Effect of Gamma Irradiation on the Quality Characteristics and Shelf Life of Pomegranate Arils during Refrigerated Storage

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ABSTRACT

This study was conducted to evaluate the effects of gamma doses (0, 0.5, 0.75, 1, 1.5, 2, and 3 kiloGray (kGy)) on some physicochemical, microbial, and sensorial characteristics of fresh pomegranate arils stored in a refrigerator for 30 days. Based on the results, the weight loss of all samples increased significantly during storage time. The total anthocyanin, and total phenolic contents in the pomegranate arils decreased with an increase in the radiation dose and storage time. Irradiation at doses ≥ 1 kGy reduced the $a^*$ values. Irradiation at ≥ 0.75 kGy doses significantly reduced the growth of bacteria and fungi. Sensory evaluation indicated that the samples irradiated at doses ≥ 1 kGy had overall scores above the acceptance limit (2.5) until day 14. However, none of the samples was acceptable after the 14\textsuperscript{th} day of storage. Overall, irradiation at 1-2 kGy is recommended for pomegranate arils based on the physicochemical, microbial, and sensory parameters.

Keywords: Punica granatum L., Quality evaluation, Total anthocyanin, Total phenolic content.

INTRODUCTION

The pomegranate fruit (\textit{Punica granatum L.}) and its products are rich sources of phenolic compounds, which affect the visual appearance and flavor of the pomegranate juice (Akhavan \textit{et al.}, 2015; Yasoubi \textit{et al.}, 2010). The edible part of the pomegranate is called arils, that their separation from the endocarp is a difficult and time-consuming process (Ashtari \textit{et al.}, 2019; Hussein \textit{et al.}, 2015). Therefore, the supply of ready-to-eat pomegranate arils with desirable sensory quality and health attributes has high economic importance in the fresh-cut fruit industry.

Pomegranate fruit and arils are subjected to continuous physiological and biochemical changes, including weight loss, microbiological decay, and aril discoloration, which can be retarded with post-harvest treatments and hurdle technologies (Mphahlele \textit{et al.}, 2014; Varasteh \textit{et al.}, 2017). The maximum shelf life of fresh pomegranate arils is around 1–2 weeks when stored at 5°C (Hussein \textit{et al.}, 2015). To overcome these concerns, post-harvest treatments, such as optimum storage temperature, modified atmosphere packaging, controlled atmosphere, coating, and drying are recommended to extend the shelf life of pomegranate arils (Hussein \textit{et al.}, 2015; Minaei \textit{et al.}, 2012; Mphahlele \textit{et al.}, 2014; Tavasoli Talarposhti \textit{et al.}, 2016).

In addition to these treatments, gamma irradiation is an effective means of processing and preserving food products (Hussain \textit{et al.}, 2019;...
2013). The FAO/IAEA/WHO joint committee on the wholesomeness of irradiated food approved the safety consumption of foodstuffs irradiated at doses up to 10 kGy (WHO, 1999). The US Food and Drug Administration seeks to allow the use of 4.5 kGy for non-frozen and non-dry products including both raw and pre-processed vegetables, fruits, and other agricultural products of plant origin (FDA, 2008). Various studies have reported the effectiveness of this technology in reducing microorganisms, and delaying physiological and biochemical harmful changes of various fruits and vegetables (Cabo Verde et al., 2013; Esmaeili et al., 2018; Hussain et al., 2013; Najafabadi et al., 2017; Shabaz et al., 2014; Tezutto-Uliana et al., 2013). However, ionizing radiation may have adverse effects on some physicochemical characteristics of food products (Alighourchi et al., 2008; Berenji Ardestani et al., 2019; Fan and Sokorai, 2011).

Application of gamma irradiation to improve the shelf life of the pomegranate fruit (Shabaz et al., 2014), juice (Alighourchi et al., 2008), and arils (Ashtari et al., 2019) has been studied. Due to the significant impact of gamma irradiation on the quality characteristics, it is necessary to evaluate narrower ranges of gamma irradiation along with market-related indicators during the extended storage period. The current study was done to investigate the effect of 0-3.0 kGy gamma irradiation on the physicochemical, microbial and sensory characteristics of pomegranate arils during refrigerated storage.

MATERIALS AND METHODS

Chemicals

Folin-Ciocalteu phenol reagent, phenolphthalein, sodium hydroxide, sodium chloride, sodium fluoride, sodium carbonate, the microbial culture media of Plate Count Agar (PCA), and yeast extract Glucose Chloramphenicol Agar (YGC) were bought from Merck Chemical Co. (Darmstadt, Germany).

Samples

The pomegranate cultivar of Alak Shir in Saveh was obtained from the mature fruit growing in the Agricultural Research Center in Saveh, Iran. Fifteen kilograms of the commercially ripe fresh fruit were harvested in November 2015. The fruits were sorted manually to get rid of the damaged ones. The acceptable ones were washed in a sodium hypochlorite (NaOCl) solution (0.2 g L\(^{-1}\)) for 3 minutes. They were cut manually, and the arils were separated manually as well. The arils (80 g) were packaged in polypropylene trays (Tabform, Tehran, Iran) of 164 mL volume (6.5×6.5×4.0 cm\(^3\)), and thermally sealed on the top with a 25 \mu m thick polypropylene film.

Irradiation Treatment

The pre-cooled and air-packaged arils were subjected to gamma irradiation at an ambient temperature (15°C). The samples were irradiated at doses of 0, 0.5, 0.75, 1, 1.5, 2.0, and 3.0 kGy with a dose rate of 2.62 Gy s\(^{-1}\) using a Gamma cell-220 irradiator (Nordion, Canada) at the Atomic Energy Organization of Iran. Dosimetry was performed using the Red-Perspex dosimeter (Harwell Dosimeters, UK) at the outer side of packaging. The overdose ratio (Dmax/Dmin) was determined and found to be 1.3. For each irradiation dose, three samples were evaluated. The irradiated samples were transported in a refrigerated truck (4–6°C) from Tehran to the target laboratory in Isfahan and then placed in a refrigerator (4°C, RH 80–85%) to be analyzed on the 1\(^{st}\), 4\(^{th}\), 8\(^{th}\), 14\(^{th}\), 20\(^{th}\), and 30\(^{th}\) days after irradiation.

Weight Loss Determination

The weight loss of the samples was calculated as the loss of weight of the pomegranate arils in each container during...
storage, and the values were reported in percentage (Hosseini et al., 2019).

**Measurement of pH, Titratable Acidity (TA) and Total Soluble Solids (TSSs)**

The pomegranate arils were manually pressed with a hand operated juice extractor (FP-2, Yongkang Junzilan Industry & Trade Co., Ltd, China). The juices (50 mL) were centrifuged (10,000×g, 10 minutes, at 4°C). The pH, TA, and TSS (%) of the juice samples were measured using the methods described earlier (Hussein et al., 2015).

**Total Phenolic Content (TPC) and Total Anthocyanin Content (TAC)**

The pomegranate arils were manually pressed with a hand device. Sodium fluoride (2 mmol L⁻¹) was added to inactivate the polyphenol oxidase activity and prevent the phenolic degradation of the juices. They were then centrifuged (10,000×g, 10 minutes, 4°C). The clear extract was used to determine the TPC and TAC of the samples (Shahbaz et al., 2014).

**Measurement of Visual Color**

The juice of arils was extracted and centrifuged as mentioned above, and used for color evaluation. The color of juices was measured using a Minolta colorimeter (Chroma Meter CR-400 Konica Minolta, Japan). The CIE L* was determined for lightness, a* for redness, and b* for yellowness (Alighourchi and Barzegar, 2009).

**Microbiological Analysis**

The PCA was used for aerobic mesophilic bacteria, while the yeast and mould counts were taken using YGC (Hosseini et al., 2019). Ten grams of each sample were obtained aseptically, and homogenized with 90 mL of sterile physiological solution. A series of 10-fold dilutions were prepared using 1.0 mL of diluents into 9.0 mL of the physiological solution. To enumerate the microbial load, 1 mL of each dilution was pour-plated in triplicate into the appropriate media. Petri dishes for the total aerobic mesophilic bacteria count were incubated at 37°C for 48 hours, while the Petri dishes for yeast and mould count were incubated at 26°C for five days. After incubation, the result was presented as a log of colony-forming units per gram (log CFU g⁻¹) of the arils (Hussein et al., 2015). The detection limit was < 1 log CFU g⁻¹.

**Sensory Analysis**

We evaluated the combined effect of the irradiation doses and the storage time on the sensory quality of the pomegranate arils (1st, 14th, 21st, and 30th days) using 12 trained panelists, including students and staff. They were regular consumers of pomegranate, and familiar with its quality attributes. The panelists were asked about the different quality attributes (aroma, color, and texture), and the overall acceptance of the arils, using a scale of 1 to 5 as follows: Color, ranging from very light red (1) to very dark red (5); texture, from very soft (1) to very firm (5); and aroma (immediately after opening the packages), from strong off-odor (1) to no off-odor (5). A final overall acceptance test was also performed using a hedonic scale, ranging from “extremely dislike” (1) to “extremely like” (5). Scores from 2.5 to 5 were considered acceptable (Moreno et al., 2007). The panelists used water and unsalted crackers as palate cleansers between tasting the samples. The rest time between every two tests was 1 minute.

Statistical tests were performed with Analysis Of Variance (ANOVA) and Duncan’s Multiple Range Test at P< 0.05 by
SAS software (Version 9.2, SAS Institute Inc., Cary, NC, USA). Triplicate samples were irradiated at each dose, and each sample was assayed in duplicate (n= 6). Results are expressed as mean±standard deviation.

RESULTS AND DISCUSSION

Weight Loss

Separation from the pomegranate makes the arils susceptible to rapid water loss, resulting in shriveling. As Figure 1A shows, all the samples demonstrated a gradual significant loss of weight during storage, irrespective of irradiation treatment (P< 0.05). The weight loss of the irradiated pomegranate arils was lower than the control during storage, but the difference was not significant. In this regard, lower weight loss was reported in 1.2–1.5 kGy-irradiated plums (Hussain et al., 2013), 1 kGy-irradiated blueberry and raspberry (Golding et al., 2014), and 900 Gy-irradiated strawberry fruits (Maraei and Elsawy, 2017) during storage, which could have happened because gamma irradiation affected the respiration rate and metabolic activity of irradiated fruits (Hussain et al., 2013; Maraei and Elsawy, 2017).

pH, Titratable Acidity, and Total Soluble Solids

The effects of gamma irradiation on the pH, TA, and TSS parameters during cold storage are shown in Figure 1 (B-D). Gamma irradiation had no significant effect on the pH and the TSS of the arils immediately after irradiation, but the TA decreased significantly at 2 and 3 kGy (P< 0.05). Other studies have shown similar changes in TA in 2 kGy-irradiated pomegranate fruit (Shahbaz et al., 2014), and in pH and TSS in 2 kGy-irradiated strawberries (Yu et al., 1995) immediately after irradiation.

The reduction trend of TA in 3 kGy-irradiated arils was significantly lower than 0 to 2 kGy-irradiated arils during 20 days of storage at 4°C (P< 0.05). Similar results were observed in the 3 and 5 kGy-irradiated arils of MalasSaveh pomegranate cultivar (Ashtari et al., 2019). Also, the pH fluctuated in a narrow range, and its increase was not significant at the end of the storage period. The decrease of TA and the increase of pH were reported in 2 kGy-irradiated strawberries after eight days (Yu et al., 1995), 1.5 kGy-irradiated raspberries after five days (Cabo Verde et al., 2013), 2 kGy-irradiated fresh raspberries after 20 days (Tezotto-Uliana et al., 2013), and 1 kGy-irradiated fresh-cut apple after 21 days (Fan and Sokorai, 2005) of storage. The trend of TA reduction in pomegranate arils could be attributed to an increase in metabolic activities, in which citric acid was used as the substrate (Hussein et al., 2015). Also, the fluctuation in TA and the pH of arils may be related to the differences in CO₂ accumulated in the passive modified packaging atmosphere during storage (Hussein et al., 2015).

The TSS of 0–2 kGy-irradiated arils significantly increased after the 20th day of storage. The control sample had the maximum value of TSS (P< 0.05). The increase in TSS is related to either a water loss or enzymatic degradation of polysaccharides or complex polysaccharides into simple sugars (Hussain et al., 2013). The increase of TSS in 3 kGy-irradiated arils was lower than other treatments at the end of the storage time. Irradiation can affect the ripening, senescence, and respiration processes (Hussain et al., 2013), as well as inactivate or reduce enzymatic and microbial activities. Similarly, the TSS of 0 to 1.5 kGy-irradiated plum fruit increased during storage (Hussain et al., 2013). In plum fruit, doses of 1.2 and 1.5 kGy significantly reduced the TSS changes compared with the treated samples at 0–1 kGy during refrigerated storage for 28 days (Hussain et al., 2013). Also, a lower TSS was observed in the 1-5 kGy-irradiated pomegranate arils
Figure 1. Effect of gamma irradiation (0-3 kGy) and cold storage (4°C for 30 days) on the weight loss, pH, Titratable Acidity (TA), and Total Soluble Solids (TSSs) of pomegranate arils. Error bars represent standard deviation between replicates (n= 6).
Changes in Total Anthocyanin Content and Total Phenolic Content

Anthocyanins are water-soluble polyphenolic compounds responsible for the red coloration in pomegranate arils (Akhavan et al., 2015). Figure 2-A shows the effect of gamma irradiation on the anthocyanin content. The TAC decreased (6–13%) with an increase in the radiation dose from 0.5 to 3 kGy. The TAC of arils significantly decreased during storage for 30 days (P< 0.05). During this period, the 2 and 3 kGy-irradiated samples had lower TAC compared with the other samples. There were no significant differences between 0 and 1.5 kGy-irradiated samples at the end of the storage period.

Similarly, the gamma irradiation at 1-5 kGy significantly reduced the TAC of pomegranate arils (cv. Malas Saveh), but unlike our results, the TAC did not change significantly during 14 days of storage (Ashtari et al., 2019). Some studies have reported a reduction of the TAC during irradiation (Alighourchi et al., 2008; Lee et al., 2011; Shahbaz et al., 2014; Tezotto-Uliana et al., 2013). The TAC of jujube fruit increased as the irradiation dose was increased from 0.5 to 2.5 kGy, and then decreased at 5 kGy (Najafabadi et al., 2017). Anthocyanin degradation can happen due to the effects of gamma radiolysis of water, which can produce various molecular species and free radicals. The free radicals can break the chemical bonds of anthocyanins, resulting in a bleaching effect (Lee et al., 2011).

Immediately after irradiation, the TPC of 3 kGy-irradiated arils significantly declined (~5%) compared with the 0.5-1 kGy-irradiated arils (P<0.05). The TPC decreased significantly for all samples during storage at 4 °C for 30 days (Figure 2-B). In the
stored 3 kGy-irradiated samples, the reduction of TPC (58%) was more than the other samples. In contrast, the control sample experienced the lowest decrease (43%) after 30 days of storage. Similarly, the TPC of 0.5–10 kGy-irradiated barberry fruit were decreased immediately after irradiation, with the exception of 7.5 kGy, at which TPC were increased (Berenji Ardestani et al., 2016). Also, the linear reduction trend of TPC with a gradual increase in the irradiation dose (0.5–2 kGy) has been reported for pomegranate, and this effect was more prominent at higher gamma doses (Shahbaz et al., 2014). Ashtari et al. (2019) reported a significant decrease of TPC in 5 kGy-irradiated arils of Malas Saveh pomegranate cultivar during 14 days of storage at 4°C; but unlike our results, the TPC reduction for doses of 1 and 3 kGy was not significant. In contrast, the TPC of 0.5–5 kGy-irradiated jujube fruit increased as the irradiation dose was increased from 0.5 to 2.5 kGy, and then decreased at 5 kGy (Najafabadi et al., 2017). Radiation treatments have been shown to either increase or decrease the TPC of fresh plant products, depending on the dose, exposure time, extraction solvents, raw material, and the time of phenolic evaluation (immediately after irradiation or later) (Alothman et al., 2009).

Color Change

The attractive color of pomegranate arils contributes to anthocyanin pigments, which influences consumer behavior. The destructive effects of gamma radiation on anthocyanins have been reported in the pomegranate fruit and juices (Alighourchi et al., 2008; Shahbaz et al., 2014). Figure 3 (A-C) shows the changes in the juice color of pomegranate arils stored at 4°C for 30 days, as given by L*, a* and b*. Immediately after irradiation, the L* values increased with an increase in the gamma doses. However, no significant differences were observed between them. Similarly, the b* values increased as gamma dosage increased from 0 to 3 kGy. This trend was significant (P< 0.05) in the samples irradiated at ≥ 2 kGy. Also, the a* values significantly decreased in the irradiated samples compared with the control (P< 0.05). Statistical analysis showed that the L* and a* values significantly changed during storage, but the b* variations were not significant. In this regard, a significant decrease in the a* values and a significant increase in the L* values were observed in all the treatments during storage (P< 0.05).

Similarly, a significant increase in the L* and a significant decrease in the a* were reported in irradiated tamarind juice (Lee et al., 2009) and both 0.5–6 kGy-irradiated and non-irradiated sour cherry juice stored at 4°C for 60 days (Arjeh et al., 2015). However, the b* values were constant in the stored sour cherry juices (Arjeh et al., 2015). In contrast, Cabo Verde et al. (2013) found no significant changes in the color indices (L*, a*, b*) in irradiated raspberries (0–1.5 kGy). But, the color indices decreased in both the irradiated and the non-irradiated raspberries stored at 4 °C for five days. Also, despite a significant reduction of betacyanin and betaxanthin contents, no significant changes in color could be observed in red beet root at 2 kGy (Latorre et al., 2010).

Thus, a color shift towards positive L* directions indicated the lightening of the surface color in pomegranate arils. A decrease in the a* of juices from the irradiated arils could be attributed to the degradation of anthocyanins (Alighourchi and Barzegar, 2009; Alighourchi et al., 2008). Irradiation produces a whole range of radical and non-radical species from ionization of intracellular water in living organisms (Latorre et al., 2010), which can break the chemical bonds of anthocyanins, resulting in a bleaching effect (Lee et al., 2011). Also, the change in color can be ascribed to a decrease in the polyphenol oxidase activity by irradiation (Shahbaz et al., 2014).
Figure 3. Effect of gamma irradiation (0-3 kGy) and cold storage (4°C for 30 days) on the color indices (L*, a* and b*) of juices from pomegranate arils. Error bars represent standard deviation between replicates (n= 6).

Microbial Analysis

The total viable counts in the fresh arils were ~1.66 log CFU g⁻¹ for the total mesophilic aerobic microorganisms and < 1 log CFU g⁻¹ for yeast and mould (Table 1). Both irradiation and storage duration significantly influenced the growth of microorganisms (P< 0.05). The initial populations of the total bacteria and fungi in the pomegranate arils went down significantly in a dose-dependent manner. This decrease was more drastic for bacteria than for fungi, resulting in an increase in the ratio of fungal to bacterial growth rate at the end of the storage periods. The growth of bacteria and fungi were significantly inhibited at 2 and 3 kGy, respectively. Also, the total viable counts gradually and significantly increased with the storage time in all treatments. The microbial population of the non-irradiated samples was higher than the irradiated samples.

The results were in accordance with the reports by Cabo Verde et al. (2013) for raspberries, Hussain et al. (2013) for plums, Hussain et al. (2012) for strawberries, and
Fan and Sokorai (2011) for fresh spinach. They showed a reduction of mesophilic aerobic bacteria, as well as yeast and mold counts after irradiation and during storage time. Ashtari et al. (2019) evaluated microbial growth of irradiated pomegranate arils only at the 14th day of storage. They reported significant reduction in microbial counts with the increase in irradiation dose from 1 to 5 kGy. Irradiation at 2.0 kGy significantly reduced the yeast and mold count (3.5 log cycle) of the strawberry fruit just after treatment. It was also below the detection level (< 1) after nine days of storage at 3 °C (Hussain et al., 2012). The inactivation mechanism of microorganisms by gamma radiation has been attributed to direct (DNA damage) and indirect (radiolysis products of water) effects, resulting in the inability of the cell to replicate, and cell mutations, respectively (Hussain et al., 2012).

**Sensory Analysis**

Sensory investigations are an important aspect of consumer satisfaction, which help measure the quality level perceived by consumers (Shahbaz et al., 2014). Twelve trained panelists were asked to assess the quality of pomegranate arils stored at 4°C on the first, 14th, 21st, and 30th day. Except for color in 2 and 3 kGy-irradiated samples, irradiation of the pomegranate aril had little effect on the other sensory attributes when compared to the control on the first day. Unlike the research of Shahbaz et al. (2014), the panelists in the current study did not feel any undesirable aroma with increasing gamma doses. Also, the gamma irradiation showed no significant effect on the firmness of the arils.

The sensory attributes of the samples at the 30th day were below the limit of acceptability. The studied samples also had the highest scores for off-odor by the end of the storage duration. Thus, Figure 4 shows the quality attributes scores (aroma, color, texture, and overall acceptance) of the examined samples for 21 days. The results showed that the sensory attributes and overall acceptance of the irradiated arils were significantly reduced during storage (P< 0.05). The scores for aroma, color,
Figure 4. Sensory attributes of irradiated (0-3 kGy) pomegranate arils during cold storage (4°C) on days 1, 14, and 21. Scoring system of, Color: Ranging from very light red (1) to very dark red (5); Texture: From very soft (1) to very firm (5); Aroma: From strong off-odor (1) to no off odor (5); Overall acceptance: From dislike extremely (1) to like extremely (5).

The color scores of the arils showed a significant reduction over time (especially in the samples irradiated at 0-0.5 and 3 kGy), which was consistent with the results of L* variations. Based on the panelists’ suggestion, the overall acceptance of the samples dropped to the minimum level and all the irradiated arils were unacceptable after the 14th day of the storage period. In general, the control and the samples exposed to < 1 kGy had lower scores than the other treatments, but the samples irradiated at 1-3 kGy were still within the acceptance limit (2.5–5) until the 14th day. In this regard, a higher sensory quality of irradiated food samples compared to the control ones was also reported by Moreno et al. (2007) and Fan and Sokorai (2011). It has also been reported that irradiation at doses up to 2 kGy may be used to enhance the microbial safety.

texture, and overall acceptance were below 2.5 in the control arils at 14th day since, at this date, the highest scores (between 3 and 4) were given to arils irradiated with the 1–2 kGy.
of fresh spinach without affecting consumer acceptance (Fan and Sokorai, 2011).

CONCLUSIONS

The total anthocyanin and total phenolic contents, as well as the color and microbial qualities of arils, were affected by irradiation and storage in a dose- and time-dependent manner. Gamma irradiation showed a potential to prevent the growth of aerobic mesophilic bacteria, yeast, and molds for 30 days of storage. Yeast and molds did not exceed the maximum acceptable limit in industry (5 log CFU g⁻¹) at doses ≥1 kGy. Based on the panelists’ suggestion, the sensorial qualities of 0–3 kGy-irradiated samples were unacceptable after the 14th day of the storage period. Therefore, the use of 1-2 kGy gamma radiation is recommended to extend the post-harvest life of fresh arils in cold storage by 14 days.

REFERENCES

اثر پرتودهی گاما بر ویژگی‌های کیفی و ماندگاری دانه‌های انار در طی تغذیری

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چکیده

در این پژوهش تأثیر تابش گاما در دانه‌های انار در دمای یخچالی و تغذیری گردید. نتایج نشان داد که میزان افت وزن تمام نمونه‌ها در طی دوره تغذیری معنادار بود. محتوای ترکیبات فنولی و آنتوسنسین کل با افزایش دوز پرتودهی کاهش یافت که در دوزه‌ای این ترکیبات در طی تغذیری نیز مشاهده گردید. پرتودهی در دوزه‌ای مساوی یا بیشتر از 1 کیلوگرم بسیار کاهش شاخص رنگ $a^*$ رود. رشد باکتری‌ها و قارچ‌ها در دوزه‌ای مساوی یا بیشتر از 75 کیلوگرم به صورت معنی‌داری کاهش یافته. بر اساس نظر ارزیابی‌ها و تا روز 14 تغذیری، امتیاز نمونه‌های پرتودهی شده در دوزه‌ای مساوی یا بیشتر از 1 کیلوگرم بالاتر از حد پذیرش (2/5) بود. با این وجود، هیچ کدام از نمونه‌ها بعد از روز 14 تغذیری از نظر حسی قابلقبول نبودند. به طور کلی، با توجه به نتایج تابش بر ویژگی‌های فیزیک‌شیمیایی، میکروبی و حسی، دوز پرتودهی در محدوده 1-2 کیلوگرم برای افزایش ماندگاری دانه‌های انار پیشنهاد گردید.