). But, these synthetic antioxidants have possible health effects and toxicity (Devatkal et al., 2010). Therefore, to resolve this problem, natural antioxidants in fish products such as cinnamon (Ojaghi et al., 2010) curry, mint (Biswas et al., 2012), oregano, rosemary (Makri, 2013), thyme, clove (Guran et al., 2015) and green tea (Yerlijaya and Gokoglu, 2010) have been used. Also, recently, Chan-Higuera et al. identified the skin extract of a mollusk called Dosidicus gigas as an antioxidant in Tuna Pâté (Chan-Higuera et al., 2019). However, these products are not as effective as synthetic antioxidants (Qin et al., 2013). Consequently, careful attention has been paid to antioxidants from inexpensive or residual sources in agriculture industries, such as apple peel (Wolfe et al., 2003), peach peel (Rossato et al., 2009), onion peel (Shim et al., 2012) and bamboo leaves (Wenjiao et al., 2013). In a literature review, Pezeshk et al. (2015) discussed the role of some natural antioxidants and anti-bacterial in sustaining the quality and increasing the shelf life of some seafood products.

Pomegranate (*Punica granatum L.*) is cultivated in many tropical and subtropical countries (Mousavinejad et al., 2009). These fruit comrises three parts: seed, juice, and peel (about 30% of the fruit weight). Pomegranate rind is not edible and is obtained upon processing of pomegranate juice. Pomegranate rind and juice are shown to possess considerable antioxidant activities due to tannins and other phenolic compounds (Devatkal et al., 2010). Currently, pomegranate juice, rind powder, and seed powder utilization in chicken, goat, fish patties and pork meat products as sources rich in natural antioxidants has been investigated (Devatkal et al., 2010; Naveena et al., 2008; Qin et al., 2013; Martínez, L et al., 2019). Because there is relatively significant level of polyunsaturated fatty acids (PUFA) in fat and filet of trout fish, oxidation can take place in a higher speed in trout fish in comparison with chicken, goat, or pork. Trout fish filet susceptibility to lipid oxidation is found to be higher than that of other meat products (Gai et al., 2014). As a result, lipid oxidation in trout fish filet must be postponed by adding antioxidants. This study focused on determination of the effectiveness of pomegranate rind and fruit extracts on the physicochemical quality of trout fish filet as measured by pH, total phenolic content, DPPH radical scavenging activity, thiobarbituric acid reactive substances (TBARS), peroxide value (PV), color, total volatile base nitrogen (TVB-N) value, and sensory evaluation in the course of storage at refrigerator temperature.

2. Materials and Methods

2.1. Sample collection

Samples of fresh pomegranate (Punica granatum L.) were purchased from a retail fruit market. The fresh rainbow trout fish samples were purchased from a fishmonger shop and the fillet was removed. The fillets were transferred to the chemistry laboratory and kept at refrigerator temperature until use.

2.2. Preparation of pomegranate rind and fruit extracts

Pomegranate rind (peel) powder was prepared based on a method by Devatkal et al.

(2010). Pomegranates were washed peeled off, and desiccated via air circulatory tray drier at 60°C and duration of 48 h. mixer grinder was used to powder the dried pomegranate peel, followed by sieving by a sieve no. 10 (1.65 mm). Then the dried product was stored at room temperature within high-density polyethylene bags. Similarly powder from pomegranate seeds was prepared by drying the pomegranate fruit seeds in a tray drier and grinding by mixer grinder and sieving by a sieve no. 10 (1.65 mm).

2.3. Ethanolic extract of pomegranate rind (EEPR)

An Ethanolic extract of pomegranate rind powder was prepared with respect to the method by Qin et al. (2013). Briefly, the extraction of 10 g powdered rind of pomegranate was done via adding 100 ml of 80% ethanol in a shaking incubator at 40°C for 24 h. The solutions were passed through the Whatman cellulose filter papers (circles and with a diameter of 110 mm), followed by vacuum evaporation using a rotary evaporator (IKA RV 10 digital). After that, some of dried pomegranate rind powder solved in ethanol with the total volume reaching to 100 ml by adding distilled water. The mixture was then maintained at 4°C until use.

2.4. Water extract of pomegranate rind (WEPR)

Preparation of the Water extract of pomegranate rind powder was performed in accordance with the method suggested by Kamkar et al. (2013), and with the use of a percolator. In this way, the extraction of pomegranate rind powder was achieved by adding distilled water in a percolator apparatus until becoming colorless. Then, the crude extract was passed through the filter and dried in a vacuum.

2.5. Ethanolic extract of pomegranate fruit (EEPF) and water extract of pomegranate fruit (WEPF)

Similarly, ethanolic and aqueous extracts of pomegranate fruit were extracted with 80% ethanol. The freshly prepared extracts (EEPR, EEPF, WEPR, and WEPF) were stored at 4°C until use (for up to 24 h).

2.6. Preparing trout fillet treatments

The rout fillet samples were split equally. After mincing, the trout fillet samples were divided into batches (100 g each), followed by their assignment to the following five groups: control (filet with no antioxidant); WEPF (10 mg WEPF per 100 g fillet); WEPR (10 mg WEPR per 100 g fillet); EEPF (10 mg EEPF per 100 g fillet); and EEPR (10 mg EEPR per 100 g fillet). The fillet samples were formed into 100 gr patties with 10 mg extract. They were smeared with 10 mg extract at aseptic conditions and then gathered in low-density polyethylene bags in the presence of air, and stored at 2-4°C for 4 days. Afterward, analyses were performed every two days (0, 2 and 4).

2.7. pH evaluation

pH of the trout fillet sample was determined with the use of a pH meter (Kent, EIL7020, Kent Industrial Measurement Limited, Surrey, England), using 5 g of the sample blended with 20 ml distilled water. Average of triplicates was reported for each treatment.

2.8. Estimation of total phenolic content

Total phenolic content was evaluated through the Folin-Ciocalteu (F-C) assay (Negi et al, 2003). Diluting 100 µL aliquot of extracts (various concentrations) was performed by adding 5 ml distilled water and 100 µL 10-fold-diluted Folin-Ciocalteu reagent. Following 5 min incubation, 300 µL of 2% sodium carbonate was added. Then, the absorbance was read at 760nm by a UV–VIS spectrophotometer (DR 5000™ UV-Vis Spectrophotometer). Tannic

acid was used as a standard sample. Results were expressed as mg/L Tannic acid equivalents.

2.9. DPPH radical scavenging activity

The scavenging effects of WEPR, WEPF, EEPR, and EEPF against DPPH radicals were measured with respect to the method by Koch et al. (2017). A mixture of 50 µL extracts (various concentrations) + 5 ml DPPH solution (0.004% methanol solution) was prepared and incubated for 30min at room temperature. UV–VIS spectrophotometer was used to measure the absorbance at 517nm. The DPPH radical scavenging activity was estimated by the following equation: Scavenging activity (%) = (Absorbance control-Absorbance sample /Absorbance control) × 100

2.10. Determining peroxide value (PV)

The extraction of Lipid from the trout fillet samples was achieved using the method by Folch et al. (1957). A mixture of extracted lipid and 10 ml chloroform-methanol (7:3 v/v) was prepared in a screw-capped test tube, followed by vortexing for 10-15s. The lipid extracts were evaporated by a rotary evaporator. Then, the PV was analyzed for the 5 g sample of recovered lipids and assessed through the measurement of the iodine released from potassium iodide whose titration was prepared in a standardized 0.01 N sodium thiosulfate solution. The PVs were expressed as the mEq of O2 per kg and calculated as:

Peroxide value (mEq/kg) = 100×(S1 – S2) × N/W, where S1 is consumption volume of sodium thiosulfate of the sample, S2 is consumption volume of sodium thiosulfate of blank, N is normalized sodium thiosulfate (0.01), and W is sample weight.

2.11. Value of thiobarbituric acid reactive substances (TBARS)

TBARS value of the trout fillet sample was determined in accordance with the approach developed by Yousef et al. (2009), with slight modifications. Briefly, Samples were precipitated in chilled 20% trichloroacetic acid (TCA). Then 2 ml extract was homogenized in 2 ml 0.1% thiobarbituric acid (TBA), incubated for 90 min in a water bath with a temperature set on 90ºC, followed by cooling down to room temperature. Then, the absorbance was read at 532 nm. Malonaldehyde was utilized as the standard for TBARS assay. TBARS values were expressed as mg of malonaldehyde per kg of the sample.

2.12. Determining total volatile base nitrogen (TVB-N)

Total volatile basic nitrogen (TVB-N) was measured based on the method by Goulas and Kontominas (2007) with some modifications. A mixture of 10g trout fillet+50ml distilled water was obtained and transferred along with 300ml distilled water to a 500cc round bottom flask. It was then distilled following addition of 2g of magnesium oxide (MgO) and a few drops of paraffin to avoid foaming. The distillate was collected in a 250cc Erlenmeyer flask which contained 25 ml of 3% aqueous solution of boric acid and 0.05 ml of methyl red and bromocresol green. Then, a titration was prepared for boric acid solution with adding 0.1 N sulfuric acid solution. The TVB-N value (mg/100g of fillet) was determined by the following equation: TVB-N(mg/100g)=(Vs-Vb)×14

Vs is the consumption volume of sulfuric acid of the sample and Vb is the consumption volume of sulfuric acid of the blank.

2.13. Instrumental measurement of color

Variations in color for the control and treated trout fillet samples at the time of storage were evaluated by colorimeter (Hunter lab Color Flex, Reston, VA, USA). The colorimeter was adjusted using a standard white tile (L*= 92.23, a*= -1.29, and b*= +1.29). a container was used to locate the trout fillet samples, followed by recording the values of L* (lightness), a* (redness), and b* (yellowness) on the outer and inner surfaces of samples.

2.14. Sensory evaluation

Sensory evaluation was determined with the use of a method presented by Devatkal et al. (2010) and Naveena et al. (2008) with some modifications. Semi-trained panels were 12 people from among the laboratory personnel who evaluated the trout fillet treatments. The panelists rated four characteristics of each sample appearance, juiciness, flavor, and general palatability) on an 8-point descriptive scale. The trout fillets were initially warmed prior to serving and water was served to rinse mouth between sensory evaluations of the samples. The experimental protocol was approved by the Ethics Committee of Tehran University of Medical Sciences (IR.TUMS.SPH.REC.1392.14023) and conformed to the ethical principles set forth in the Declaration of Islamic Republic of Iran.

2.15. Statistical analysis

Spss software was employed in this study. All the experiments were performed in triplicate for each of the five groups. Data of total phenolic and DPPH radical scavenging activity were analyzed using a one-way Analysis of Variance (ANOVA). The collected data related to pH, color, TBARS, TVB-N and PV were analyzed using two-way ANOVA considering treatment and storage time as the leading factors. Statistical significance was determined at 95% confidence level ($p < 0.05$).

3. Results and discussion

Figures 1 to 9 demonstrate the results of total phenolic, DPPH, pH, PV, TBARS, TVB-N and color value. Changes in sensory quality of the trout fillet are also shown in Table 1.

3.1. Total phenolic contents and antioxidant activity of pomegranate extracts

Aiming to compare the total phenolic content of extracts, the normality of the observations for each group was evaluated by ANOVA test. All groups showed normalization. The total phenolic contents of WEPR, EEPR, WEPF and EEPF were 5.2±0.23, 4.24±0.21, and 3.02±0.19 and 2.91±0.06 mg/L Tannic acid, respectively (figure 1). Anti-oxidant, antimicrobial and anti-cancer activity has already been observed in Phenolic compounds of pomegranate (Mousavinejad et al., 2009, Afaq F et al., 2009). There are more phenolic and antioxidant compounds in Pomegranate peel than in other parts of the fruit. In this study, the highest and lowest phenolic compounds were observed in WEPR (5.2 ± 0.2 mg/L Tannic acid) and EEPF (2.91 ± 0.6 mg/L Tannic acid) groups respectively. Water and Ethanolic extracts of pomegranate were obtained in similar studies. Devatkal et al. (2010) reported total phenolic of pomegranate rind powder (PRP) and pomegranate seed powder (PSP) to be 4476.2 and 2590.6 (µg/g powder), respectively (Devatkal et al., 2010). Tehranifar et al. (2010) found 295.79-985.37 mg/100g in total phenolic content of twenty Iranian pomegranate juice cultivars (Tehranifar etal, 2010).

3.2. DPPH free radical scavenging activity

The DPPH free radical scavenging activity (% scavenging activity) is depicted in Figure 2. The results revealed that the BHT group had the greatest activity in this regard. WEPR and EEPR groups had the highest capability of neutralizing free radicals, while the EEPF group had the least ability. Results showed an enhanced radical scavenging activity as the extract concentration increased, which was comparable to that of BHT. In this aspect, anti-radical activity of the rind extracts (WEPR and EEPR) were significantly stronger than the fruit extracts (WEPF and EEPF), which was similar to that in BHT ($p<0.05$). The anti-radical function of extracts has been linked to the amount of total phenolic compounds (Mousavinejad et al., 2009).

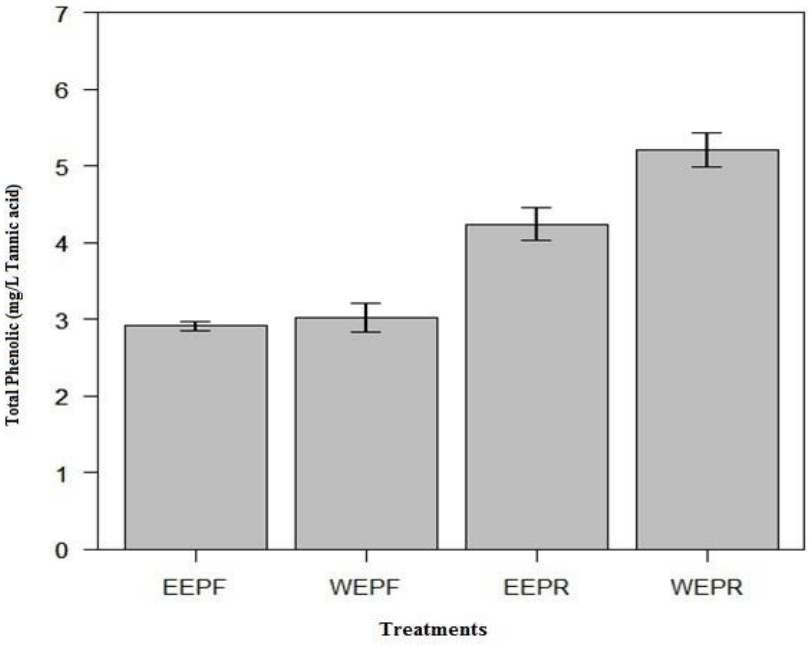

Figure 1. Total phenolic contents in pomegranate extracts.

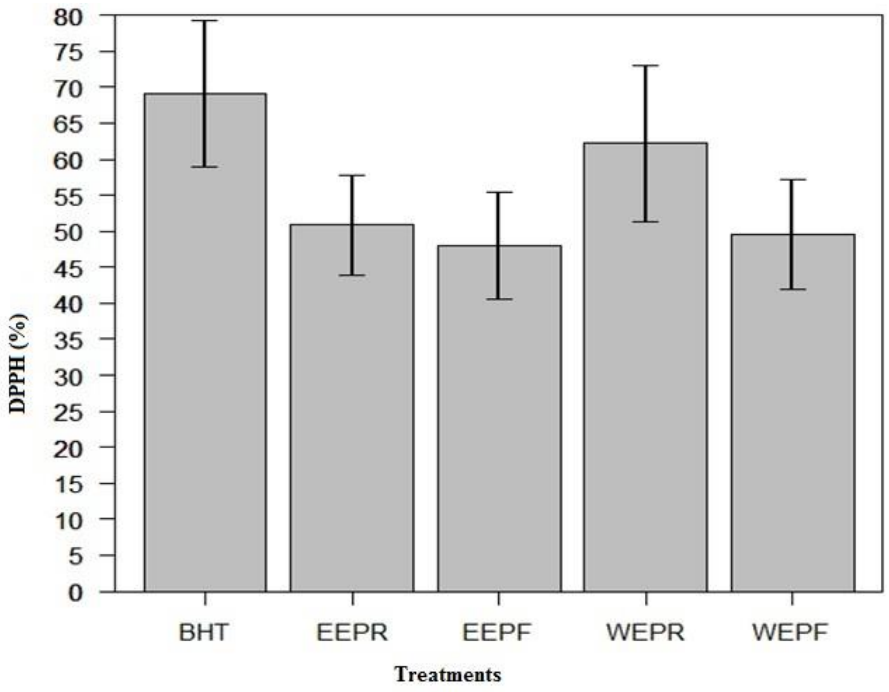

Figure 2. The antioxidant activities of pomegranate extracts and BHT in DPPH assay.

Among four extracts, WEPR had significantly ($p<0.05$) higher phenolic content and anti-radical activities compared to the other extracts. The results were in agreement with what Martínez L et al. (2019) reported, pointing out the potential of pomegranate extract as an antioxidant and antimicrobial compound in vitro (Martínez L et al., 2019). Similarly, Devatkal et al. (2010) and Naveena et al. (2008) indicated free radical scavenging activity in pomegranate juice, rind and seed extracts (Devatkal et al., 2010; Naveena et al., 2008).

3.3. Changes in pH

Figure 3 shows the changes in pH of trout fish fillet mediated by water and ethanolic extraction of rind and fruit pomegranate. pH values of the control group significantly increased to 6.96 during storage ($p<0.05$). Attempting to investigate the effects of the time factor and the interaction between time and extract, Mauchly's sphericity test was used. Results of the current study revealed a significant effect of storage time on pH. However, no meaningful difference was found in pH among different extracts. The pH in fresh fish is almost neutral. Upon death, nitrogenous compounds in fish are decomposed by proteolytic enzymes activity and increasing pH in fish meat during storage (Gokoglu et al., 2004). Increase in pH represents poor quality.

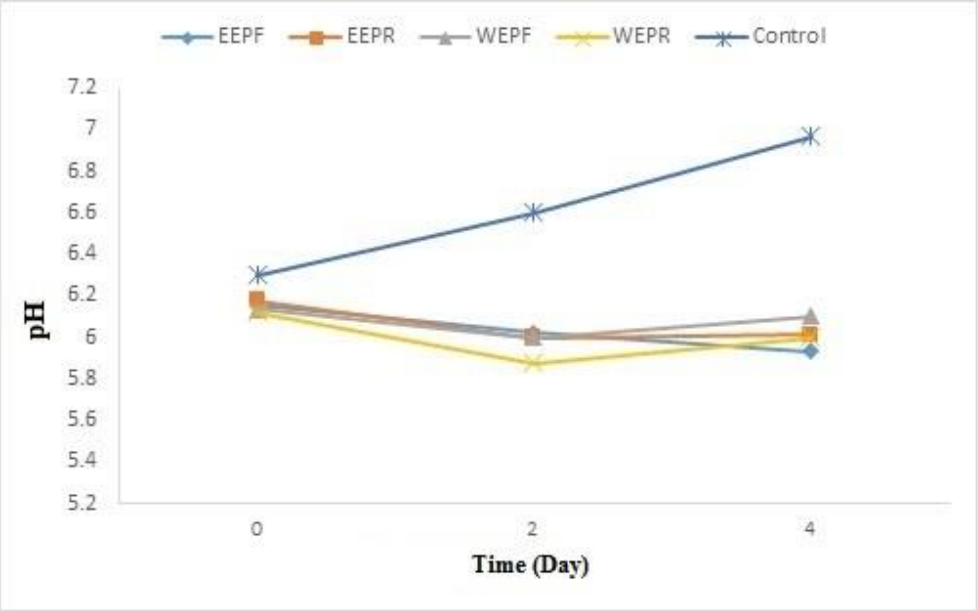

Figure 3. pH values of pomegranate extract samples during refrigerated storage (4°C) in different

A maximum pH of 6.8–7.0 is reported to be desirable during storage at refrigerator (Gai et al., 2014). El Marakchi et al. (1990) found a pH value of 6.1 in raw sardine. During the storage of fish fillet with pomegranate extracts, pH decreased to 5.93. A significant difference ($p<0.05$) in pH was found between the control and treatment groups; pH values of the EEPF treatment were lower than that of other extracts. However, no meaningful changes was found in the pH values of the treatments samples ($p>0.05$) during storage. pH of the trout fish fillet decreased due to the addition of the extracts. Similar values of pH were reported by Gokoglu et al. (2009) in marinated anchovy with pomegranate sauce.

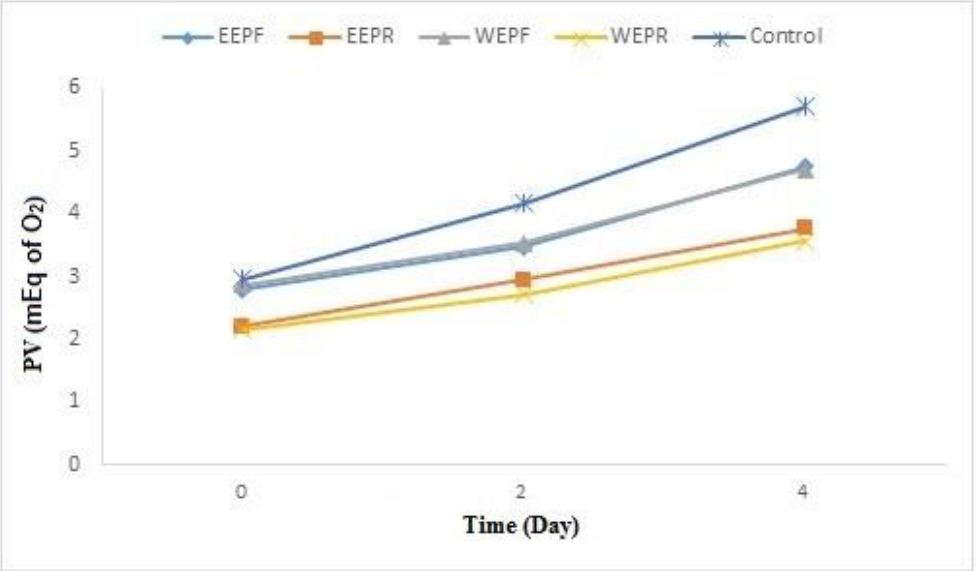

Figure 4. Peroxide value of pomegranate extract samples during refrigerated storage (4°C) on different days.

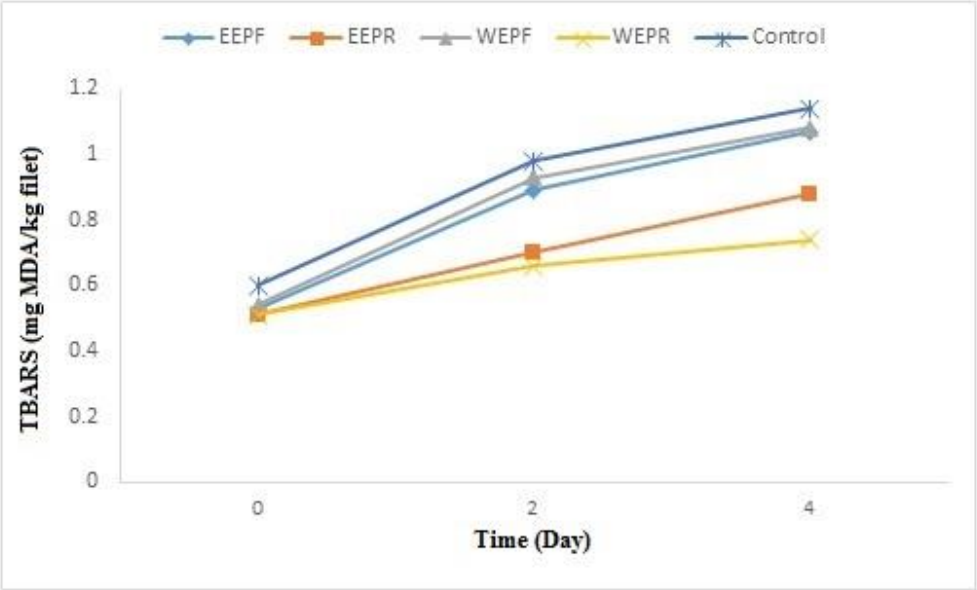

Figure 5. TBARS value of pomegranate extract samples during refrigerated storage (4°C) on different days

3.4. Changes of Peroxide value (PV)

Figure 4 describes the changes in PV value. According to the findings, PV levels of control group were greater than other extraction groups. WEPR and EEPR had the lowest PV value. The results of the Mauchly's sphericity test demonstrated that there was a meaningful difference between storage time and PV value. Polyunsaturated fatty acids are the main Components of fish fillet, making fish fillet extremely susceptible to lipid oxidation (Maqsood and Benjakul, 2010). Peroxide value (PV) is a representative of the primary stages of oxidative change (Shahidi and Zhong, 2005). In this study, a significant increase in PV values was found in both control and treated samples ($p<0.05$) following an increase in storage time, which was due to faster generation of new hydroperoxide which overweighs its degradation. The samples with extractions showed significantly ($p<0.05$) reduced peroxide level in comparison with the control group. WEPR and EEPR reduced the formation of peroxide more efficiently than WEPF and EEPF did, highlighting stronger antioxidant activity of compounds of pomegranate peel. Our results also demonstrated that the pomegranate extracts, especially rind extracts, were capable of PV production in the trout fillet stored in refrigerator (4±10C), which were consistent with those of Pezeshk et al. (2011), and Mexis et al. (2009).

3.5. Thiobarbituric acid reactive substances

Variation in values of TBA during storage are presented in Figure 5. The concentration of TBARS was calculated using the standard curve, obtained by a commercial Malonaldehyde bis (dimethyl acetal) regent (Merck Schuchardt OHG). The following formula was used: $y = 0.0348X + 0.0153$. The highest and lowest TBA values were found in the control and WEPR samples, respectively. Thiobarbituric acid reactive substances (TBARS) have been utilized to measure secondary oxidation products (Shahidi and Zhong, 2005). In the present study, there was a marked increase in TBA values for both control and treated samples ($p<0.05$) as the storage time increased, and control samples showed the highest TBA value. TBARS values were also slightly increased in the WEPR treated sample, being at its minimum rate (<0.74 mg MDA/kg sample) up to 4 days. However, a remarkably hampered TBARS production ($p<0.05$) in the trout fillet treated with WEPR, EEPR, EEPF, and WEPF was found compared to the control group. Reduction in the amount of T-BARS was significantly associated with the total phenolic contents, and he highest content was found in the rind pomegranate extracts (WEPR and EEPR). These result demonstrated that the pomegranate extracts, especially rind extracts, were efficient in slowing down the increase in TBARS levels of trout fillet during refrigeration (4±10C) storage. Similar finding was reported by Yerlikaya et al. (2010) and Ozen et al. (2011).

3.6. Total volatile base nitrogen (TVB-N)

The findings of this study showed that there was a higher level of TVB-N in the control group than the treatment groups. WEPR and then WEPF also had the lowest amount of TVB-N (figure 6).

In this study, the TVB-N values of the control and treated samples significantly increased ($p<0.05$) with storage time ($p<0.05$) Similar trend was also observed in Zhuang, S. et al. (2019) study. However, no significant difference ($p>0.05$) in TVB-N value was observed on day 0 between the control and treated groups. TVB-N is a quality index for fish, which is mainly attributed to trimethylamine, dimethylamine, ammonia, and other volatile basic nitrogenous compounds produced for the activity of spoilage bacteria and endogenous enzymes (Kilinc and Cakli, 2005). Viji et al. (2020) proposed the value of 30 mg N per 100 g fillet as the maximum acceptable level. At the end of the storage period, the lowest amount of TVB-N was found in fish fillet containing WEPR (13.3 ± 1.77), which had a

significant difference with other extracts. Also, the highest TVB-N was observed in control sample (30.1 ± 0.99), which was higher than acceptable limit. The initial TVB-N values in the studied samples were in accordance with results achieved by Gokoglu et al. (2004) and Pezeshk et al. (2011). Our findings also demonstrated that in the end of storage, control sample showed a significantly higher TVB-N values ($p<0.05$) than those of the treated samples (30.1 ± 0.99 mg N per 100g fillet). Minimum TVB-N values were reported in the WEPR samples at the end of storage (13.3 ± 1.77 mg N per 100 g fillet). Low TVB-N in the treated samples can be attributed to the total phenolic content and antioxidant compounds found in rind and fruit pomegranate. Mexis et al. (2009) also reported similar events during the refrigerated storage of oregano essential oil treated rainbow trout fillets.

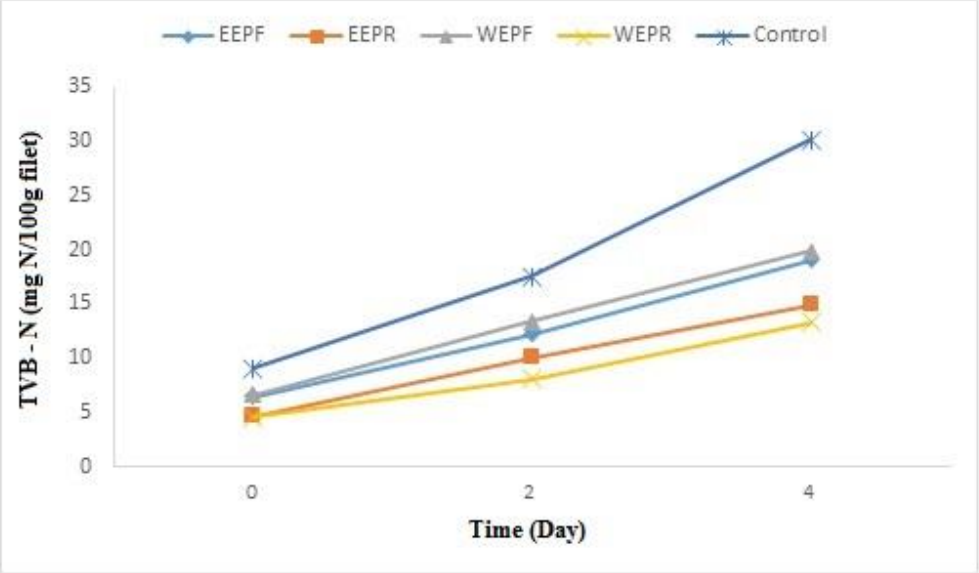

Figure 6. TVB – N value of pomegranate extract samples during refrigerated storage (4°C) on different days.

3.7. Changes of color value

Changes in L*, a*, and b* values of trout fillet with and without antioxidants are presented in Figure 7, 8 and 9, respectively. Analysis of variance of colors showed significant difference ($p<0.05$) in terms of the effects of extracts and storage time.

According to the findings, the amount of Lightness (L* value) on day 0 for the control and EEPF samples was more than that in other samples. The Lightness (L* value) of the control group in the three storage periods (0, 2 and 4 days) was significantly ($p<0.05$) higher than the treated samples with extracts, which was consistent to results of Qin et al.'s (2013) study. Significantly minimum lightness (L* value) was ($p<0.05$) found in the rind pomegranate extracts (WEPR and EEPR). Losses of lightness of the WEPR and EEPR samples during storage might be linked to high turbidity and impurities in the rind of pomegranate. In terms of the Lightness (L* value) , many studies have shown that extracts of plants rich in phenolic compounds (turmeric, mint, black currant, almond) make the meat and food less transparent. (Jia N et al., 2012; Lorente-Mento et al., 2020).

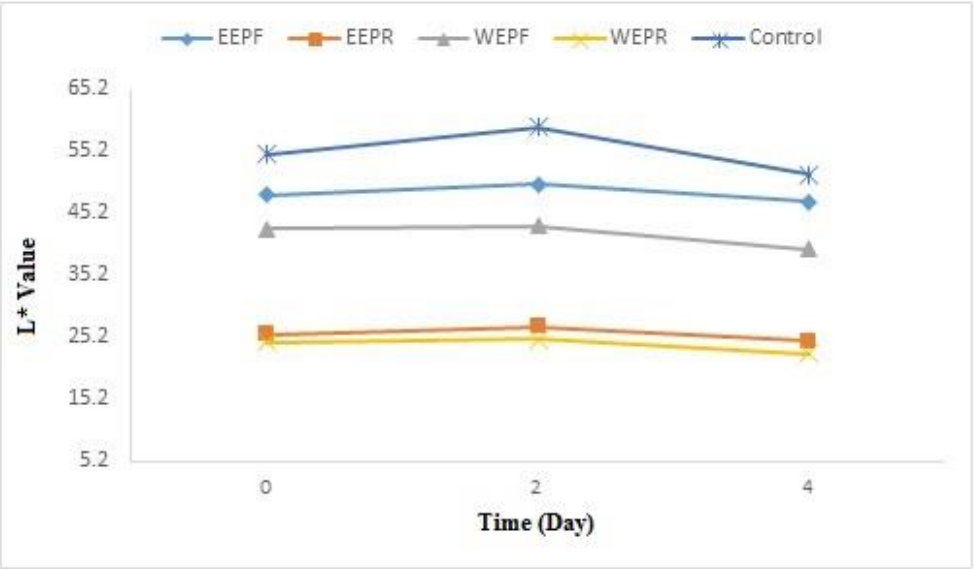

Figure 7. Changes of L* (lightness) value of the trout fillet treatments during refrigerated storage (4°C) on different days.

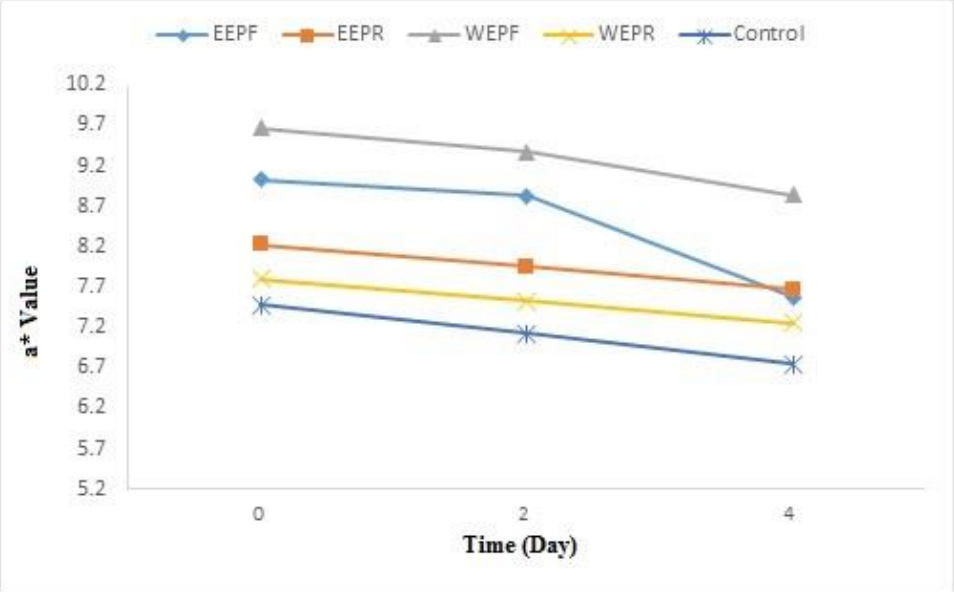

Figure 8. Changes of a* (redness) value of the trout fillet treatments during refrigerated storage (4°C) on different days.

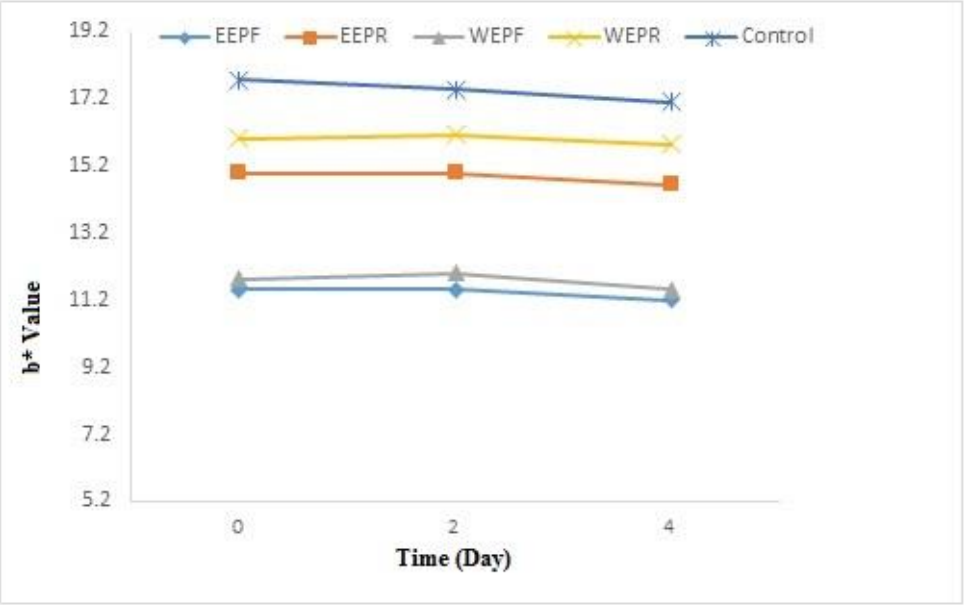

Figure 9. Changes of b* (yellowness) value of the trout fillet treatments during refrigerated storage (4°C) on different days.

According to figure 8, Redness (a* value) was significantly ($p<0.05$) higher in the WEPF sample than the control and other groups, with the minimum level (a* value) being found in the control sample during storage time. In all samples, a * value significantly ($p<0.05$) decreased at the end of storage. Oxidation of lipids could lead to the loss of redness (a* value) of the control sample during storage (Ozen et al., 2011). The main reason for the increased redness (a* value) and reduced lightness (L* value) was attributed to the presence of pomegranate extracts. Increases of the redness of the samples containing pomegranate extracts could be due to the formation of the main pigment (anthocyanins) in pomegranate extracts (Qin et al., 2013).

As shown in figure 9, a significantly higher b* value (yellowness) was found in the control group than the extract groups ($p<0.05$). Minimum b* value (yellowness) ($p<0.05$) was observed in the EEPF group. In all samples, the b * value in the day 1 of storage period increased slightly, and then decreased in the last storage day. Similar results were reported by Biswas et al. (2012) and Naveena et al. (2008). In overall, the degree of color change depended on the extracts and their composition.

3.8. Sensory evaluation

Sensory quality of the trout fillets was evaluated by panelists, as shown in Table 1. The panelists scored appearance, juiciness, flavor, and overall palatability of the trout fillets from 1 to 8. Using Mann Whitney test with Bonferroni correction for data analysis, significant ($p<0.05$) difference was found between the extracts and sensorial quality. Scores of all the parameters were significantly ($p<0.05$) greater in the WEPF group than the others. The flavor and juiciness scores were significantly lower ($p<0.05$) in the control group than that in the sample groups. Also, appearance and overall palatability scores were lowest in the EEPR group. We failed to demonstrate any meaningful difference between the WEPR and WEPF groups in this regard. The highest sensorial quality was observed in the aqueous extracts of pomegranate. Effects of

natural antioxidant such as turmeric, shallot (Pezeshk et al., 2011), and grape seed extract (Moradi et al., 2011) have been also reported in recent studies.

Table 1. Sensory evaluation scores of the trout fillet samples treated with pomegranate extracts

Parameters				
Samples	Appearance[a]	Juiciness[b]	Flavor[c]	Overall palatability[d]
Control group	5.58±1.31	5.25±1.48	5.00±1.82	5.75±0.93
	5.33±0.65	6.00±0.73	5.67±0.42	5.25±0.57
	5.50±1.00	6.50±0.64	6.00±0.54	5.50±0.45
	7.16±0.94	6.92±0.80	6.83±0.51	7.08±0.99
	7.58±0.51	7.41±0.24	7.42±0.26	7.58±0.63

a Appearance: 0 = extremely poor to 8 = excellent.
b Juiciness: 0 = extremely dry to 8 = extremely juicy.
c flavour: 0 = extremely intense odor or flavour to 5 = no flavour or odor.
d Overall palatability: 0= extremely palatable to 8 = extremely unpalatable.

4. Conclusions

The current study demonstrated that utilizing pomegranate extracts as natural antioxidants could delay lipid oxidation in fish fillet during refrigerated storage, with pomegranate rind showing the greatest ability. In the pomegranate extracts-receiving samples, the magnitude of change in TVB-N, TBA, and PV was less than in the control sample. These results indicated that pomegranate extracts could retard the fish spoilage and may be considered as an alternative for synthetic antioxidants in food industry as natural and cheap antioxidant sources to minimize lipid oxidation of fish products.

5. References

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Acknowledgments

This study was funded by Tehran University of Medical Sciences grant no. 93-02-27-25049. The authors declare that there is no conflict of interest.