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Effects of Intermittent Warming and Prestorage Treatments (Hot Water, Salicylic Acid, Calcium Chloride) on Postharvest Life of Pomegranate Fruit cv. 'Shishe-Kab' during Long-Term Cold Storage

Farid Moradinezhad[1]* , Mehdi Khayyat [2]

1,2.Department of Horticultural Sciences, College of Agriculture, University of Birjand, Birjand, Iran

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Abstract
We examined the effectiveness of intermittent warming (IW), hot water (HW), salicylic acid (SA), and calcium chloride ($CaCl_2$) on the postharvest life of pomegranate fruit cv. 'Shishe-Kab' and extending fruit shelf life during cold storage (3°C). Fruit were subjected to cycles of 1 d at 17 ± 1°C every 6 d of storage at 3°C under IW conditions. Pre-storage treatments were HW (50°C) for 3 min, SA (2mmol L^{-1}), or a combination of SA (2 mmol L^{-1}) and $CaCl_2$ (2%) solutions for 3 min at 20°C. Weight loss was not affected by HW or chemical treatments, but firmness decreased significantly in HW-treated fruit when they were subjected to IW. The lowest quantity of unmarketable fruit was observed in fruit treated with a combination of SA and $CaCl_2$, regardless of whether stored in cold storage (CS) or exposed to IW. Regardless of HW and chemical treatments, IW significantly albeit slightly increased shelf life and reduced fruit decay compared with the control. However, the longest fruit shelf life (19 wk) was obtained with a combination of HW, SA, and $CaCl_2$ compared with the control (11 wk), especially under long-term cold storage.

Keywords: chemical treatment, chilling injury, postharvest, shelf life.

Abbreviations: $CaCl_2$, calcium chloride; **CI**, chilling injury; **CS**, cold storage; **cv**, cultivar(s); **g**, gram(s); **HW**, hot water; **IW**, intermittent warming; **LDPE**, low-density polyethylene; **N**, newton; **RH**, relative humidity; **SA**, salicylic acid; **TA**, titratable acidity; **TSS**, total soluble solids.

Introduction

Pomegranates are cultivated around the world in subtropical and tropical regions in different microclimatic zones such as in Iran, Turkey, Italy, India, Chile, Spain, and California. World pomegranate production amounts to approximately 1.5 million tons with Iran contributing 47% to the total (FAO, 2010). The market has steadily grown, presumably because of increasing consumer awareness of the potential health benefits attributed to pomegranate phytochemicals. 'Shishe-Kab' is a commercial pomegranate widely cultivated in Iran, especially in the South Khorassan province. However, its postharvest life and consumption is still limited due to the quantitative and qualitative losses which occur during postharvest handling because of chilling injuries, weight loss, decay, and husk scald of fruit as reported for most pomegranate cultivars (Caleb *et al*., 2012).

The storage temperature recommended for pomegranates varies from 0 to 10°C with a shelf life ranging from 2 wk to 7 m depending on the cultivar (Koksal, 1989; Sayyari *et al*., 2009), or from 6 to 10°C with a maximum 12-wk shelf life (Knee, 2002). At these temperatures ripening processes and decay are slowed, but storage time is still limited, and temperature cannot be lowered further or chilling injury will develop (Knee, 2002). Elyatem and Kader

*Corresponding author
Email: f_moradinezhad@yahoo.com

(1984) showed that the storage of pomegranates at 5°C or lower resulted in chilling injury (CI) with symptoms of brown discoloration of the skin, surface pitting, and susceptibility to decay organisms. Most of the time, these symptoms reach the arils such that both internal and external fruit quality is reduced (Elyatem and Kader, 1984). To prevent or delay the onset of chilling injury and to extend the maximum storage period without compromising fruit quality, a number of temperature manipulations and chemical treatments have been tested for different commodities including the pomegranate.

Heat treatment causes changes in fruit ripening, such as the inhibition of ethylene synthesis and action of cell wall degrading enzymes, due to changes in gene expression and protein synthesis (Paull and Chen, 2000). Heat treatment increased postharvest life by delaying softening in plums (Tsuji *et al*., 1984) and improved the flavor of a number of fruit without affecting concentrations of soluble solids in apples (Klein *et al*., 1990) or strawberries (Garcia *et al*., 1995). Prestorage hot water treatment increased fruit quality and reduced fruit weight loss and chilling injury in pomegranate cv. 'Malas-e-Saveh' (Talaie *et al*., 2004; Mirdehghan and Rahemi, 2005).

Salicylic acid (SA) is a simple phenolic compound recognized as a plant growth regulator because of its effects on plant physiological processes (Raskin, 1992). The effect of SA on resistance to diseases has been discussed by several researchers. SA is known to be the signalling molecule in plant systemic acquired resistance (SAR) induction (Raskin, 1992). SA application, either preharvest (Yao and Tian, 2005) or postharvest, reduced fungal decay in sweet cherries through the induction of the defense resistance system (Chan and Tian, 2006) and stimulation of antioxidant enzymes (Xu and Tian, 2008). In addition, pre-treatment with SA reduced CI in peaches (Wang *et al*., 2006) and pomegranates (Sayyari *et al*., 2009).

Calcium chloride ($CaCl_2$) has been widely used as a preservative and firming agent in the fruit and vegetable industry for whole and fresh-cut commodities. Manganaris *et al*. (2007) suggested $CaCl_2$ immersion as a potential postharvest treatment for whole peaches, since it increased tissue firmness and reduced susceptibility to physiological disorders. Studies on fruit ripening processes have also shown that tissue calcium content usually affects certain senescence characteristics such as respiration rate (Bangerth *et al*., 1972). Pre- and postharvest applications of calcium in strawberries prevented postharvest disorders, retarded fruit ripening, and decreased postharvest fruit weight loss (Lara *et al*., 2004) and decay (Hernandez-Munoz *et al*., 2006). In pomegranates, satisfactory results were shown by pre- and postharvest $CaCl_2$ treatments (El-Kassas *et al*., 1995).

In attempts to reduce CI and extend the shelf life of pomegranate fruit, several postharvest technologies have been tested including benzyl adenine (Mirdehghan and Rahemi, 2005), polyamines (Mirdehghan *et al*., 2007; Barman and Asrey, 2011), SA (Sayyari *et al*., 2009), controlled and modified atmosphere storage and intermittent warming (IW) (Artés *et al*., 1998; 2000), hot water (HW) and wrapping (Talaie, *et al*., 2004), and shrink film wrapping and coatings (Nanda *et al*., 2001). However, no information has been reported on the combination effects of prestorage HW dipping, IW, SA, and $CaCl_2$ on the storage life of pomegranates, particularly in the Iranian 'Shishe-Kab' cultivar. Thus, the aim of this research was to evaluate the effects of HW alone or combined with these chemicals under IW or cold storage (CS) conditions on the quality maintenance and shelf life extension of pomegranate fruit during long-term cold storage.

Materials and methods

Plant material and preparation

Fully mature, medium-sized (250-310 g) pomegranate (*Punica granatum*) fruit cv. 'Shishe-Kab' were harvested in October 2011. 240 clean, free-of-defects, and uniformly-sized fruit were used to apply the following treatments (10 fruit per replicate, three replicates). Fruit were immersed in a hot water (50°C) water bath for 3 min and then immediately dipped in salicylic acid (2 mmol L^{-1}) or a combination of salicylic acid (2 mmol L^{-1}) and calcium chloride (2%) for 3 min at 20°C. Control fruit was washed only with distilled water at 20°C. Following treatments, the fruit were completely air-dried at room temperature (20 ± 1°C). Thereafter, they were divided into two lots and placed into low-density polyethylene (LDPE) bags that were left slightly open. In order to stimulate the occurrence of CI and decay, they were stored in a cool room at 3°C and 80% relative humidity (RH) for 22 wk. Fruit intermittent warming (IW) cycles of 1 d at 17 ± 1°C every 6 d of storage at 3°C were studied. Warming cycles were applied to the first lot by removing the corresponding boxes from the cold room to another room at 17°C and 70% RH by wk 10. From wk 11, IW fruit were stored at 3°C until the end of the experiment. The second lots of fruit were kept in cold storage (CS) at 3°C for 22 wk. After 10 wk of storage, the fruit were assessed for physico-chemical parameters, while decay, CI, shelf life, and sensory assessments were evaluated after 22 wk of storage. The fruit were kept for a further 3 d at 18 ± 1°C before quality parameters and decay were assessed.

Fruit weight loss and firmness measurement

To calculate weight loss, fruit weights were measured just after harvest and after 22 wk of storage; data was expressed as a percentage of the initial value. Fruit firmness was measured using a digital penetrometer (Extech Co., Fruit Hardness Tester, Model FHT 200, USA) fitted with a 3 mm diameter tip, and data was recorded as Newton.

Total soluble solids (TSS), titrable acidity (TA), TSS/TA ratio and pH

Total soluble solid in the extracted juice of fruit was measured by a hand-held refractometer (Extech Co., Model RF 10, Brix, 0–32 %, USA), and the results were expressed as ºBrix. To measure titrable acidity, 5 ml of extracted fruit juice was diluted to 40 ml with distilled water and titrated with 0.1 N sodium hydroxide. Titrable acidity was calculated as percentage of citric acid by the following formula (Nielsen, 2010):

$$\% \text{ acid (wt/vol)} = N \times V1 \times Eq\ wt / V2 \times 10$$

where N = normality of titrant (usually NaOH (mEq/ml)), V1 = volume of titrant (ml), Eq. wt. = Equivalent weight of predominant acid (mg/mEq), V2 = volume of sample (ml).

pH of the juice was also evaluated using a digital pH meter (Extech Co., USA).

Chilling injury (CI), unmarketable fruit, and shelf life

Chilling injury indices were individually evaluated in each fruit on a 4-point hedonic scale based on the percentage of husk surface affected by CI symptoms (dehydration, browning, and pitting): 0 (no symptom), 1 (1–25% of surface damaged), 2 (26–50% of surface damaged), and 3 (>51% of the surface damaged).

Unmarketable fruit was evaluated as a result of CI or other undetermined parameters based on undesirable fruit after 22 wk storage. The percentage of unmarketable fruit was calculated with the following formula:

Number of fruit in each treatment that were undesirable / number of fruit in each treatment × 100.

Shelf life was based on the physical appearance of the fruit as judged by the retention of freshness, color, and glossy appearance without any desiccation, pathogenic decay, and/or chilling injury.

Organoleptic evaluation

Sensorial quality for aril color, taste and juiciness of fruit was evaluated by a panel of five subjects after 22 wk of storage. The evaluation was scored on a scale of 1–5, where a score of 5 indicated the fruit was very good (evident harvest freshness, bright pink juicy arils, and absence of off-flavor), and a score of 1 was considered a very bad degree (complete dislike, desiccated fruits with tough brown peel, brown color arils with low juiciness and becoming dry). A score of 3 (like moderate with retention of freshness, color, and juiciness of arils) and above was considered acceptable for commercial purposes.

Statistical analysis

The experiments were conducted using a completely randomized design with three replicates. Data from the analytical determinations were subjected to analysis of variance (ANOVA). Mean comparisons were performed using the least significance difference (LSD) at P<0.05. All analyses were performed with GenStat (Discovery Edition, Version 7.2, 2008, VSN International Ltd., UK).

Results

Fruit weight loss and firmness

HW treatment alone or in combination with SA and $CaCl_2$ had no significant effect on fruit weight loss compared with the control in both CS and IW condition after 10 wk of storage (Table 1). After 10 wk, the firmness of the control fruit decreased significantly (Table 1). Fruit treated with a combination of HW and SA or HW, SA, and $CaCl_2$ had significantly greater firmness compared with fruit treated with HW alone when stored with IW.

Table 1. Effects of prestorage treatments and storage conditions on weight loss and firmness of 'Shishe-Kab' pomegranate after 10 wk of storage

Prestorage Treatments	Storage condition*	Weight loss (%)	Firmness (N)
At harvest		-	51.5a
Control	CS	3.05a	25.06b
	IW	2.33a	24.76b
Hot water (HW)	CS	2.47a	20.38bc
	IW	2.47a	17.16c
HW + SA	CS	2.38a	21.24bc
	IW	2.29a	23.34bc
HW + SA + $CaCl_2$	CS	2.40a	23.08bc
	IW	3.28a	21.10bc

Columns with different letters indicate significant differences at P <0.05 according to LSD test (n =15). * CS (Cold storage), IW (Intermittent warming storage).

Total soluble solids (TSS), titratable acidity (TA), TSS/TA ratio and pH

The HW treatment alone or in combination with SA and $CaCl_2$ had no significant effect on TSS as compared to the control (Table 2). At the end of both CS and IW storage, there was a slight decrease in pH in the control and treated fruit compared with fruit at harvest (Table 2). However, HW, combined treatments, and also IW conditions had no significant effects on fruit pH after 10 wk of storage.

Similarly, HW and chemical treatments had no significant effects on TA and the TSS/TA ratio. However, TA was increased and the TSS/TA ratio decreased in treated fruit compared with fruit at harvest. Overall, the results showed that HW alone or in combination with chemicals had no significant effects on TSS, TA, TSS/TA ratio, or pH of juice.

Table 2. Effects of prestorage treatments and storage condition on chemical changes of 'Shishe-Kab' pomegranate juice after 10 wk of storage

Prestorage treatments	Storage condition*	TSS (%)	TA (%)	TSS/TA	pH
At harvest		15.9a	0.62b	25.6a	3.86a
Control	CS	15.62a	1.66a	9.74b	3.41b
	IW	17.16b	1.78a	9.89b	3.40b
Hot water (HW)	CS	15.72a	1.77a	9.14b	3.29b
	IW	16.04a	1.72a	9.56b	3.47b
HW + SA	CS	16.61a	1.89a	8.85b	3.23b
	IW	15.98a	1.70a	9.58b	3.47b
HW + SA + $CaCl_2$	CS	16.66a	1.78a	9.67b	3.38b
	IW	16.50a	1.85a	9.12b	3.41b

Columns with different letters indicate significant differences at P <0.05 according to LSD test (n =15). * CS (Cold storage), IW (Intermittent warming storage).

Chilling injury (CI), unmarketable fruit and shelf life

As expected, CI increased during the storage period but was affected by treatment (Table 3). After 22 wk of cold storage, the control fruit had significantly higher CI symptoms than treated fruit. The HW treatment alone decreased CI significantly compared to the control. Combined treatments were more effective than the HW treatment alone in reducing CI (Table 3).

Table 3. Effects of prestorage treatments and storage condition on unmarketable fruit, chilling injury (CI), and shelf life of 'Shishe-Kab' pomegranate fruit after 22 wk of storage

Prestorage treatments	Storage condition*	CI	Unmarketablefruit (%)	Shelf life (wk)
Control	CS	3a	100a	11g
	IW	2.9a	100a	12f
Hot water (HW)	CS	2.3b	88.8b	13e
	IW	2.1b	73.2c	15d
HW + SA	CS	1.1c	69.0d	15d
	IW	0.95c	66.6de	17c
HW + SA + $CaCl_2$	CS	0.3d	47.8g	18b
	IW	0.5d	59.2f	19a

Columns with different letters indicate significant differences at P <0.05 according to LSD test (n =15). * CS (Cold storage), IW (Intermittent warming storage).

After 22 wk of storage, all control fruit were unmarketable in both CS and IW storage (Table 3). HW dips alone or in combination with SA or $CaCl_2$ treatments significantly decreased the percentage of decay compared with the control fruit. Combination treatments of chemicals with HW were more effective in reducing the number of undesirable fruit than the HW treatment alone (Table 3), particularly when treated with both SA and $CaCl_2$.

Regardless of the treatments, IW-treated fruit had a significantly greater shelf life than fruit stored in CS conditions (Table 3). However, fruit treated with chemicals combined with an HW treatment had a significantly greater shelf life than fruit which had been treated with HW alone, especially when treated with both SA and $CaCl_2$ after the HW dip. The greatest shelf life (19 wk) was obtained with this combined treatment compared to the control (11 wk).

Organoleptic evaluation

There was a decrease in aril color, taste, and juiciness of the control fruit (compared with at harvest) when stored in both CS and IW conditions that resulted in unacceptable fruit as scored by panelists (Table 4). HW dips increased fruit quality compared to the control in organoleptic aspects, although the taste and juiciness were not acceptable. However, fruit that were treated with HW combined with SA or SA and $CaCl_2$ had acceptable sensory properties as judged by panelists.

Table 4. Effects of prestorage treatments and storage condition on sensory traits of 'Shishe-Kab' pomegranate fruit after 22 wk of storage

Prestorage treatments	Storage condition*	Organoleptic score* Aril color	Taste	Juiciness
At harvest		4.7a	4.6a	4.3a
Control	CS	1.3c	1.1d	1.4d
	IW	1.2c	1.2d	1.3d
Hot water (HW)	CS	3.1b	2.4c	2.8c
	IW	3.0b	2.6c	2.9c
HW + SA	CS	4.1ab	3.8b	3.5b
	IW	3.9b	3.7b	3.3b
HW + SA + $CaCl_2$	CS	4.5a	4.2ab	3.9ab
	IW	4.3a	4.1ab	3.7b

* Score: 1, very bad; 3, acceptable; 5 very good

Columns with different letters indicate significant differences at P <0.05 according to LSD test (n =15). * CS (Cold storage), IW (Intermittent warming storage).

Discussion

The results demonstrated that the combination of HW, SA, and $CaCl_2$ was more effective in extending shelf life and reducing fruit decay than the application of each treatment alone during long-term cold storage. This is in agreement with previous reports of pomegranate fruit treated separately with HW (Talaie *et al*., 2004; Mirdehghan and Rahemi, 2005), IW (Artés *et al*., 1998), SA (Sayyari *et al*., 2009), and calcium (El-Kassas *et al*., 1995). The results not only indicated that combined treatments had a greater positive effect on the quality maintenance of pomegranates, as previously reported for strawberries (Shafiee *et al*., 2010) and peaches (Cao *et al*., 2010), but also confirmed our recent report regarding the combined effects of chemical treatments on the quality improvement of pomegranate fruit (Moradinezhad *et al*., 2013). CI significantly decreased when pomegranates were treated with a combination of chemical treatments after an HW dip, which is in agreement with the findings of Wang *et al*. (2006) for peaches and Sayyari *et al*. (2009; 2011) for pomegranates. These benefits were probably achieved due to an improvement in the defense resistance system of pomegranates against postharvest pathogens, the reduction of susceptibility to chilling injury, or both. Calcium chloride and/or the induction of the defense system by SA application have been widely used as a preservative and firming agent by fruit and vegetable industries (Chan and Tian, 2006). The results showed that SA had significant protective and preventive activity on main postharvest pathogens in pomegranate fruit, as decay by various pathogens is a major cause of postharvest loss during long-term storage (Caleb *et al*., 2012). Fruit treated with a combination of HW, SA, and $CaCl_2$ had the greatest shelf life and minimum amount of decay. Lara *et al*. (2004) also reported that calcium treatments prevented postharvest disorders and retarded fruit ripening and decay in strawberries. Calcium is involved in maintaining the textural quality of produce since calcium ions form cross-links or bridges between free carboxyl groups of the pectin chains, thus strengthening the cell wall (Garcia *et al*., 1996).

Heat treatments have been reported to induce tolerance to low temperatures in many commodities (Lurie, 1998; Ferguson *et al*., 2000; Fallik, 2004) and to cause the inhibition of ethylene synthesis (Paull and Chen, 2000). The effect of temperature has been shown to be of major importance in the results of dipping-washing treatment. Garcia *et al*. (1996) reported that the use of warm temperatures (40-60°C) increased the beneficial effects of the treatment because of higher washing solution retention inside the product (Garcia *et al*., 1996).

The HW dip showed similar results, but intermittent warming during CS storage had no significant effect on the reduction of chilling injury of fruit husk in contrast with results reported by Artes *et al*. (2000). In their case, pomegranate fruit remained in cold storage for 90 d, whereas in our research fruit was subjected to cold storage for a longer period (154 d) and, consequently, a greater impact of chilling injury occurred.

After 10 wk of storage, firmness of the control fruit decreased. The postharvest storage of fruit is accompanied by loss of cell wall integrity due to the breakdown of pectic substances, which leads to an increase in soluble pectin and a decrease in fruit firmness. However, HW treated fruit, when stored in IW conditions, had the largest loss of firmness. This result is in agreement with the findings of Girardi *et al*. (2005) who reported that IW caused a significant decrease in the firmness of peach fruit. HW and chemical treatments had no significant effects on the TSS of pomegranate fruit compared to the control. As expected for a non-climacteric fruit, slight changes in pH, TA, and TSS were detected. Values of these parameters confirmed previous reports (Kader *et al*., 1984; Artes *et al*., 1998; 2000).

Conclusion

Combined prestorage treatments of HW and SA and $CaCl_2$ have more benefits than their individual application on maintaining quality and extending pomegranate fruit shelf life in prolonged cold storage. Under this combined treatment, 'Shishe-kab' pomegranate fruit could be stored for at least 18 wk, effectively extending their marketing period with less decay. The control fruit, however, spoiled totally by wk 11. It is therefore concluded that a combination of HW, SA, and $CaCl_2$ treatment is a simple and low cost method that has the ability to improve quality and postharvest life of pomegranate fruit cv. 'Shishe-Kab' during cold storage. However, more research is needed in this regard.

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