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PHYSICOCHEMICAL TRAITS OF SEEDLESS BARBERRY (*BERBERIS VULGARIS* L.) FRUITS STORED UNDER REFRIGERATION AS AFFECTED BY HEAT AND CALCIUM CHLORIDE TREATMENTS

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ABSTRACT. The loss of chemical characteristics and quality of the fresh seedless barberry fruit during storage and qualitative losses of its dried fruit are the most important postharvest challenges in barberry industry and its exports. The fresh harvested fruit samples were dried using an electrical drier at 50°C to 50% moisture content. Thereafter, the effects of hot water alone (65°C for 45 sec), and hot water + 2% calcium chloride were carried out on the quality maintenance and chemicals during the cold storage of seedless barberry. The results showed that the samples treated with calcium chloride stored at 2°C had the highest TSS over time, whereas the titratable acidity of barberry fruits was not significantly affected by postharvest treatments. Hot water alone or in combination with calcium chloride treatment increased redness and chroma values result in better appearance quality than control. In addition, the treatments reduced the variable *L** and thereby enhanced fruit lightness. The highest antioxidant content (% 77.92) was observed in hot water treated samples and the lowest (% 54.28) was obtained on control. Also, the highest amount of anthocyanins and antioxidants were obtained from samples treated with hot water. Only calcium chloride treatment had a significant effect on Ca content of the samples. The results revealed that postharvest application of hot water and calcium chloride treatments improved the appearance quality and nutritional values of fresh seedless barberry fruit, as well as extend the cold storage life, likely due to reduced pathogen contamination.

Keywords: anthocyanins; antioxidants; hot water treatment; quality; postharvest.

INTRODUCTION

Seedless barberry tree (*Berberis vulgaris* L.) belongs to the family of *Berberidaceae* (Zargari, 1993).

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Barberry is one of the important fruits owing to its extensive medicinal properties, its resistance to a wide range of adverse water and soil conditions, its role in the industry, and its environmental impact in Iran. Fresh barberry fruits have high water content (about 80%) at harvest time. Therefore, normally, the main part of the harvested fruit is dried to reduce microbial contamination leading to postharvest losses along the supply chain. In this respect, the main problems for the barberry industry are related to its traditional drying methods and its improper handling and packaging (Kafi *et al.,* 2002). The drawbacks of traditional methods of barberry processing, including the extensive reduction of the fruit moisture for its longevity and the long duration of its drying by traditional methods, reduce its postharvest quality and safety. In addition, demand for fresh small fruits, like barberry and cranberry, has been raised in the market during the last decade because of consumers awareness about the high nutritional value of fresh produce, needed for human well-being. Hence, postharvest treatments are essential to minimize microbial spoilage and reduce the risk of pathogen contamination for fresh fruits.

Calcium chloride ($CaCl_2$) is widely applied to fruits and vegetables, in either full or cut forms, as a preservative and tissue hardening agent (Martin-Diana *et al.,* 2007). It is known that aging rate depends on the tissue Ca content, so that higher Ca content influences the parameters underpinning senescence, such as respiration, protein content, chlorophyll, and membrane fluidity (Poovaiah, 1986). A study on the effect of Ca on mechanical properties of potatoes and the cell wall reported that Ca application alleviated the stress of degradation of potato tissue and cell wall (Cybulska *et al.,* 2011). Similarly, Moradinezhad and Khayyat (2014) reported that the treatments with hot water, 2% $CaCl_2$ and 2 mM salicylic acid (SA), extended the shelf life and inhibited the decay of pomegranates, compared to the control. It has been shown that postharvest Ca treatment extended the shelf life of strawberries, so that the dipping of strawberries in 1% $CaCl_2$ solution increased Ca content of the fruit tissue, maintained fruit firmness, and controlled postharvest decay of the fruits (Garcia *et al.,* 1996). Heat treatment has been applied in different studies, as an alternative to chemical treatments for harvested fresh fruits and vegetables (Mahajan *et al.,* 2014). Heat treatment, especially in the form of hot water, to remove pests and fungal diseases in plants has commenced being commercially applied since the early 20th century (Escribano and Mitcham, 2014).

Then, interests aroused to this safe technique, that merely uses heat as an alternative to chemical treatments to control postharvest decay of the harvested fresh fruits (Fallik, 2004). The heat treatment may be applied in several ways to fresh fruits and vegetables, but hot water

dip is most preferred because it is more efficient than air in heat transfer (Fallik, 2004).

A recent study focused on the effect of pre-storage treatment, including the immersion of stone fruits in water with various temperature (24-70°C) on the control of brown rot and found that immersion in 60°C hot water for 60 sec. reduced the disease by as high as 73% in peach and nectarine fruits during cold storage (Karabulut *et al.,* 2010).

Previous studies have revealed that hot water dip is adequately effective on maintaining the quality of some freshly cut crops, like lettuces (Murata *et al.,* 2004; Moreira *et al.,* 2006), eggplants (Barbagallo *et al.,* 2012), and onions (Siddiq *et al.,* 2013).

As the literature shows, there is little information about the effects of hot water dip and $CaCl_2$ treatments and their combination on the postharvest response of fresh seedless barberry fruits held at cold storage.

Postharvest treatments may make it possible to maintain the nutritional quality of barberry, even when it has higher moisture content because fresh fruits possess the highest chemical quality, but they can be stored only for a short time or in frozen form.

Therefore, in this report, we examined the effect of hot water and $CaCl_2$ treatments on semi-moist barberry fruit in order to reduce losses and to extend storage life, as well as to improve fruit quality.

MATERIAL AND METHODS

Preparation of plant material and treatments

Uniform branches from determined trees were harvested from Research Orchard of the University of Birjand, Birjand, Iran, and transferred to the physiology laboratory on October 23, 2015. Fresh fruits were then separated from the branches and stored at 10°C before treatment.

The fresh fruit samples were dried using an electrical drier (Suzuki Food Dehydrator, ZFD-1200, China) at 50°C to 50% moisture content. Thereafter, fruits were immersed either in a solution of 65°C hot water + 2% $CaCl_2$ or in hot water (65°C), alone for 45 sec. The control samples were immersed in distilled water at 25°C and for 45 sec. Afterward, they were packed in LDPE bags (10 × 15 cm, 0.02 mm thickness) with normal atmosphere and were stored in a refrigerator at 2±0.5°C.

The physical traits (color components) and chemical traits (total soluble solids, titratable acidity, anthocyanin, antioxidant activity, phenolic compounds, and fresh fruit Ca content) were measured after 45 days of cold storage.

Color measurement of the samples

The following color components of the fruits were determined with a colorimeter (TES-135 A, Taiwan), after three weeks of cold storage: L^* (lightness), a^* (-greenness to + redness) and b^* (-blueness to + yellowness). The indicator C (Chroma) and h° (Hue) was determined by equations *(1)* and *(2)*, respectively (Hosseini and Moradinezhad, 2018).

$$C = \sqrt{a^2 + b^2} \quad (1)$$

$$\text{hue}^\circ = \text{arc tang}\ \frac{b}{a} \quad (2)$$

Measurement of total soluble solids

The fruit samples were oven-dried at 50°C and were then ground. Thereafter, 25 g was taken from each sample and mixed with 100 mL water and was extracted by a cloth filter with tiny mesh. The total soluble solids of the extract was measured with a hand-held refractometer (RF 10, 0-32%, Extech Co., USA) at 25°C, and expressed as °Brix.

Titratable acidity

To measure titratable acidity (TA), 5 mL of the extract was poured into a volumetric flask and was adjusted to 100 mL, by adding distilled water.

Then, the diluted extract was titrated with 0.1 N NaOH in the presence of phenolphthalein as an indicator (2-3 drops). Titration was terminated when a light pink color emerged for few seconds.

Fruit total acidity (in mg 100 mL^{-1} fruit juice) was calculated by the following equation *(3)* and was reported on the basis of malic acid (the dominant acid in barberries).

$$\text{Titrable acidity (mg mL}^{-1}\text{ fruit juice)} = \frac{\text{acid equivalent weigh} \times \text{NaOH normality} \times \text{volume of NaOH used in titrati on} \times 100}{\text{sample size} \times 10} \quad (3)$$

Total anthocyanin

Total anthocyanin content was quantified spectophotometrically by the pH differential method. A volume of 2 mL of the fruit extract was added with 25 mL of buffer solution (pH = 0.1), that contained a mixture of 0.2 M potassium chloride and 0.2 M hydrochloric acid. Then, another 2 mL of the plant extract was adjusted to 25 mL, by adding buffer solution (pH = 4.5), containing a mixture of 1 M sodium acetate and 1 M hydrochloric acid.

The absorption by the samples was recorded at 510 nM (Rapisarda *et al.,* 2000). Anthocyanin concentration was determined by equation *(4)*.

$$C \text{ mg } L^{-1} = (\text{abs pH 2} - \text{abs pH 4.5}) \times 484.82 \times 1000/24825 \times DF \quad (4)$$

Measurement of antioxidant activity

Antioxidant activity (%) was determined by DPPH (2, 2-diphenyl-1-picrylhydrazyl) method. Briefly, a quantity of 1 g of the dried sample was separated and mixed with 10 ml of 70% methanol. Then, it was placed on a shaker for 10-15 min and was centrifuged at 150 rpm, after 20 min. The supernatant was collected and refrigerated for 24 hrs. Afterward, 500 µL of the extract was mixed with 1.5 ml of DPPH solution, and was placed in darkness for 60 min, and recorded at 517 nm. The following equation (5) was used for the calculation.

$$\text{Antioxidant activity (\%)} = \frac{\text{absorbance of control} - \text{sample of absorbance} \times 100}{\text{absorbance of control}} \quad (5)$$

Measurement of phenolic compounds

Total phenolic compounds (TPH) were measured by Folin-Ciocalteau's (FC) assay (Makkar *et al.,* 1993). The absorbance was measured at 725 nm, using a spectrophotometer (Biospec 1601, Shimadzu, Tokyo, Japan). A calibration curve was also prepared using gallic acid (Merck KGaA, Darmstadt, Germany) and results were expressed as mg gallic acid per 100 g dry matter.

Measurement of calcium content

Calcium (Ca) content of the extracts was measured by atomic absorbance device (Li *et al.,* 2007).

Statistical design and data analysis

The study was laid on a Randomized Complete Block Design with four replications. Data were analyzed by the GenStat (version 12.1, VSN, International, Ltd., UK, 2009) statistical program. Means were compared by Fisher's LSD test at the 5% level.

RESULTS AND DISCUSSION

Color properties

Analysis of variance for fruit color components (*a**, *b**, *C*, and *L**) revealed that the effect of treatments was significant ($p< 0.01$) on lightness (*L**), redness (*a**), and color intensity (*C*).

According to means comparison, the treatments of hot water alone and hot water + $CaCl_2$ did not differ significantly and both improved color components in the stored fruits, compared to the control (*Table 1*). Accordingly, both treatments reduced the variable *L** and thereby enhanced fruit lightness (*Table 1*) after 45 days of cold storage. Also, hot water increased redness, yellowness, and color intensity of the fruits by increasing *a**, *b**, and *C*, respectively (*Table 1*). It has been reported that the *L** and *hue* values of cherries gradually decreased in storage, but Ca treatment postponed this loss for four weeks (Wang *et al.*, 2015).

Brambilla *et al.* (2011) also found that the redness (*a**) of blueberry fruits was increased when they were treated with hot water, that was in consistence with the presented results in our study. However, in a study on the immersion of freshly-cut papaya in 0.1% cinnamaldehyde and 0.75% calcium chloride, it was reported that *L** and *hue* values were increased in compared to control (Albertini *et al.,* 2016). Also, after six days of storage, Chroma in samples treated with 0.75% calcium chloride was higher than those of control and it was constant until the end of storage, whereas it was decreased in other treatments (Albertini *et al.,* 2016), that was in agreement with our results.

Table 1 - **Effect of immersion treatments on the studied color components of fresh seedless barberries**

Postharvest treatments	Color components				
	*L**	*a**	*b**	*C*	*h°*
Control	27.49 a	35 b	8.03 a	36.02 b	14.22 a
Hot water	24.09 b	43.6 a	9.12 a	44.62 a	11.74 a
Hot water + 2% $CaCl_2$	23.01 b	40.4 a	8.87 a	41.44 ab	12.56 a

Similar letters in each column show insignificant differences at $p< 0.05$ level.

Total soluble solids

Analysis of variance indicated that total soluble solids (TSS) were influenced by immersion significantly (p<0.01). Means comparison of postharvest treatments showed that the treatment with hot water + 2% $CaCl_2$ significantly changed TSS, compared to untreated control. The highest TSS (6.84 °Brix) was observed in treated fruits with hot water + 2% $CaCl_2$ (*Fig. 1*).

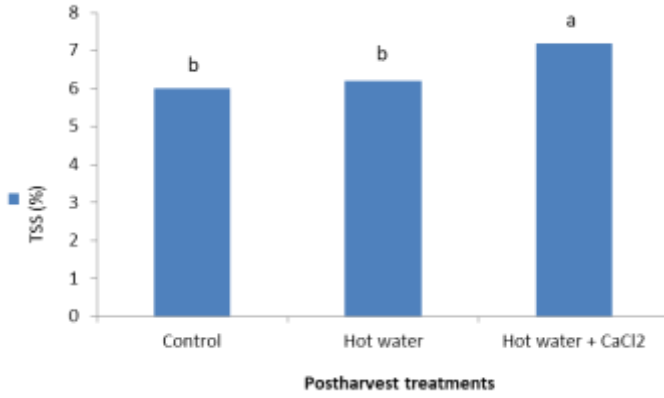

Figure 1 - Effect of hot water alone and hot water + 2% $CaCl_2$ treatments on total soluble solids of fresh seedless barberry fruit stored at 2°C for 45 days

The increase in sugar content during storage can be partially related to the loss of fruit juice and the increased concentration of fruit juice contents (Sayari *et al.,* 2009). Since sugar and acids act as the main substrate for respiratory metabolism, the loss of TSS during storage is mainly due to the respiration and the conversion of sugars to carbon dioxide and water. There is a report showing that the treatment with hot water (50°C for 3 min) and hot water + 2% $CaCl_2$ improved TDS in pomegranate fruits 'Shishe-Kab', as compared to control (Moradinezhad *et al.,* 2014).

Titratable acidity (TA)

According to the results of analysis of variance, the titratable acidity of barberry fruits was not significantly affected by postharvest treatments.

The fruits lose their acids over the storage due to the decomposition of organic acids (Rabiei and Rahmani, 2014). Organic acids may be mostly respired, as a carbon source during the tricarboxylic acid cycle. Thus, TA concentration is reduced during storage (Kays *et al.,* 2004). The loss of cherry taste during the loss of fruit acids impairs the potential shelf life of this fruit. Therefore, inhibition of the loss of fruit acid is the main goal to extend the shelf life and its marketability (Mattheis *et al.,* 1997). In contrary to our results, in previous studies on fig and pomegranate fruits, the TA significantly increased in treated fruit with $CaCl_2$. It is reported

that TA of fig fruits was increased during storage when they were treated with calcium chloride and calcium lactate (Irfan *et al.,* 2013). Similarly, a study on the foliar application of different rates of calcium chloride and urea on pomegranate fruits showed that 2% $CaCl_2$ had the highest impact on increasing the TA of the fruits (Ramezanian *et al.,* 2009).

Moradinezhad *et al.* (2014) reported that the treatment with hot water (50°C for 3 min) and hot water + 2% $CaCl_2$ enhanced the TA of pomegranate fruits 'Shishe-Kab' cultivar, as compared to control.

Anthocyanin

Analysis of variance revealed the significant impact of postharvest immersion treatments on the anthocyanin content of the samples. Means comparison indicated that the highest anthocyanin (0.39 mg L^{-1}) was observed in treated fruit with hot water and the lowest (0.24 mg L^{-1}) value was obtained on control *(Fig. 2)*.

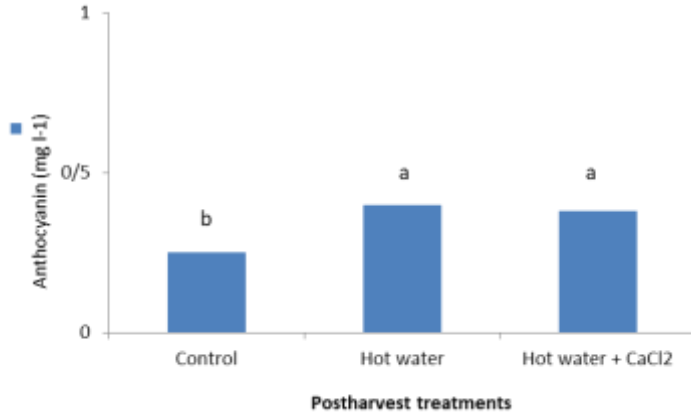

Figure 2 - Effect of hot water alone and hot water + 2% $CaCl_2$ treatments on anthocyanin content of fresh seedless barberry fruit stored at 2°C for 45 days

Anthocyanins are highly instable molecules in foods whose color stability is strongly determined by metal ions and sugars, pH, temperature, structure, oxygen, light, enzymes, and other accompanying compounds (Rein, 2005).

Like most reactions, the decomposition of the anthocyanins is considerably accelerated with temperature rise. So, the storage temperature impacts anthocyanin content remarkably, so that lower storage temperature increases anthocyanin content of *Arbutus unedo* L. fruits (Guerreiro *et al.,* 2013).

Aghdam *et al.* (2013) reported that $CaCl_2$ at various rates enhanced anthocyanin content of cornelian cherry (*Cornus mas*) fruits. In addition, Giovanelli *et al.* (2012) conducted a similar study on blueberries and reported that hot water treatment increased fruit anthocyanin content

slightly, which is consistent with our findings. In contrast, Brambilla *et al.* (2011) reported the loss of anthocyanin content in blueberry by postharvest hot water treatment.

Antioxidant

As the analysis of variance showed, antioxidant was influenced significantly ($p< 0.01$) by the hot water alone, and hot water + 2% $CaCl_2$ treatments. The highest antioxidant content (% 77.92) was observed in hot water treated samples and the lowest (% 54.28) was obtained on control (*Fig. 3*).

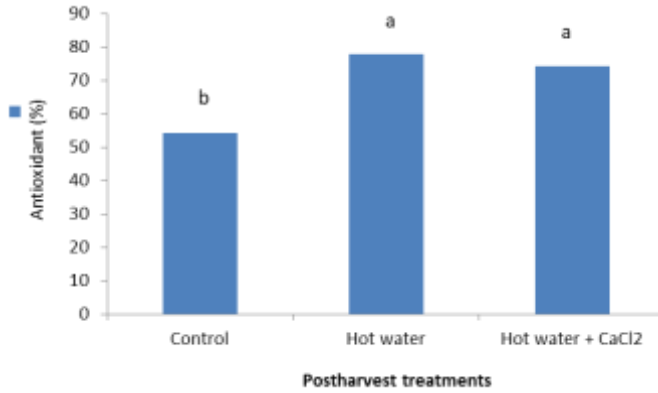

Figure 3 - Effect of hot water alone and hot water + 2% $CaCl_2$ treatments on antioxidant content of fresh seedless barberry fruit stored at 2°C for 45 days

The loss of antioxidant capacity at higher temperatures may be partially related to the decrease in ascorbic acid content as a non-enzymatic antioxidant factor (Javanmardi and Kubota, 2006).

Lower antioxidant capacity at higher temperatures and during storage is likely to associate with the fact that a great part of antioxidant compounds is consumed to defend free radicals (Gulen and Eris, 2004). Different rates of $CaCl_2$ increased considerably the antioxidant capacity of comelian cherry fruits, compared to control (Aghdam *et al.,* 2013). It has been reported that Ca in carrots augments antioxidant activity in response to the increased capacity of free radicals (Jacobo-Velázquez *et al.,* 2011), which is similar to our results.

Total phenolic compounds

The results show that postharvest dipping treatments affected significantly total phenolic compounds of fruits, compared to control. The highest phenol content (4.1 mg g DW) was observed in fruits treated with hot water + 2% $CaCl_2$, and the lowest (3.88 mg g DW) was obtained with hot water alone treatment (*Fig. 4*). Phenols are representative of a large group of secondary compounds (flavornoids, anthocyanins, tannins, and lignins) in plant tissues (Grace

and Logan, 2000). The antioxidant property of polyphenols is associated with their high responsiveness as electron or hydrogen donor, the establishment of stability and the removal of paired electrons due to their chemical structure, their capability in the transport of metallic ions, the capability of flavonoids in changing peroxidation trend by changing the fats, and the reduction of membrane fluidity (Arora *et al.,* 2000). These changes can inhibit the penetration of free radicals and limit peroxidase reactions. These compounds are strong antioxidants in stressful plant tissues and this feature is associated with the skeleton structure of a phenolic group of these metabolites. Aromatic ring-binding hydroxyl groups can scavenge free radicals and mitigate oxidative damages, thereby protecting cell structure against the adverse effects of stress (Al-Amier and Craker, 2007). Similar results showed that $CaCl_2$ at various rates increased total phenols in comelian cherry fruits during storage and the samples treated with higher $CaCl_2$ rates displayed a more remarkable increase in fruit phenol content (Aghdam *et al.,* 2013). In addition, it has been reported that treatment of raspberry and strawberry fruits with 2% $CaCl_2$ dip had a positive effect on the retention of the total phenolic contents, during the storage period (Turmanidze *et al.,* 2017). Hot water treatment is known to have desirable impacts on the maintenance of polyphenol components in fruits, maybe because of the inactivation of endogenous polyphenol oxidase enzyme of the fruits (Rossi *et al.,* 2003; Sablani *et al.,* 2010; Skrede *et al.,* 2000). However, postharvest hot water treatment of blueberries resulted in the loss of total phenols of the fruits over the storage (Giovanelli *et al.,* 2012). Similarly, Brambilla *et al.* (2011) reported that total phenol compounds of blueberries were decreased after the application of hot water.

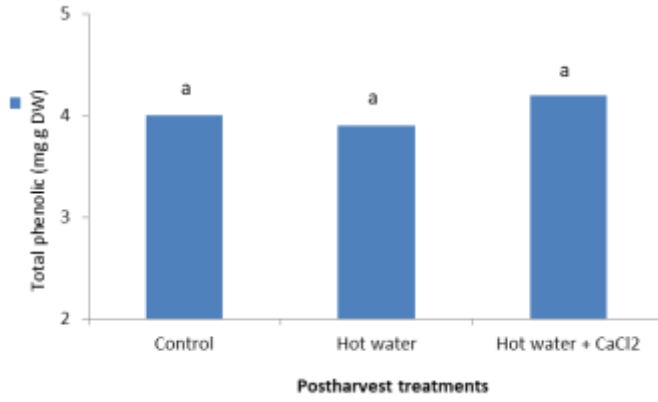

Figure 4 - Effect of hot water alone and hot water + 2% $CaCl_2$ treatments on total phenolic compounds of fresh seedless barberry fruit stored at 2°C for 45 days

Calcium content

The treatment of the fruits with hot water + 2% $CaCl_2$ significantly ($p< 0.01$) increased Ca content of the tissues as analysis of variance revealed. The results indicated that the highest Ca content (6.039 mg L^{-1}) was related to the treatment with hot water + 2% $CaCl_2$ and the lowest (2.457 mg L^{-1}) was observed in control (*Fig. 5*).

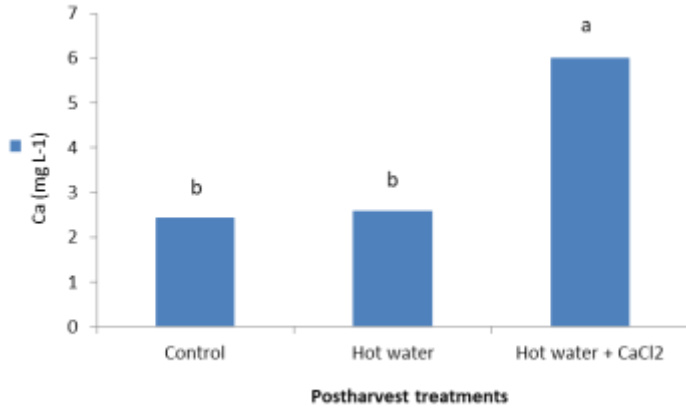

Figure 5 - Effect of hot water alone and hot water + 2% $CaCl_2$ treatments on Ca content of fresh seedless barberry fruit stored at 2°C for 45 days

Ca ion is likely to enter into fruit tissue through the cracks in cuticle and epidermis (Glenn and Poovaiah, 1985). A significant increase was observed in Ca content of the fruits immersed in cold water containing Ca (Wang *et al.,* 2014). Similarly, a study on the application of $CaCl_2$ at four rates of 0.5, 1, 1.5, and 2% showed that the highest Ca content of the tissues after the storage time was obtained from the treated samples with 2% $CaCl_2$ (Madani *et al.,* 2014).

Likewise, the postharvest application of $CaCl_2$ considerably enhanced Ca content of the fruit tissue in raspberries and strawberries (Carlos, 2014; Turmanidze *et al.,* 2017) and apple (Chardonnet, 2003; Torres *et al.,* 2017) stored under refrigeration.

CONCLUSION

Owing to less attention to use postharvest technologies in barberry industry in Iran, the main part of harvested fruit are dried. However, demand for fresh barberry fruits raised and so there is a need to prepare fresh produce with safety for marketing. The results of this study revealed that postharvest application of hot water and calcium chloride treatments improved the appearance quality and nutritional values of fresh seedless barberry fruit, as well as extend the cold storage life, likely due to reduced pathogen contamination. However, further research is needed to recommend for commercial application.

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