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Influence of Methyl Jasmonate on Inducing Chilling Tolerance in Pomegranate Fruits (Malas Save)

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Abstract: Susceptibility to chilling injury in pomegranate is the main limiting factors for storage fruits in low temperature. Inducing chilling tolerance make possible to storage fruits in low temperature for long time. In this study effect of different concentrations of methyl jasmonate (8, 16 and 24 μL L^{-1}) as comparison with control on inducing chilling tolerance of pomegranate fruits (Malas Save) was investigated. Qualitative attributes of treated fruits was investigated in different times during low temperature storage and also after transferring that to high temperature as a shelf life. The results showed that methyl jasmonate suppressed chilling injury and water loss and preserved external appearance in pomegranate fruits without abnormal effects on internal fruits quality. In general, with increasing methyl jasmonate concentration, fruits chilling injury reduced significantly. The highest chilling injury index was found in control fruits and the lowest one was for 24 μL L^{-1} during storage in low temperature and also after placing in high temperature. Furthermore, no significant differences was found between treated and control fruits for internal fruit characteristics at end of storage.

Key words: Pomegranate (*Punica granatum* L.), chilling injury, quality attributes, pitting, weight loss, methyl jasmonate

INTRODUCTION

Pomegranates (*Punica granatum* L.) are widely grown in many tropical and subtropical countries, especially in the moderate climate of the Mediterranean region (Salaheddin and Kader, 1984). The first pomegranate production in the world is belonging to Iran but little attention is found for prolonging postharvest life of pomegranate fruits (Asghary Sarcheshme, 1990). In general, the major cause limiting the storage potential of pomegranates is the development of decay, which is often caused by the presence of fungal inoculums in the blossom end of the fruit (Hess-Pierce and Kader, 2003). This problem is aggravated at temperatures higher than 5°C, which are recommended for pomegranates to avoid chilling injury. In the other word, using of cold storage is the only effective method for enhancing storage life of pomegranate but fruits exposed below 5°C for more than 2 months manifest chilling injury (Kader *et al*., 1984). Therefore, susceptibility to chilling injury is the main limiting factors for long term storage in low temperature. Symptoms of CI in pomegranates are surface pitting, brown discoloration of the skin, husk scald, pale color of the arils, brown discoloration of the white segments separating the arils and a higher sensitivity to fungal development (Artes, 1992). This phenomenon limits storage life and leads to substantial degradation of produce quality. There are different methods to alleviate chilling injury in pomegranate fruits. It has been reported that heat treatments such as intermittent warming during low temperature storage and pretreatments with moderate temperature (15-25°C) as a conditioning and curing with 33°C can successfully delay chilling injury in fruits (Artes, 1995).

CI can be result from oxidative stress caused by Reactive Oxygen Species (ROS) (Evans *et al*., 1991) When it appears that superoxide, hydrogen peroxide and hydroxyl radicals increase from scavenging capacity of tissues (Paull, 1990). Postharvest heat treatments can reduce CI in different commodities (Wang, 1993). Plant cells respond to heat shock with production special small polypeptides (HSPs) (Sabehat *et al*., 1998) that these proteins protect cell against environmental stress and repair cell damage during the stress. There is a correlation between heat shock proteins and pathogen related proteins (PR-Proteins), therefore the treatments that led to increasing chilling injury resistance but also control decay incidence in fruits (Meir *et al*., 1998). Induction of heat shock proteins (HSPs) also induced by application of some signaling molecules. Salicylate and jasmonates are endogenous signal molecules that play essential roles in regulating stress responses and plant development

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(Turner *et al*., 2002). Jasmonic Acid (JA) and its methyl ester, methyl jasmonate (MeJA), have been found to occur naturally in a wide range of higher plants (Creelman *et al*., 1997). It is a final product of the enzymatic oxidation of unsaturated fatty acids and lipoxygenase (LOX) is a pivotal enzyme in this pathway (Vick and Zimmermann, 1984). This compound, defined as a natural plant growth regulator, was found to be active in many physiological systems. Jasmonate has been shown to increase the chilling tolerance of several plant species (Wang and Buta, 1994; Meir *et al*., 1998). Protection plant cells against environmental stress by Jasmonates may also associate with involving special gene expression (Ding *et al*., 2001). Exposure of avocado (Gonzalez-Aguilar *et al*., 2003) and tomato (Ding *et al*., 2001) fruits to low concentration of jasmonates suppressed CI and decay in low temperature storage fruits. Today, due to safety, application of these compounds for reducing environmental stress progressively increased and can be promising. However, the mechanism of MeJA treatment used to protect against decay and chilling injury is unclear.

Therefore, this study was undertaken to determine if Methyl Jasmonate could reduce CI and extend shelf life of pomegranate fruit stored at low temperatures.

MATERIALS AND METHODS

Fruit characteristics and handlings: Pomegranate fruits (*Punica granatum* L. cv Malas Save) were harvested at 30 September 2005 from a commercial orchard in Ghom province, Iran. Fruits were transported to the laboratory and were sorted based on uniformity in size, color and freedom from defects.

Post harvest treatments: For evaporation treatments, different concentration of Methyl Jasmonates (8, 16 and 24 μL L^{-1}) were spotted on the filtrated pepper that placed into plastic jar (20 L) that containing 20 fruits. Then the plastic container was incubated in dark place with temperature about 25°C for 16 h. Afterwards, the containers were opened, ventilated for 4 h and then lots of 20 fruits were placed in open boxes. Control fruits were placed in low temperature without ant treatments. Each concentration was repeated with three times. At last fruits were stored in storage with temperature 2°C and 85-90% RH for 3 months. Different characteristics were investigated in three stages. Stage 1, immediately after harvest and before treatments and three stages later were carried out during low temperature storage periods, thereafter fruits were transferred to shelf life condition for one week for measuring the latest stage of measurements.

Chilling injury: CI incidence was evaluated during low temperature storage and an additional one week in high temperature. CI (pitting and husk-scald) were determined for each fruit and scored on the following scale: 0 = no pitting; 1 = 1-25% pitting; 3 = 26-50% pitting 4 = extensive pitting covering than 50% of the fruit surface. The average extent of CI damage was expressed as a CI index, which was calculated using the following formula (Funga *et al*., 2004):

$$\text{CI index} = \frac{\sum(\text{ni} \times \text{i})}{\text{N}}$$

Juice characteristics: The arils were homogenized in a commercial blender and the juice analysed for Soluble Solids Content (SSC), Titratable Acidity (TA) and vitamin C. Amount of SSC was determined in a digital hand refractometer and TA was determined by titrating of juice with 0.1 M NaOH to pH 8.1. Vitamin C content in the juice was measured by titrating with dechlorophenol endophenole. Total anthocyanins in juice were calculated by recording light absorption of juice at 510 nm. For this purpose, fruit juice was diluted with water with 1:3 ratio and the absorption factors was determined by spectrophotometer.

Sensory analysis: Visual appearance was evaluated by different trained people at shelf life period. Evaluation was scored with five different scales.

Statistical analysis: The experimental design was completely randomized. Analysis of variance (ANOVA) and Duncan's multiple range tests for comparison of means were performed with the data using the SAS system.

RESULTS AND DISCUSSION

Chilling injury: CI in pomegranate was manifested as peel pitting and husk-scald, scald, confirming previous reports (Artes *et al*., 2000). CI in pomegranate fruits increased considerable during storage (Fig. 1). After shelf life, an increase in pitting and husk scald was observed in all treatments. The higher development of husk scald at higher temperature was in agreement with results reported by Artes *et al*. (2000).

Fruits treated with methyl jasmonate showed some less CI index that is agreement with Gonzalez Aguilar *et al*. (2003) and Ding *et al*. (2001). As the Fig. 2 showed, with increasing methyl jasmonate concentration CI suppressed significantly. Thus the lowest CI was found in fruits that treated with 24 μL L^{-1} methyl jasmonate. Therefore,

Table 1: Change in internal qualities of pomegranate juice at harvest, during 90 days storage and after one additional week at shelf life condition

Treatments	Weight loss (%)	TSS (%)	TA (%)	TSS/TA	Vitamin C (mg/100 mL)	Antocyanin absorbtion (510 nm)
At harvest	0	17.53a	1.45a	12.06d	22.04a	1.75a
1 month	3.2d	17.2a	1.32b	13.03c	17.02b	2.04a
2 month	4.52c	17.22a	1.2b	14.37b	14.92bc	2.2a
3 month	5.24b	17.1a	1.07d	15.98a	13.03bc	2.39a
Shelf life	5.5a	17a	1.02e	16.55a	12.07c	2.48a

* Mean separation within each column is by Duncan's multiple test at $p = 0.05$

Table 2: Change in internal qualities of pomegranate fruit juice treated with different concentration of methyl jasmonate (8, 16 and 24 $\mu L\ L^{-1}$) and untreated control fruits

Treatments	Weight loss (%)	TSS (%)	TA (%)	TSS/TA	Vitamin C (mg/100 mL)	Antocyanin absorbtion (510 nm)
Control	7a	17.22a	1.21a	14.23a	12.45b	2.13a
8 $\mu L\ L^{-1}$	5.36b	17.10a	1.17a	14.61a	16.46a	2.22a
16 $\mu L\ L^{-1}$	5.48b	17.35a	1.32a	13.14ab	16.96a	2.16a
24 $\mu L\ L^{-1}$	5.54b	16.95a	1.19a	12.24b	17.38a	2.2a

* Mean separation within each column is by Duncan's multiple test at $p = 0.05$

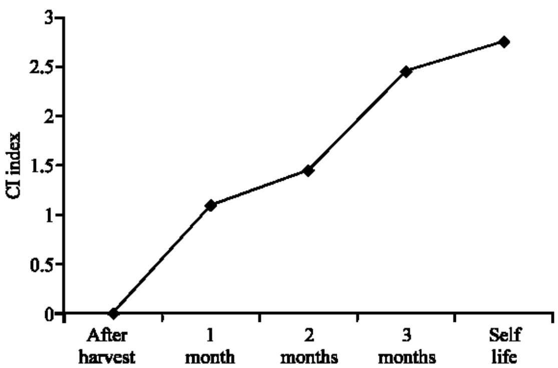

Fig. 1: CL index of pomegranates after different storage time in low temperature (2°C) and 85-90% RH and after one additional week at high temperature

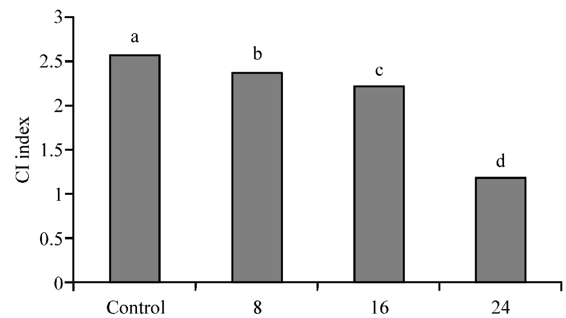

Fig. 2: Effect of various methyl jasmonate concentrations (8, 16 and 24 $\mu L\ L^{-1}$) and control on chilling injury of pomegranate cv, Malas Save, values labeled with the same letter are not different at the 5% significance level

treatment of pomegranate with low concentrations of MeJA could induce some defense-mechanism responses that indirectly provide protection against chilling damage, rather than compounds themselves producing a direct effect. It was reported that pre-treatment with MeJA at low concentrations induces protection against chilling injury and resistance to pathogens in tomato fruit, most likely by inducing the transcription of PR-protein genes (Ding *et al*., 2001).

CI when happen that amount of activated oxygen species was more than cell antioxidant capacity. Thus application signaling molecule such as jasmonates can changed antioxidant capacity of cells and followed by reduced stress damage (Eszter *et al*., 2002; Evans *et al*., 1999). In addition, heat shock protein production in fruits that exposed to methyl Jasmonate evaporation treatments can be related with acquired resistance to CI (Eszter *et al*., 2002).

Fruits weight loss: With increasing storage period, fruits weight loss increased progressively that led to significant differences among storage time. The Higher weight loss was found when fruits transferred to high temperature (Table 1). However, no symptoms of shriveling were observed after either cold storage or shelf life condition. These results are the same of previous report (Artes *et al*., 2000) where weight loss after shelf life reached to 5% of fruit.

As the Table 2 showed, Fruits evaporated with methyl jasmonate lost less weight than untreated control fruits, although there were no significant differences between different concentrations of methyl jasmonate but a significant difference was found between treated and untreated fruits (Table 2). It was recently reported that MJ treatment did not affect the rate of water loss of mango Tommy Atkins at low temperature (78°C) and shelf life (5 days at 208°C) Period (Gonzalez-Aguilar *et al*., 2000).

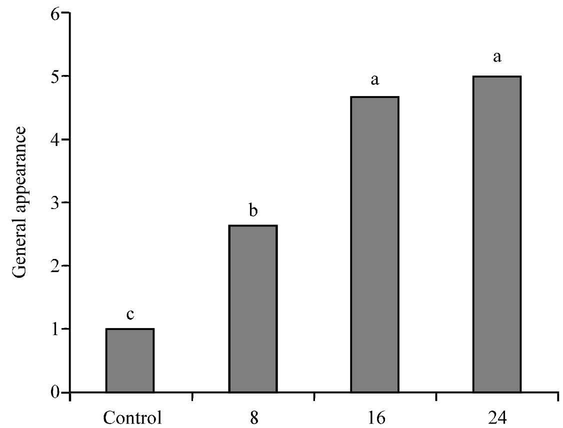

Fig. 3: Visual appearance of fruits treated with different concentrations of methyl jasmonate (8, 16 and 24 μL L^{-1}) and control fruits after 3 months storage at low temperature and one additional week in shelf life condition. Values labeled with the same letter are not different at the 5% significance level

Changes in chemical quality attributes: As expected in a non-climacteric fruit, a slight, but not significant decrease in SSC was found after cold storage in almost all treatments (Table 1) in agreement with Artes *et al.* (2000) but there was a significant difference among different storage time for TA and SSC and TA ratio. After long term storage at low temperature and an additional week in high temperature, TA percent decreased progressively that resulted in increase SSC to TA ratio (Table 1). Vitamin C in the juice also declined after long term storage as compared with fresh fruits (Table 1).

Although vitamin C in juice of fruits that stored in shelf life condition was the lowest but no significant difference was found with fruits that stored for 2 and 3 months in low temperature (Table 1).

No significant difference was found among treatments for SSC and TA in storage fruits but SSC o TA ratio significantly changed during the storage (Table 2). Amount of vitamin C and antocyanin content in juice slight, no significant increased during the storage (Table 2). Increasing light abortion at 510 nm by diluted juice can be due mainly to different anthocyanin together especially the one with higher absorbance (Artes *et al.*, 2000). These results correspond to slight or negligible changes in aril color as measured directly by spectrophotometer.

Visual quality: In general there was a reduction in visual quality with storage duration, being greater in fruit after raising temperature at end of storage, the visual appearance of Pomegranate fruits and arils were less acceptable in untreated fruits stored at 2°C (Fig. 3). The best results were obtained in evaporative fruits with methyl jasmonate (24 μL L^{-1}).

Overall, application of methyl jasmonate can delayed chilling injury incidence in pomegranate fruits with undesirable effect on the internal quality. Although there are many methods to reduce chilling injury in various horticultural crops (Wang and Buta, 1999), we have shown MeSA and MeJA treatments are inexpensive, easy to set up and applicable to various fruit produce (Ding *et al.*, 2001).

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