Acta Alimentaria 51 (2022) 4, 523–533
DOI: 10.1556/066.2022.00141

AKADÉMIAI KIADÓ

Effects of gamma irradiation, osmotic and freezing processes on chemical, microbial, and pest characteristics of dried Iranian barberry fruit during storage

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ORIGINAL RESEARCH PAPER

Received: July 26, 2022 • Accepted: November 5, 2022
Published online: November 30, 2022

ABSTRACT

Seedless barberry is a medicinal shrub and has been cultivated in Iran for more than two centuries. It is perishable with short shelf-life. Irradiation has shown to improve microbial safety and expands durability of raw fruits. Undoubtedly, current food processes undesirably affect bioactive compounds such as anthocyanins. Fresh barberry fruit was harvested in Birjand city by methods including "cutting branches" and "collecting fallen fruit under shrubs", which locally are known as "puffy barberry" and "jewel barberry", respectively. Some of the fresh barberries were treated by osmotic solution and then they have been dried. Untreated dried fruit was processed by freezing. Osmotic and frozen treatments were packed in polyamide film. Some of the dried jewel/puffy barberries packed in polyamide film were irradiated at doses of 0, 3, 5, and 10 kGy. All samples were stored at 4 and 25 °C for 6 months. Effects of barberry types (puffy/jewel), processes, storage time and temperature on chemical, microbial, and pest characteristics of dried barberry fruit were evaluated. Puffy barberry gamma irradiated with 5 kGy after 6 months of storage at 4 °C showed acceptable properties. Irradiation and storage at 4 °C were reported as optimal processing and storage conditions for barberry fruit.

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KEYWORDS

irradiation, osmotic process, freezing, chemical, microbial, pest

1. INTRODUCTION

Iran is the main producer of *Berberis vulgaris* fruit as a plant of *Berberidaceae* family in the world. Barberry has been used in medicine and food industry since 3,000 years ago. *B. vulgaris* bright red fruit has no seeds and are 8–10 mm long. *B. vulgaris* fruit contains anthocyanin functional pigments (Berenji Ardestani et al., 2013). South Khorasan province has been the only production hub of *B. vulgaris* in Iran and around the world for more than 250 years (Anonymous, 2020).

Dried barberry fruit is a food additive, and is used to make sauces, syrups, and desserts. Export issues and lack of knowledge about processing, packaging, marketing, and nutritional properties are the reasons for its low consumption (Berenji Ardestani et al., 2013). Fresh barberry fruit shelf-life is only 15–75 days in refrigerator because of mechanical damages, microbial, and fungal spoilage (Akhavan et al., 2017). Drying increases the shelf life of barberry fruit, but dried fruit also can be contaminated by air, dust, pests, and microorganisms. Heat treatment affects nutritional and quality properties. Residues of chemical preservatives in products lead to allergies and diseases in consumers and to environmental pollution (Bideli et al., 2015).

Freezing diminishes the rates of chemical reactions, stops the growth of microorganisms, and inhibits oxidizing enzymes, but defrosting will result in the loss of water-soluble compounds (Berenji Ardestani et al., 2015).

Osmosis is a non-destructive process based on moisture release through the fruit cell membrane by the osmotic pressure generated by concentrated sugar and salt solutions (Rastogi et al., 2002).

Irradiation is a food preservation method with no radioactive residues and a tool to fight foodborne diseases and death and to improve food safety. At allowed radiation doses, it is not easy to distinguish irradiated food. Irradiation is a non-thermal process equivalent to pasteurisation in terms of preservation efficiencies, but irradiated food is still "raw". Radiation processing is applicable in packaged products without the possibility of environmental pollution or secondary contamination. The cost of radiation is economical compared to conventional methods due to limited energy consumption. Neither radiation nor other food processing can turn spoiled food to consumable. Currently, more than half a million tons of 40 types of food (1% of consumed food) are irradiated annually in more than 40 countries (Akhavan et al., 2017).

This study investigated the effects of gamma irradiation on chemical, microbial, and pest characteristics in dried *B. vulgaris* fruit and compared with osmotic and freezing processes to determine desirable processing and storage conditions for up to 6 months.

2. MATERIALS AND METHODS

2.1. Materials

Chemicals and microbial culture media were purchased from Merck Chemical Co. (Darmstadt, Germany).

2.2. Sample preparation and processing

Fresh barberry fruit was harvested by two methods of "cutting branches" and "collecting fallen fruit under shrubs" in Mahmoei village of Birjand city in November. Some of the fresh barberries were treated immediately by osmosis (immersion in osmotic solution containing salt 1 g L^{-1} + sodium meta-bisulphite 3 g L^{-1} + citric acid 3 g L^{-1} + sugar 40 g L^{-1}) at 50 °C for 15 min and then excess solution was removed at ambient temperature on a clean shelf (Lerici et al., 1985). Then osmotic treated branches of barberry fruits were dried in a warehouse, which is locally called "Bargah" under shadow at ambient temperature. This product in the region is called "puffy barberry". The fruits fallen under the shrubs were collected and dried on a clean cloth outside under sunlight after osmotic treatment, and they are locally known as "jewel barberry". Ten grams of samples were packed in polyamide film. The drying time of the sun method was 336 h, until the desired humidity 18–20% was reached. The outside temperatures were minimum 0 ± 2 °C and maximum 25 ± 2 °C during 24 h (Ahmadi-Roshan et al., 2022). In addition, non-osmotic "puffy" and "jewel" dried barberries were processed by freezing through placing packages containing 10 g of dried barberry fruit at −20 °C for 72 h. Then packages were removed from the freezer and their contents were placed on a tissue or absorbent paper for 30 min. Afterwards, samples were ready to analysis. Also 10 g samples of non-osmotic "puffy" and "jewel" dried barberry in polyamide film packages were exposed to gamma rays at doses of 0, 3, 5, and 10 kGy. Irradiation was performed in a Gamma cell 220 (Nordion, Canada) ^{60}Co source at ambient conditions, with a dose rate of 1.97 Gy s^{-1} at Nuclear Science and Technology Institute in Tehran. Dosimetry was performed with a chemical ferric dosimeter. Packages were coded and stored away from light at ambient (25 ± 2 °C) and refrigerator (4 ± 1 °C) temperatures for evaluating quality characteristics at day 0 and after 6 months (Berenji Ardestani et al., 2016).

2.3. Determination of moisture content

Moisture content (gram per 100 g) was determined by drying at 70 ± 1 °C for 6 h (Berenji Ardestani et al., 2013).

2.4. Evaluation of titratable acidity

Titratable acidity was evaluated by titration with 0.1 M NaOH (Berenji Ardestani et al., 2013).

2.5. Measurement of total soluble solids (TSS or °Brix)

TSS of samples was measured by a refractometer (portable refractometer, Gerber line 0–32%, USA), at 20 °C (Berenji Ardestani et al., 2013).

2.6. Monomeric anthocyanin content

Differential pH method was used (Berenji Ardestani et al., 2016).

2.7. Microbial tests

Microbial analyses consisting of total aerobic mesophilic bacterial count (ISO, 2014), spore forming bacteria count (Corbo et al., 2004), coliforms count (ISO, 2006), presence or absence of

Escherichia coli (ISO, 2005), and total yeast/mould count (ISO, 2008) were done in triplicate. Results were reported as CFU g^{-1}.

2.8. Estimation of pests

The percentage of pest (any damage caused by the activity of insects and mites that was visible to the naked eye) was determined (ISO, 2021).

2.9. Statistical analysis

Chemical, microbial, and pest characteristics were investigated in a completely randomised design by factorial test with 3 factors including time, drying method, and temperature (not included in pest test) in 2 levels, and factor of processing in 6 levels. The trial was performed in triplicate. In this design, softwares SPSS ver.19, MINITAB ver.17, and SAS ver. 9.1 were used. ANOVA analysis of variance test and mean comparison by LSD test were done at the significance level of 0.05.

3. RESULTS AND DISCUSSION

3.1. Chemical properties

The results in ANOVA of chemical properties showed significant differences at least between the mean of two treatments from the main effects including barberry harvest method (barberry type), processing, temperature and time ($P < 0.05$). Most multiple interactions were not significant ($P > 0.05$).

3.1.1. Effect of barberry type on chemical properties. According to results in Table 1, type of barberry had significant ($P < 0.05$) effect on all chemical properties except TSS:Acid. The results showed that the values of moisture, titratable acidity, anthocyanin contents, and TSS were higher in jewel than puffy barberry fruit. These differences were attributed to differences in post-harvest processing method (Berenji Ardestani et al., 2013).

3.1.2. Effect of processing on chemical properties. The effects of different processing methods are illustrated in Table 2. In a similar study, authors reported no significant difference between control (0 kGy) and irradiated samples during storage. However, irradiated samples at doses >1 kGy showed less weight loss ($P < 0.05$). Higher moisture and weight loss values in the control sample can be due to microbial spoilage and fruit respiration; while radiation reduces respiration

Table 1. Effect of barberry type on chemical properties

Barberry type	Moisture (%)	Titratable acidity (%)	Anthocyanins (mg L^{-1} $_{DW}$)	TSS %	TSS:Acid
Jewel	25.43 ± 0.9[a]	0.39 ± 0.01[a]	220.39 ± 28.1[a]	11.68 ± 5.6[a]	30.72 ± 3.8[a]
Puffy	21.97 ± 1.6[b]	0.38 ± 0.02[b]	205.18 ± 24.3[b]	11.13 ± 4.5[b]	30.26 ± 4.0[a]

TSS: Total soluble solids. Results were given as mean ± standard deviation ($n = 3$); Different letters indicate significant difference ($P < 0.05$) in a column.

Table 2. Effect of osmosis, freezing, and gamma irradiation on chemical properties

Processes	Moisture (%)	Titratable acidity (%)	Anthocyanin (mg L^{-1} $_{DW}$)	TSS (%)	TSS:Acid
Osmosis	21.44 ± 1.2^{c}	0.24 ± 0.02^{d}	245.21 ± 95.2^{b}	10.75 ± 1.2^{c}	44.93 ± 3.8^{a}
Freezing	23.64 ± 4.7^{b}	0.42 ± 0.01^{b}	272.74 ± 46.3^{a}	11.16 ± 4.3^{b}	26.77 ± 6.1^{d}
0 kGy	24.63 ± 5.0^{a}	0.44 ± 0.01^{a}	280.45 ± 70.1^{a}	11.78 ± 2.6^{a}	27.06 ± 4.2^{cd}
3 kGy	24.50 ± 3.1^{a}	0.42 ± 0.11^{b}	201.10 ± 19.1^{c}	11.62 ± 5.4^{a}	27.30 ± 5.1^{cd}
5 kGy	24.60 ± 6.3^{a}	0.42 ± 0.13^{b}	162.98 ± 52.0^{d}	11.63 ± 3.5^{a}	27.92 ± 44^{c}
10 kGy	23.40 ± 4.2^{b}	0.40 ± 0.16^{c}	114.22 ± 36.6^{e}	11.51 ± 1.2^{a}	28.97 ± 0.8^{b}

Results were given as mean ± standard deviation ($n = 3$); Different letters indicate significant difference ($P < 0.05$) in a column.

rate and microbial activity (Akhavan et al., 2017). These results are in the same line with our findings for irradiated samples at 3 and 5 kGy.

The amount of water elimination in osmotic treated banana samples increased, because the osmotic force increases with increasing concentration of osmotic solution and, as a result more water is released from the tissue of fruit during immersion in osmotic solution. The osmosis process produces a product with low water activity and higher durability (Lerici et al., 1985). The above results are consistent with the results of the present study that the highest decrease (~13%) in moisture content was for osmotic treatments.

Preservation of moisture during storage has economic effect on weight of product (Berenji Ardestani et al., 2015). In the present study, the lowest moisture reduction (4%) was observed in frozen samples. Irradiated samples at 3 and 5 kGy did not differ significantly from the control.

The rate of titratable acidity reduction in irradiated raspberry was higher than in the control sample, which indicated aging of the fruit and it was not related to respiration rate. Also, after harvest, organic acids are used as a substrate in synthesis of compounds with 12 carbon skeletons, especially volatile compounds. There were no significant differences among the acidity values of irradiated barberry fruits at the end of storage at 4 °C (Akhavan et al., 2017). Likewise, we observed a decreasing trend of acidity in irradiated samples compared to the control in this study.

The lowest value of acidity in frozen and stored dates at −20 °C could be due to the reduction of microbial growth and rate of chemical reactions (Al Jasser, 2010).

In treatment of grapefruit in 55% w/w osmosis solution of sucrose, the acidity was reduced because some acids were released from the fruit tissue during osmosis treatment (Shakiba and Mohammadpour Karizki, 2015). Both above results are consistent with our outcomes.

Just like found in the present study, osmosis reduced the level of anthocyanins because of their migration into the osmosis solution (Kucner et al., 2013). Freezing affects anthocyanin content differently in different varieties of a fruit (Berenji Ardestani et al., 2016). We found that freezing did not cause significant change in anthocyanin content compared to the control.

Free radicals resulted from water radiolysis during irradiation break chemical bonds in anthocyanins, open the ring structure of anthocyanins, so the colour will fade and their content will decrease (Berenji Ardestani et al., 2016). Reduction up to 25% during freezing has been reported in phenolic contents (Berenji Ardestani et al., 2015). In the present study, anthocyanin content showed decreasing trend with freezing and with increasing radiation dose compared to the control.

The lowest percentage of TSS was related to dates stored at −20 °C, the moisture content of which was kept well in frozen samples (Tefera et al., 2007). Similarly, TSS values of the frozen samples were lower ($P < 0.05$) than samples stored at 4 or 25 °C in the present study.

Similarly to present research, increasing trend of TSS in irradiated samples was found significantly lower compared to the control sample by the end of storage, because irradiation inhibits microbial and enzymatic activities, thus slows down TSS enhancement ($P > 0.05$) (Akhavan et al., 2017).

3.1.3. Effect of temperature on chemical properties. The effects of different storage temperatures are shown in Table 3.

In agreement with the results of the present study, increasing temperature was reported to lead to increase in respiration rate and decrease in organic acids content, which is attributed to their greater use as a respiration precursor in fruit (Znidarcic and Pozrl, 2006). Increasing temperature causes more water to evaporate, which makes the solution more concentrated, eventually increasing TSS (Azeredo et al., 2006).

3.1.4. Effect of storage time on chemical properties. The results in Table 4 show that moisture, titratable acidity, anthocyanin contents, and TSS:Acid decreased, however, TSS conversely increased after 6 months. This increase is attributed to water depletion and enzymatic decomposition of large polysaccharides to simple sugars during storage (Akhavan et al., 2017). Likewise, a gradual and significant reduction was seen in the weight of fresh barberry fruit during storage due to moisture loss (Akhavan et al., 2017). Also the decrease in amount of titratable acidity with increasing storage time may be due to the conversion of acidic substances into sugars. Organic acids, by interaction with carbohydrates, are likely to provide a substrate or carbon skeleton for the production of phenolic compounds, including anthocyanins (Berenji Ardestani et al., 2015).

Table 3. Effect of temperature on chemical properties

Temperature (°C)	Moisture (%)	Titratable acidity (%)	Anthocyanins (mg L^{-1} $_{DW}$)	TSS (%)	TSS:Acid
4 ± 1 °C	24.52 ± 2.2^a	0.39 ± 0.04^a	229.39 ± 10.4^a	11.24 ± 2.3^b	30.49 ± 2.9^a
25 ± 2 °C	22.90 ± 3.5^b	0.38 ± 0.01^b	195.75 ± 8.3^b	11.58 ± 1.5^a	30.49 ± 1.7^a

Results were given as mean ± standard deviation ($n = 3$); Different letters indicate significant difference ($P < 0.05$) in a column.

Table 4. Effect of storage time on chemical properties

Storage time	Moisture (%)	Titratable acidity (%)	Anthocyanins (mg L^{-1} $_{DW}$)	TSS (%)	TSS:Acid
Beginning of the storage	24.31 ± 5.30^a	0.40 ± 0.01^a	234.03 ± 82.80^a	11.37 ± 1.21^b	30.90 ± 5.60^a
After 6 month of storage	23.09 ± 2.90^b	0.39 ± 0.02^b	191.54 ± 48.10^b	11.45 ± 2.20^a	30.11 ± 1.40^b

Results were given as mean ± standard deviation ($n = 3$); Different letters indicate significant difference ($P < 0.05$) in a column.

The weight and moisture content drops of irradiated plum fruit during 28 days refrigerated storage were less than of the control sample, which was attributed to the effect of irradiation on respiration rate. By increasing the irradiation dose, the weight and moisture losses of irradiated raspberries increased during storage (Akhavan et al., 2017).

These findings confirm the results of this study that the moisture content decreases during 6 months of storage. Significant dual interaction of process × time showed that the moisture content of control sample at the beginning of the storage was the highest and there was no significant difference between irradiated samples and control. After 6 months of storage, the moisture content of control decreased but it was the highest among all treatments. There were no significant differences between irradiated samples up to 5 kGy compared to control, but at the dose of 10 kGy, moisture content decreased significantly ($P < 0.05$).

3.2. Microbial assessment

According to analysis of variance, main effects including process, temperature, time, barberry type, and quadruple interaction were significant, of which the latter will be studied.

Microbial counts (Fig. 1 A–D) in control (0 kGy) and osmotic samples at 4 °C were more than at 25 °C after 6 months, meaning that moisture content and TSS can affect the growth and reproduction of microbial population in osmotic treatment. At higher temperature, the effect of moisture reduction in the osmotic treatment was enhanced and the microbial count decreased. Conditions become unfavourable faster for microbial growth and reproduction at 25 °C due to the higher growth rate at the beginning of storage, production of higher amounts of inhibitory substances by the bacteria, and reduction of nutrients.

In the present study, the count of aerobic mesophilic bacteria in frozen, osmotic, and control of jewel barberry samples after 6 months of storage at both 4 and 25 °C were >300 CFU g^{-1} (allowed CFU g^{-1} in food). The counts of spore forming bacteria in osmotic treatment at 4 °C, frozen, and control of jewel barberry treatments at 25 °C after 6 months of storage were higher than 300 CFU g^{-1} (maximum allowed CFU g^{-1} in food). The number of aerobic mesophilic and spore forming bacteria in irradiated treatments at different doses reached zero or below the detection limit or went below the minimum allowed limit (<30 CFU g^{-1}). Mould and yeast counts in frozen and control of jewel barberry fruit after 6 months of storage at 4 °C and frozen puffy barberry fruit after 6 months of storage at 25 °C were >150 CFU g^{-1} (food max. CFU g^{-1} permitted).

Furthermore, coliforms in control and irradiated with 3 kGy treatments of puffy barberry fruit at the beginning of the storage time at 4 and 25 °C and control of puffy barberry fruit after 6 months of storage at 4 °C were >300 CFU g^{-1} (maximum permitted CFU g^{-1} in food). With the exception of the above, the count of mould/yeast and coliforms in most treatments reached zero or below the detection limit or were under the minimum permitted limit (15 and 30 CFU g^{-1}, respectively).

In barberry fruit, moulds and yeasts are more likely to grow due to its acidic nature. Irradiation and refrigerated storage effectively decreased the microbial and fungal population ($P < 0.05$). Fungal contamination of control and irradiated samples increased during storage. Akhavan et al. (2017) found that the microbial load of irradiated raspberries and cherry tomatoes did not change during 14 days of storage at 4 °C. Immediately after irradiation of strawberries and cherry tomatoes the fungal population was significantly reduced (3.5 logarithmic cycles) and after 9 days of storage at 3 °C, the microbial burden was below the detection

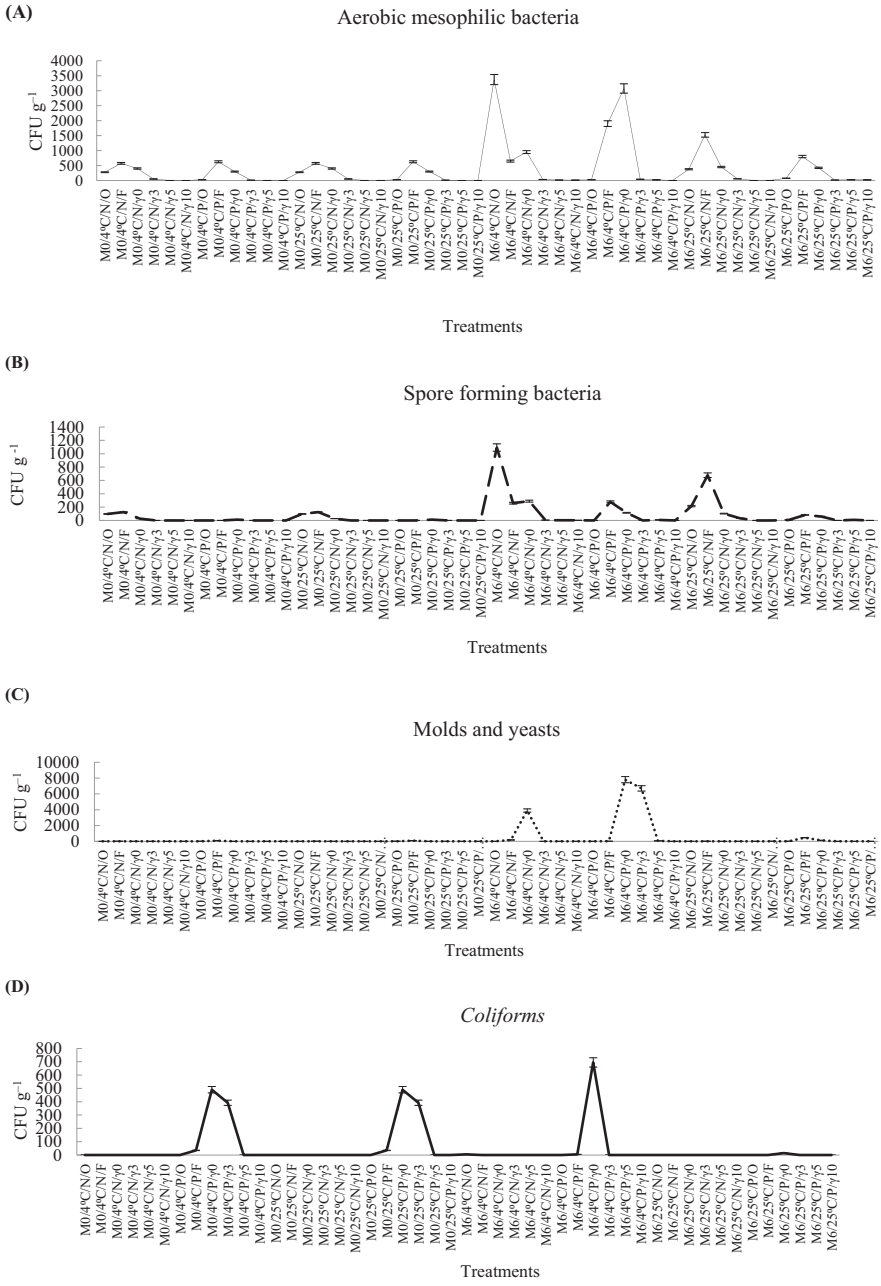

Fig. 1. Microbial contamination of non-irradiated and irradiated (3, 5, and 10 kGy) jewel/puffy barberry fruit samples during 6 months of storage at 4 and 25 °C. (A): aerobic mesophilic bacteria; (B): spore forming bacteria; (C): moulds and yeasts; (D): coliforms. M0: at the beginning of storage; M6: after 6 months of storage; 4 °C and 25 °C: storage temperatures; N: jewel barberry; P: puffy barberry; O: osmotic treatment; F: frozen treatment; γ_0, γ_3, γ_5 and γ_{10} are gamma irradiated at 0, 3, 5, and 10 kGy

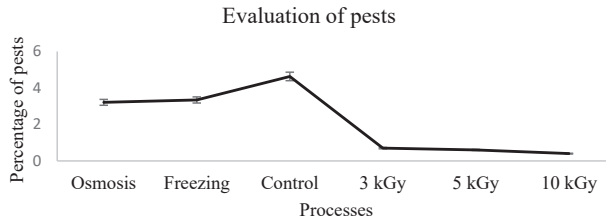

Fig. 2. Pests evaluation of non-irradiated and gamma irradiated samples (3, 5, and 10 kGy)

limit. The effect of gamma radiation on inactivation of microorganisms is attributed to direct (damage to DNA structure) or indirect (compounds resulting from water radiolysis) mechanisms, which in turn leads to cell mutation and reproduction inability (Akhavan et al., 2017). At a_w 0.6 (after osmotic treatment) only osmophilic moulds and a limited number of fungi can grow (Rahman, 2007). These confirm the results of this study that osmotic treatments (with the exception of three treatments) reduced the number of most groups of microorganisms. In most cases the microbial loads after 6 months of storage at 4 °C in irradiated samples were zero or less than permitted limits. The population of the studied microorganisms increased during 6 months of storage at 25 °C. The number of aerobic mesophilic bacteria was higher in jewel barberry fruit and of other types of microorganisms were higher in puffy barberry fruit.

3.3. Evaluation of pests contamination

According to analysis of variance, the main effect of processing had significant effects on the mean of at least two treatments ($P < 0.05$), but other main effects, double, and triple interactions were not significant ($P > 0.05$). According to the results shown in Fig. 2, the highest percentages of pest were observed in the control sample (0 kGy), then in frozen and osmotic treatments. In contrast, the lowest percentage of pests was found in sample irradiated with 10 kGy. Although there were no statistically significant differences between the three irradiated samples ($P > 0.05$), the percentage of pests has decreased with increasing dosage. Given that the percentages of pests in barberry samples at 25 °C were 5 times higher than in samples stored in the refrigerator after six months, and these values were outside the permissible range for human consumption, they were excluded from the research cycle and only statistical analysis of pest results at 4 °C was performed.

Living pests including living organism that are visual with naked eye such as insects or mites in any growth stages that feed on barberry or grow on it can cause infection and will reduce its quality properties. Pests can be detected in the form of cavities, where insects and mites feed, by the presence of strains, faeces, and similar things in barberry. The rate of pest percentage should not be more than one percent. According to Fig. 2 and the permitted amount of pest percentage (one percent) in dried barberry, irradiated samples with pest percentage less than one percent are suitable for consumption (ISO, 2021).

4. CONCLUSIONS

Considering the microbial properties (aerobic mesophilic bacteria, spore forming bacteria, moulds, yeasts, and coliforms counts) and the percentage of pests, which are the main concerns

for consumer health in terms of safety, as well as acceptable chemical properties such as moisture, acidity (desirable barberry flavour), and anthocyanin contents, optimal recommended conditions to process and store have been gamma irradiation of puffy dried barberry with dose of 5 kGy and storage up to 6 months at 4 °C.

ACKNOWLEDGMENTS

The authors acknowledge the supports provided by both Khorasan Razavi Agricultural and Natural Resources Research Center and Nuclear Science and Technology Research Institute.

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