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Effects of salicylic acid, jasmonic acid and calcium chloride treatments on reduction of chilling injury in pomegranate fruit

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Abstract. The aim of this experiment was to investigate the effects of salicylic acid, jasmonic acid and calcium chloride on reduction of chilling injury in pomegranate fruits cvs. 'Malas Yazdi' and 'Malas Ashkezar'. Salicylic acid (1 and 2 mM from source of acetyl salicylic acid), jasmonic acid (0.3 and 0.4 mM from source of n-propyl dihydrojasmonate), calcium (1 and 2 % from source of calcium chloride) and control (distilled water) were used as treatment. After the treatments fruits were stored in cold storage at 1.5 ^{o}C±0.5 and 85±5% relative humidity for 2 months. The results revealed that treatments with salicylic acid (SA), jasmonic acid (JA) and calcium chloride ($CaCl_2$) significantly reduced the percentage of chilling injury of pomegranate fruits. The lowest chilling injury was observed in 0.4 mM of JA-treated fruits and the highest one was for untreated fruits. Treatments of fruits with 0.3 mM JA and 2 mM SA increased the electrolyte leakage of fruits but it was not significantly affected by other treatments. It was also showed that treatments of fruits with SA, JA and $CaCl_2$ had no significant effect on pH, ripening index, but the total soluble solids of fruit juices was increased. The results evidenced that pomegranate fruits treated with 2 mM SA had the lowest ascorbic acid, but it was not significant difference between other treatments and untreated fruits. In conclusion, cultivar of 'Malas Ashkezar' exhibited higher tolerance to cold storage compared to 'Malas Yazdi', therefore cultivar of 'Malas Ashkezar' recommended to keep in cold storage for long time.

Keywords. Arils – Phenolic compounds – Antioxidants – Browning.

I – Introduction

Pomegranate (*Punica granatum* L.) belonging to the family punicaceae is one of the favorite table fruits of tropical and subtropical regions. The edible part of the fruit is called arils, which contain around 80% juice and 20% seed. The fresh juice contains 85.4% moisture and considerable amounts of total soluble solids, total sugars, reducing sugars, anthocyanins, phenolics, ascorbic acid and proteins (El-Nemr *et al.*, 1990) and has also been reported to be a rich source of antioxidants (Gil *et al.*, 2000; Kulkarni *et al.*, 2004).

To reduce the occurrence of chilling injury (CI) in pomegranate several techniques have been applied, including intermittent warming (Artes *et al.*, 2000; Mirdehghan *et al.*, 2007b), polyamine (Mirdehghan *et al.*, 2007a), salicylic acid (Sayyari *et al.*, 2009).

Salicylic acid (SA) is known for its induction of plant defense against biotic and abiotic stress and is reported to increase chilling tolerance in peach fruit (Wang *et al.*, 2006), tomato fruit (Ding *et al.*, 2002) and sweet peppers (Fung *et al.*, 2004. Exogenous SA treatment may also induce the expression of pathogenesis-related (PR) protein (Malamy *et al.*, 1990) and establish systemic acquired resistance (SAR) (Gaffney *et al.*, 1993).

In recent research, methyl jasmonate (MeJA) has been applied to reduce the development of chilling injury symptoms in a number of horticultural crops, including Zucchini squash (Wang and Buta, 1994), mango (Gonzalez-Aguilar *et al.*, 2000), avocado, grapefruit and peppers (Meir *et al.*, 1996). Reduction in chilling injury by MeJA might be due to enhanced antioxidant enzyme activity and a higher unsaturated/saturated fatty acid ratio (Cao *et al.*, 2009).

The postharvest application of calcium to some horticultural commodities has been demonstrated to reduce the incidence of chilling-induced disorders. Application of calcium significantly reduces the severity of chilling injury in avocados (Chaplin and Scott, 1980), peaches (Wade, 1981) and tomatoes (Moline and Teasdale, 1981). Lester and Grusak (1999) have shown that calcium application in plums was effective in terms of membrane functionality and integrity maintenance, with lower losses of phospholipids and proteins and reduced ion leakage. The aim of this study was to determine the effects of SA, JA and $CaCl_2$ on reducing CI in pomegranates stored at 1.5± 0.5°C followed 3 days at 20°C.

II – Material and methods

1. Plant material and treatment

Pomegranate fruits cvs. 'Malas Yazdi' and 'Malas Ashkezar' at ripe stage were harvested on October 14, 2008 from the Agricultural Research Center of Yazd province. Fruits were immediately transported to the laboratory. Pomegranates were sorted based on size and the absence of physical injuries or sunburn, then were randomly divided into 7 lots of 128 fruits for the following treatments: lot 1 and 2 were immersed into solution of 1 mM and 2 mM ASA, pH 3.5 for 5 min. lot 3 and 4 were immersed into the solution of 0.3 mM and 0.4 mM PDJ at 25ºC for 15 min. lot 5 and lot 6 were dipped in 1% and 2% $CaCl_2$ solution containing tween-20 (2ml l^{-1}) for 5 min. The last lot of fruits was dipped for 5 min in distilled water, which served as the control. After immersion, the fruits were air-dried and then stored at 1.5± 0.5ºC and 85± 5% relative humidity (RH) for 63 days. Fruit samples were taken after immersion (0) and at 21-day intervals during storage and further stored at 20ºC for 3 days.

2. Chilling injury and electrolyte leakage

CI index was evaluated according to external skin browning, as follows: 0 (no symptom); 1 (20% of the browning lesion); 2 (40% of the browning lesion); 3 (60% of the browning lesion); 4 (80% of the browning lesion); 5 (100% of the browning lesion). The severity of CI was calculated by the following formula: CI= Total No × 100/ 5 × N. of fruits

The rate of electrolyte leakage was determined as described by McCollum and McDonald (1991). For each husk, six discs (10 mm) of peel tissue were cut with a cork borer. Conductivity after incubation in 25 ml of 0.4 M Manitol was measured with a conductivity meter after 4h of incubation under constant shaking. After reading was taken, the vials were autoclaved at 121ºC for 20 min, held overnight and conductivity was measured again for total electrolytes. The rate of electrolyte leakage was expressed as a percentage of total: (initial/total) × 100.

3. Total soluble solids, titratable acidity and ascorbic acid

Total soluble solids concentrations (TSS) were measured with refractometer and expressed as ºBrix. Titratable acidity (TA) was assayed by titration with 0.2 N NaoH to pH 8.2 and expressed as gram of citric acid equivalent per 100 g^{-1} fresh weight. Ascorbic acid content was determined by titration with Iodine and expressed as (mg^{-1} 100g of fresh fruits).

4. Antioxidant activity and total phenolic compounds

The arils of each replicate were combined and frozen in liquid N_2, were milled to obtain homogeneous samples, and were stored at -20ºC until analysis. For each sample, 5 g of arils was homogenized in 10 ml of 50 mM phosphate buffer pH 7.8 and then centrifuged at 4800 rpm for 15 min at 4ºC. The supernatant was used for total antioxidant activity and total phenolic compounds quantification in duplicate, as previously described (Serrano *et al.*, 2005). For TAA, L-ascorbic acid was used for calibration curve, and the results were expressed as mg ascorbic acid equivalent 100 g^{-1} fw (fresh weight). The total phenolic compounds were quantified using the Folin-Ciocalteu reagent and results were expressed as mg gallic acid equivalent 100 g^{-1} fw.

5. Statistical analysis

The experimental design was factorial complete randomized design (CRR) with four replicates. Data for the analytical determinations were subjected to analysis of variance (ANOVA). Mean comparisons were performed by Duncan's Multiple Rang Test. Differences at $p< 0.05$ were considered as significant. All analyses were performed with MSTATC.

III – Results and discussion

1. Chilling injury symptoms and electrolyte leakage

In pomegranate fruits CI**,** manifested as skin browning, increased during storage but was affected by treatment. The highest chilling injury was observed for control fruit at 63 days, and the lowest one was for 0.4 mM JA and 1 mM SA at 21 days (Fig. 1). These results were agreement with Ding *et al.* (2001) who reported that MeSA and MeJA treatments reduced chilling injury in tomato fruit. The effect of MeSA and MeJA on alleviating chilling injury cold storage may be attributed to its ability to induce the accumulation of heat shock protein (HSP) (Ding *et al*., 2001) and antioxidant systems (Wang *et al*., 2006; Evans *et al*., 1991).

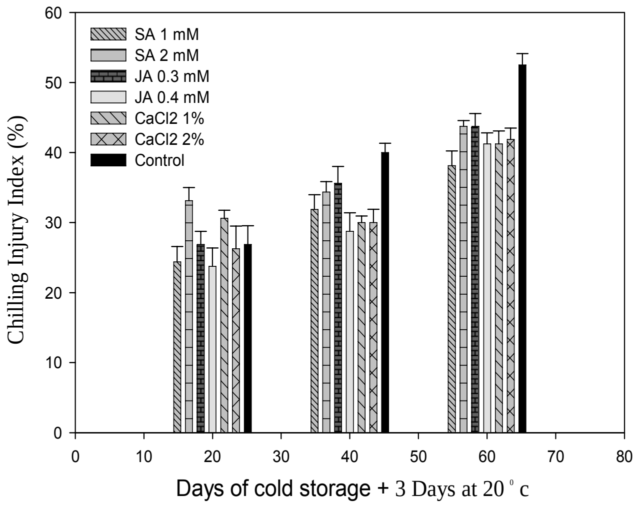

Fig.1. Chilling injury index of control and treated pomegranates after several periods of cold storage +3 days at 20ºC (shelf-life). Data are means +S.E.

With respect to electrolyte leakage (EL), there were no significant differences between treated fruits and control, whereas EL in fruits treated with 0.3 mM JA and 2 mM SA was higher than that of control (Fig. 2). Results in Fig. 2. Were showed that EL gradual increase within 42 days, followed by a decrease from 42 to 63 days. contrary to our findings Meng *et al.* (2009) who had observed that MeJA treatment in peach fruit could reduce cell membrane electrolyte leakage by maintaining membrane integrity. Also Sayyari *et al.* (2009) have reported that SA treatment were effective in reducing electrolyte leakage in pomegranate.

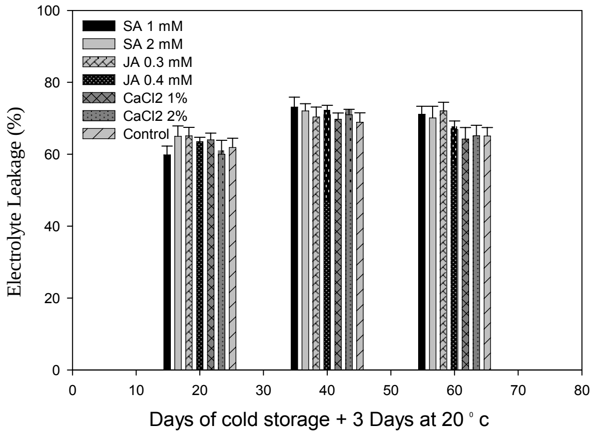

Fig. 2. Electrolyte leakage of control and treated pomegranates after several periods of cold storage +3 days at 20 ºC (shelf-life). Data are means +S.E.

2. Total soluble solids, total acidity and ascorbic acid

The influence of all treatment on total soluble solids (TAA) is shown in Table 1. SA applied at 1 mM had significantly higher TSS than non-treated fruits. These results are in line with Serivastava and Dowidy (2000) who reported that treatment with SA increased TSS in banana fruits.

Table 1. Changes in total soluble solids (TSS, ºBrix), total acidity (TA, g 100 ml^{-1}), ascorbic acid (AA, mg 100 ml^{-1}) and total phenolic compounds (mg eq. gallic acid 100 g^{-1}) in control and treated pomegranate during storage

	TSS	TA	AA	Total phenolic compounds
SA 1 mM	13.27 a	0.88 a	15.62 ab	65.69 a
SA 2 mM	13.00 ab	0.90 a	14.01 b	62.79 a
JA 0.3 mM	12.92 ab	0.89 a	15.68 ab	59.44 a
JA 0.4 mM	12.83 ab	0.90 a	15.75 ab	63.13 a
$CaCl_2$ 1%	13.09 ab	0.92 a	15.75 ab	62.78 a
$CaCl_2$ 2%	13.13 ab	0.89 a	16.48 a	62.62 a
Control	12.75 b	0.90 a	15.57 ab	62.71 a

For each parameter, similar letter within rows are not significantly at p< 0.05 level.

The results showed that total acidity (TA) were not influenced by any treatments (Table 1). Similar observation has been reported by Ding *et al.* (2007) and Biten Court De Souza *et al.* (1999) who had described that TA were not affected by SA or MeJA or $CaCl_2$, respectively. Results evidenced that pomegranate fruits treated with 2 mM SA had the lowest ascorbic acid (AA), but it was not significant difference between other treatments and untreated fruits (Table

1). Our results are consistent with the finding of González-Aguilar *et al.* (2004) who reported that ascorbic acid was not affected by the MJ treatment.

3. Total phenolic compounds and total antioxidant activity

Total phenolic compounds were not influenced by all treatments (Table 1). These results are agreement with González-Aguilar *et al.* (2004) who had described that Total phenolics were not affected by the MJ treatment. While, Rudell *et al.* (2002) reported that treatments of MJ in apple Fuji induced the accumulation of chlorogenic acid. The explanation for this behavior can be due the fact that we measured changes in total phenols. According to previous reports punicalagin has been described as the major compound in pomegranate arils contributing to total antioxidant activity (TAA) (Kulkarni *et al.*, 2004). Therefore, further research is necessary to assay amount of punicalagin separately.

Total antioxidant activity (TAA) increased at the midpoint of cold storage, then gradually decreased (Fig. 3). The results revealed that treatments with 1, 2 mM SA and 0.3 mM JA decreased TAA, but it was not significantly affected by other treatments. Contrary to our findings Huang *et al.* (2008) reported that application of SA could increase antioxidant enzyme activity and thus delay membrane lipid peroxidation. Therefore, they suggested that pretreatment with SA in combination with low temperature may be a useful strategy for prolonging orange postharvest life and maintaining nutritional conditions during storage. In pomegranate cultivars, anthocyanin, ascorbic acid and phenolics are responsible for the TAA, alone or in combination (Kulkarni *et al.*, 2004). In our experiment the content of total phenolics did not change during storage, and the amount of ascorbic acid reduced throughout storage period. While, TAA increased with prolonging storage time that may be due to increased of anthocyanin or punicalagin as the major phenolic compound that contributes to TAA.

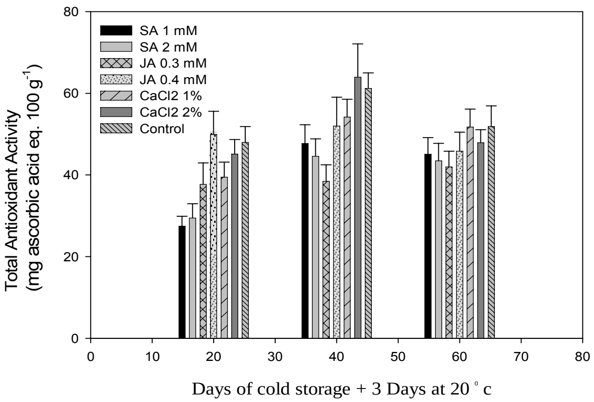

Fig. 3. Total antioxidant activity in arils during cold storage +3 days at 20ºC (SL) of control and treated pomegranates.

Based on the data it was concluded that treatment with 0.4 mM JA was the most effective for reducing CI. The reduction in chilling injury by JA may be due to enhanced antioxidant enzyme activity (Cao *et al.*, 2009). However, further research is necessary to carry out with different postharvest applications (pressure-infiltration and vaccum infiltration) and concentrations for reducing CI in pomegranate fruits and maintaining antioxidant activity and nutritional conditions during storage.

In conclusion, cultivar of 'Malas Ashkezar' had higher total soluble solids, total acidity, ascorbic acid, phenolic compounds and total antioxidant activity than that of 'Malas Yazdi'. Moreover, cultivar of 'Malas Ashkezar' exhibited higher tolerance to cold storage compared to'Malas Yazdi', therefore cultivar'Malas Ashkezar is recommended to keep in cold storage for long time.

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