DETERMINING THE PHYSIOCHEMICAL CHANGES AND TIME OF CHILLING INJURY INCIDENCE DURING COLD STORAGE OF POMEGRANATE FRUIT

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Abstract: Intermittent warming (IW) is a good postharvest technique to prevent or alleviate chilling injuries during cold storage. Performing the warming treatment at the period of time before chilling injury is irreversible during storage, and it is the first prerequisite for a successful IW treatment. In order to determine the fruit physiochemical changes and time of irreversible chilling injury incidence during cold storage of pomegranate fruit (cv. Rabab-e-Neyriz), this research was conducted. Fruits were stored at 2 ± 0.5 °C and 90 ± 5% relative humidity for 90 days. At 15-day intervals, 40 fruits (four replicates and 10 fruits in each replicate) were sampled and further stored at 20°C for 3 days (shelf life). Chilling injury (CI) index and weight loss (WL) in intact fruits, electrolyte leakage (EL) and K leakage (KL) in peel samples, total soluble solids (TSS), titratable acidity (TA), TSS/TA ratio and pH in fruit juice were measured. With respect to quality parameters, TSS did not change significantly under cold storage. According to TA changes, the TSS/TA ratio was decreased up to 30 days but subsequently increased and the highest ratio was detected at the end of storage, which was significantly higher than the TSS/TA ratio at the harvest time. Results related to CI index, WL, EL and KL showed that pomegranate fruits could be stored cold without significant chilling damages up to 30 days. It was suggested that performing the IW treatment during this period could be concomitant with desired effects in long-term storage of this commercial cultivar.

Key words: chilling injury, cold storage, electrolyte leakage, K leakage, pomegranate, titratable acidity, total soluble solids, weight loss.

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Introduction

Pomegranate (*Punica granatum* L.) is considered one of the oldest known edible fruits and probably originated in a region of Iran, where it was first cultivated in about 2000 B.C. (Valero et al., 2015). According to statistics, Iran is the main producer whose production exceeded 500,000t in 2007 (Valero et al., 2015). ‘Malas-e-Saveh’, ‘Naderi-e-Budrood’, ‘Malas-e-Yazdi’, ‘Sishe Kape-Ferdos’, and ‘Rabab-e-Neyriz’ are five commercially important Iranian cultivars whose fruit characteristics and their potential for further breeding were discussed by Varasteh et al. (2006). These cultivars are late ripening, medium to large size with thick red rind and red arils.

Pomegranate fruit is a highly perishable commodity (Mirdehghan and Rahemi, 2002), with the major storage problems being water loss and browning symptoms in both peel and arils (Valero et al., 2015). However, to avoid excessive desiccation and decay, storage at low temperatures is necessary, although it is confirmed that long periods of cold storage lead to occurrence of a wide range of alterations known as chilling injury (CI) symptoms, manifested as skin browning, surface pitting and higher susceptibility to decay (Kader, 2006; Valero and Serrano, 2010). Furthermore, depending on duration of storage, these symptoms could reach the arils and the white segments, resulting in a reduction in both internal and external fruit quality (Mirdehghan et al., 2007).

In order to alleviate CI symptoms in sensitive fruits, different postharvest treatments such as intermittent warming (IW) have been effectively used (Toivonen, 2003). IW consists of exposing fruits to one or more periods of high temperature during cold storage (Wang, 1993). It has been shown that IW treatments could ameliorate membrane changes and injuries associated with chilling temperatures (Toivonen, 2003). For example, the effectiveness of IW in delaying or alleviating fruit CI in oranges (Schirra and Cohen, 1999), limes (Kluge et al., 2003), plums (Kluge et al., 1997) and peaches (Fernández-Trujillo and Artés, 1997; Zhu et al., 2010) has been reported. However, there are few and contradictory reports on IW treatment of pomegranate fruits during cold storage. Abda et al. (2010) stated that IW had no positive effect on reducing chilling injury, electrolyte leakage and fruit decay in pomegranate. On the other hand, Artés et al. (2000) concluded that IW of 1 day at 20°C every 6 days at 2°C storage was very beneficial for minimizing CI and maintaining fruit quality. Also, Mirdehghan and Rahemi (2002) reported that intermittent warming of 12 h at 25°C every 4 or 6 weeks during cold storage significantly reduced CI and fruit decay. Evaluating the IW ability on maintaining the fruit quality during cold storage could be an interesting scientific field for commercially important cultivars of pomegranate. The reason is the importance
of pomegranate fruit as a rich source of bioactive compounds applicable in human health diet and related industries. Determining optimum storage conditions incorporating IW that will favour commercial adoption is challenging (Biswas et al., 2012). Inconsistent reports regarding the effectiveness of IW in reducing CI could be due to its dependence on the cultivar and time–temperature regimes (Biswas et al., 2012). The first prerequisite for a successful IW treatment is to perform the intended warming treatment at the period of time before chilling injury becomes irreversible during storage time (Mirdehghan and Rahemi, 2002). So, it is required to determine the time of irreversible CI incidence during cold storage of important pomegranate cultivars. To the best of our knowledge, there is no any previous report on physiochemical changes of ‘Rabab-e-Neyriz’ pomegranate fruits during cold storage. In this research, we studied the changes and determined the exact time of CI incidence, too.

**Material and Methods**

Pomegranate (cv. Rabab-e-Neyriz) fruits were picked on October 20, 2012 in a commercial orchard in Neyriz (Fars province, Iran). Fruits were harvested when fully mature according to commercial practice and immediately transported to the laboratory. Those with defects (sunburn, crack, bruise and cut in the husk) were discarded and the remaining fruits were stored at 2 ± 0.5 °C and 90 ± 5% relative humidity for 90 days.

After 15, 30, 45, 60, 75 and 90 days, 40 fruits (four replicates and 10 fruits in each replicate) were sampled and further stored at 20°C for 3 days (shelf life). Evaluations of CI index and weight loss (WL) were conducted on intact fruits. Afterwards, some of the randomly selected peel tissues of each replicate were used for measuring the electrolyte leakage (EL) and K leakage (KL). Finally, total soluble acids (TSS), titratable acidity (TA), TSS/TA ratio and pH of fruit juice were measured.

CI index was individually evaluated in each fruit with a 4-point hedonic scale based on the percentage of husk surface affected by CI symptoms (dehydration, browning and pitting) (Sayyari et al., 2009): 0 (no symptom), 1 (1–25% of the damaged area), 2 (26–50% of the damaged area) and 3 (≥51% of the damaged area). CI index was calculated using the following formula:

$$\text{CI} = \frac{\sum (\text{value of hedonic scale} \times \text{number of fruits with the corresponding scale number})}{4 \times \text{total number of fruits in the sample}}.$$  

WL was calculated as: \(\text{WL} = \frac{\left(W_i - W_t\right)}{W_i} \times 100\), where \(W_i\) is the weight (g) of the fruit at the beginning of storage and \(W_t\) (g) is the weight of the fruit at the sampling date. Weight loss of pomegranate fruits in each replicate was the mean value of 10 fruits.
To determine the rate of EL, the method described by McCollum and McDonald (1991) was used. For each replicate, ten similar discs (10 mm) of peel tissue were randomly cut with a cork borer. Conductivity was measured after 4 h of incubation in 25 mL of 0.4 M mannitol under constant shaking, using a conductivity meter (644 Conductometer, Metrohm, Herisau, Switzerland). Then, the vials were autoclaved at 121°C for 20 minutes, held overnight and conductivity was measured again for total electrolytes. The rate of EL was expressed as a percentage of the total: \((initial/total) \times 100\).

Determining the rate of KL was performed according to Lurie and Klein (1991). The sample preparation method was the same as described for EL evaluation. Initial and total K contents in the solution were measured by flame photometry (Model Jenway PFP7, Bibby Scientific Ltd, Staffordshire, UK) and the rate of KL was expressed as a percentage of the total: \((initial/total) \times 100\).

TSS was determined with an Atago PR-101 (Atago Co. Ltd., Tokyo, Japan) digital refractometer at 20°C and expressed as percent (° Brix). TA was measured by titration method using 0.1 N NaOH up to pH 8.1, according to Sayyari et al. (2010). TSS/TA ratio was calculated by dividing TSS by TA. Juice pH was measured by a pH meter (744, Metrohm, Herisau, Switzerland).

All the data were expressed as means ± standard deviation (SD) of 4 replicates, and subjected to analysis of variance (ANOVA) using the SAS 9.1.3 service pack 4 software (SAS institute, Cary, NC, USA). Furthermore, the means were separated by least significant difference (LSD) test at \(P \leq 0.05\).

Results and Discussion

As shown in Figure 1, cold storage up to 1 month had no significant effect on CI index, but afterwards, an increasing trend was measured in which all differences were statistically significant. Also, as storage time passed, the rate of WL significantly increased but there was no significant difference between days 15 and 30 (Figure 2). Compared to harvest time, low temperature caused an increase in EL, but at days 15 and 30, statistically similar values were detected. Afterwards, the rate of EL was significantly increased up to 60 days and subsequently decreased, as evaluated fruits at days 75 and 90 had significantly lower EL compared to fruits at day 60 (Figure 3). The pattern of changes in KL during storage was exactly similar to what was stated for EL, too (Figure 4).

Significant increases in weight loss, membrane permeability and ion leakage are integral parts of the behavior of chilling sensitive tissues encountered at chilling temperatures. Normally, these changes happen before a significant incidence of CI symptoms (King and Ludford, 1983; Murata, 1990; Murata and Tatsumi, 1979, Saltveit, 2002).
Figure 1. Chilling injury (CI) index of pomegranate (cv. Rabab-e-Neyriz) fruit after several periods of cold storage (at 2 ± 0.5°C and 90 ± 5% relative humidity) + 3 days at 20°C (shelf life). Data are means of 4 replicates (10 fruits in each replicate) ± SD. Storage times with the same letter are not significantly different by LSD test at p ≤ 0.05.

Figure 2. Weight loss of pomegranate (cv. Rabab-e-Neyriz) fruit after several periods of cold storage (at 2 ± 0.5°C and 90 ± 5% relative humidity) + 3 days at 20°C (shelf life). Data are means of 4 replicates (10 fruits in each replicate) ± SD. Storage times with the same letter are not significantly different by LSD test at p ≤ 0.05.
Figure 3. Electrolyte leakage of pomegranate (cv. Rabab-e-Neyriz) fruit peel after several periods of cold storage (at 2 ± 0.5°C and 90 ± 5% relative humidity) + 3 days at 20°C (shelf life). Data are means of 4 replicates (10 fruits in each replicate) ± SD. Storage times with the same letter are not significantly different by LSD test at p ≤ 0.05.

Figure 4. K leakage of pomegranate (cv. Rabab-e-Neyriz) fruit peel after several periods of cold storage (at 2 ± 0.5°C and 90 ± 5% relative humidity) + 3 days at 20°C (shelf life). Data are means of 4 replicates (10 fruits in each replicate) ± SD. Storage times with the same letter are not significantly different by LSD test at p ≤ 0.05.
Results related to CI index, WL, EL and KL showed that fruits stored up to 1 month had no significant incidence of CI symptoms (Figures 1, 2, 3 and 4). So, it can be concluded that these changes are reversible and consequently the IW treatment during this period could be concomitant with desired effects. Mirdehghan and Rahemi (2002) indicated that performing the IW treatment before the incidence of significant CI symptoms during cold storage could be a useful technique to reverse the undesirable damages and to keep the quality of pomegranate fruits.

The significant increases of EL and KL in peels from day 30 to the highest amounts at day 60 could be related to occurrence of severe damages to cell membranes during this period (Figures 3 and 4). As a result, lower leakages were detected in the remaining days of storage (Figures 3 and 4). These data confirmed the previous finding that storage time longer than 1 month at temperatures below 5°C can be concomitant with different CI symptoms in pomegranates (Valero and Serrano, 2010).

As expected in a non-climacteric fruit, pomegranates have slight changes in TSS, TA and pH during cold storage (Artes et al., 2000). The taste of pomegranate is determined mainly by TSS and the ratios between TSS and TA (Fawole and Opara, 2013). With respect to quality parameters, TSS did not change significantly under cold storage (Figure 5). These data were in agreement with Sayyari et al. (2009, 2010). It was likely that the moisture loss in the pomegranate fruit during storage was mainly from the peel and not from the arils; hence there was no increase in TSS due to concentration.

On the other hand, TA was increased up to 1 month of storage and afterwards, a decreasing pattern was recorded (Figure 6). So, the TSS/TA ratio was decreased up to 30 days but subsequently increased and the highest ratio was detected at the end of storage, which was significantly higher than that at harvest time (Figure 7). This data was in agreement with Meighani et al. (2014), who reported an increase in TSS/TA ratio after long-term storage of ‘Malase Torshe Saveh’ pomegranate as compared to harvest time.
Figure 5. Total soluble solids (TSS) of pomegranate (cv. Rabab-e-Neyriz) fruit juice after several periods of cold storage (at 2 ± 0.5°C and 90 ± 5% relative humidity) + 3 days at 20°C (shelf life). Data are means of 4 replicates (10 fruits in each replicate) ± SD. Storage times with the same letter are not significantly different by LSD test at p ≤ 0.05.

Figure 6. Titratable acidity (TA) of pomegranate (cv. Rabab-e-Neyriz) fruit juice after several periods of cold storage (at 2 ± 0.5°C and 90 ± 5% relative humidity) + 3 days at 20°C (shelf life). Data are means of 4 replicates (10 fruits in each replicate) ± SD. Storage times with the same letter are not significantly different by LSD test at p ≤ 0.05.
Figure 7. TSS/TA ratio of pomegranate (cv. Rabab-e-Neyriz) fruit juice after several periods of cold storage (at 2 ± 0.5°C and 90 ± 5% relative humidity) + 3 days at 20°C (shelf life). Data are means of 4 replicates (10 fruits in each replicate) ± SD. Storage times with the same letter are not significantly different by LSD test at p ≤ 0.05.

Figure 8. pH of pomegranate (cv. Rabab-e-Neyriz) fruit juice after several periods of cold storage (at 2 ± 0.5°C and 90 ± 5% relative humidity) + 3 days at 20°C (shelf life). Data are means of 4 replicates (10 fruits in each replicate) ± SD. Storage times with the same letter are not significantly different by LSD test at p ≤ 0.05.
Organic acids are the main respiratory substrates during cold storage of pomegranates (Sayyari et al., 2011). Since pomegranate is a non-climacteric fruit, reduction in TA level is a strong indicator of the ongoing metabolism in the fruit during cold storage (Fawole and Opara, 2013). The pattern of changes in pH included a decrease in the first 30 days and afterwards, a continuous increase until the end of storage (Figure 8). Elyatem and Kader (1984) also reported an increase in pH of pomegranate stored at 0–10°C for 16 weeks. The change in pH is associated with changes in TA and might be due to the biochemical condition of the fruit and the rate of respiration and metabolic activity during cold storage (Jitareerat et al., 2007).

Conclusion

It was concluded that pomegranate (cv. Rabab-e-Neyriz) fruits could be stored without significant chilling damages up to 30 days at 2 ± 0.5°C and 90 ± 5% relative humidity. It might be worthwhile to investigate the efficacy of performing the IW treatment (as a potentially beneficial treatment) in alleviating the CI incidence and keeping the fruit quality in this commercial cultivar during the first month of storage. For this purpose, there is a need to design and conduct an accurate study in order to examine the best warming temperature and its frequency during cold storage.

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UTICAJ DUŽINE ĆUVANJA PLODOVA NARA NA MOMENAT POJAVE OŠTEĆENJA NASTALIH POD UTICAJEM NISKIH POZITIVNIH TEMPERATURE KAO I NA FIZIČKO-HEMIJSKE PROMENE PLODOVA

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Rezime

Povremeno grejanje (IW, engl. intermittent warming) je tehnika koja se primenjuje posle berbe kako bi se sprečila ili ublažila oštećenja uzrokovana čuvanjem plodova na niskim pozitivnim temperaturama tokom skladištenja. Ovo istraživanje je sprovedeno kako bi se utvrdile fizičko-hemijske promene i vreme pojava oštećenja uzrokovanih niskim temperaturama tokom čuvanja plodova nara (sorta Rabab-e-Neyriz) u hladnjaci. Plodovi su bili skladišteni na 2± 0.5°C i 90 ± 5% relativne vlažnosti tokom 90 dana. U petnaestodnevnim intervalima, 40 plodova (četiri ponavljanja i 10 plodova u svakom ponavljanju) su bili uzorkovani i dalje skladišteni na 20°C tokom 3 dana (rok trajanja). Ispitivan je indeks oštećenja plodova uzrokovani niskim temperaturama (CI, engl. chilling injury), gubitak mase ploda ili kalo (WL, engl. weight loss) kod netaknutih plodova, curenje elektrolita (EL, engl. electrolyte leakage) i curenje K (KL, engl. K leakage) kroz pokožicu ploda, sadržaj ukupne rastvorljive suve materije (TSS, engl. total soluble solids), sadržaj ukupnih kiselina (TA, engl. titratable acidity), indeks slasti i pH vrednost u soku ploda. U pogledu parametara kvaliteta, indeks rastvorljive suve materije se nije značajno menjao tokom čuvanja plodova u hladnjaci. Zbog početnog povećanja sadržaja ukupnih kiselina indeks slasti se smanjivao tokom prvih 30 dana čuvanja plodova. Nakon 30 dana, zbog smanjenja sadržaja ukupnih kiselina, indeks slasti se povećao i bio je najveći na kraju skladištenja. Rezultati povezani sa indeksom CI, WL, EL i KL su pokazali da se plodovi nara mogu skladištit u hladnjaci do 30 dana bez značajne štete uzrokovane niskim temperaturama. Sugestirano je da se sa izvođenjem tretmana za povremeno grejanjem tokom ovog perioda, može produžiti kvalitet čuvanja kod ove komercijalne sorte.

Ključne reči: oštećenje uzrokovano niskim pozitivnim temperaturama, hladnjača, curenje elektrolita, curenje K, nar, titraciona kiselost, ukupne rastvorljive suve materije, gubitak mase.

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