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Methyl jasmonate and polyamines reduce chilling injury symptoms of orange (*Citrus sinensis*) fruit during cold storage

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Abstract

Chilling injury is one of the most important problems of tropical and subtropical fruits during storage that can occur if the temperature falls below 5 °C. Polyamines and methyl jasmonate (MJ) are believed to prevent and inhibit chilling injury (CI) during storage. In order to find a suitable treatment to reduce CI of oranges (*Citrus sinensis*) during cold storage, a research was conducted with two concentrations of MJ and two concentrations of spermidine (Spd) and putrescine (Put) (1 and 1.5 mgL^{-1}), applied as pre-storage treatments and fruits were stored at 2 °C for 1.5 months. Application of MJ and PAs reduced percentages of CI, decay, pitting, physiological decay (PHD), ion leakage, potassium leakage, and weight loss (WL) and firmness in the fruit as compared to control after the storage period. Put at 1 mgL^{-1} had significantly lower percentage ion leakage and pH although CI in this treatment was similar to other treatments. Fruit juice density was not affected by any of the treatments.

Key words: Jasmonic acid, polyamines, chilling injury, citrus, cold storage.

Introduction

Cold storage not only reduces the biosynthesis of ethylene in the fruits but also reduces the response of fruits to ethylene (Wills *et al.*, 2007). Cold storage is an effective method for controlling pathogenic decay and maintaining quality. However, citrus and many tropical fruits are sensitive to low temperatures and CI may occur at temperatures below 5 ºC, limiting storage life (Jin *et al.*, 2009). Low temperatures close to chilling, change structure and action of the cell membrane, stop the cytoplasmic movements, change the respiration rate and ion leakage from the cell (Ganji Moghadam, 1994).

Methyl jasmonate (MJ) is a substance used in plant defense system and many diverse developmental pathways such as seed germination, root growth, flowering, fruit ripening and senescence. MJ is derived from jasmonic acid and the reaction is catalyzed by S-adenosyl-L-methionine: jasmonic acid carboxyl methyltransferase. Plants produce jasmonic acid and MJ in response to many biotic and abiotic stresses and wounds (Cheong and Yang, 2003). Ghotbi (2009) reported that application of MJ before storage reduced CI in pomegranates stored in cold storage.

Polyamines (PAs), on the other hand, are bio-compounds with low molecular weight having N-alyphatic groups which exist in all living organs of animals and plants (Valero *et al.*, 2002). Both natural (self-produced) and synthetic PAs (applied exogenously) improve the storage life of fruits (Mirdehghan *et al*, 2007; Valero *et al.*, 2002). The most important roles of PAs in fruits includes delaying in color, increasing fruit firmness, reducing respiration rate, induction of mechanical resistance and decreasing chilling symptoms (Valero *et al.*, 2002). Mirdehghan *et al.* (2007) reported that pre-storage application of PAs on pomegranates, improved storage life of fruits by increasing internal polyamines. Our goal in this study was to evaluate the impact of polyamines and methyl jasmonate (MJ) on chilling injury and various quality attributes in sweet orange (*Citrus sinensis*) during cold storage.

Materials and methods

Orange (*C. sinensis* cv. Valencia) fruit were collected from a commercial orchard near Darab, Fars, Iran and were transferred to the postharvest laboratory of Horticultural Sciences Department, Shiraz University, Iran in April 2011. Fruits were washed, sprayed with ethanol (95%) and were dipped in treatment solutions for four minutes. Treatments included methyl jasmonate (MJ) (0.3 and 0.4 mM) and PAs (spermidine (Spd) and putrescine (Put) each at 1 and 1.5 mgL^{-1}) and distilled water as control. Then fruits were packed in plastic nets and were placed in the incubator (2 °C, 80% RH) for 1.5 month. The temperature and relative humidity were checked daily.

Decay, CI, and pitting and physiological disorders (PHD) such as red spot were rated on a scale of 1 (low incidence) to 5 (severe incidence) and percentage of each disorder was calculated.

Fruit weight loss (WL) was measured as:

$$W.L.(\%) = \frac{\text{Initial weight-final weight}}{\text{Initial weight}} \times 100$$

Six 10-millimeter disks of flavedo were collected from each treatment and were shaken in the 25 milliliteres 0.4 M manitol for 4 hours. Their initial electrolyte content was measured by EC meter (METROHM-644 conductometer, Swiss) and the initial rate of K^+ leakage was measured by the Flame photometer (Jenway, pep 7, UK). Then the solutes were autoclaved (1atm, 20 min). After 24 hours again the EC and K^+ leakage were measured. EC percentage as ion leakage percent and K^+ leakage percentage were calculated as:

$$EC\% = \frac{\text{Initial Electrical Conductivity}}{\text{Final Electrical Conductivity}} \times 100$$

$$\text{Ion Leakage } \% = \frac{\text{Initial } K^+ \text{ in the Solute}}{\text{Final } K^+ \text{ in the Solute}} \times 100$$

Firmness was measured by the Penetrometer (STEVENS-LFRA texture analyser, GY-2, England). Juice volume and percentage of juice volume per fruit weight were measured.

Juice pH was detected by pH meter (Jenway, 3510- EU) and soluble solids concentration (SSC) by a hand refractometer (BLEEKER N-52436, Holland). Vitamin C was measured by indophenol titration method and the following equation was used for its actual content:
Vitamin C (mg/100cc) = Correct Volume x F x 40

Titratable acidity (TA) was measured by titration method with 0.1 N NaOH and using phenolphthalein. The actual content of acid was detected by below equation:

$$\text{Citric acid in mg per 100 mL Juice} = \frac{\text{Used NaOH mL x NaOH N x 0.0064}}{\text{Sample mL}} \times 100$$

The experimental design was a completely randomized design with seven treatments and three replications for each treatment. There were five fruits in each replication. Data were analyzed by SAS software V 9.1 and the means were compared with LSD at 5%.

Results and discussion

Application of MJ and PAs reduced percentages of CI, decay, pitting, PHD, ion leakage, potassium leakage, and WL and firmness in the fruit as compared to control (Table 1). Put at 1 mgL^{-1} had significantly low percentage ion leakage and pH although CI in this treatment was similar to other treatments (Table 1). MJ plays effective role in signaling defense responses in chilled plants and the onset of the tolerance has been often correlated with the accumulation of defense-related enzymes and compounds (Creelman and Mullet, 1997). Previous studies have shown that exogenous application of MJ resulted in an improved chilling tolerance and reduced incidence of CI in several fruits (Ding *et al.* 2001; Fung *et al.* 2004). It has been reported that postharvest application of MJ significantly reduced CI after cold storage of various chilling-sensitive fruits and crops such as avocado, grapefruit and pepper (Dorby *et al.* 1999) and different mango cultivars (González-Aguilar *et al.* 2000). MJ could effectively reduce flesh leatheriness and internal browning, the typical CI symptoms in loquat fruits (Cao *et al.*, 2009). In our study, the chilling tolerance of orange fruits was also enhanced by applications of postharvest MJ treatment, which is in agreement with previous studies. Evidences suggest that oxidative stress from excess production of ROS, such as O^{2-}, singlet oxygen, H_2O_2 and hydroxyl radical, may contribute to the development of CI and that antioxidant enzymes, SOD, CAT and APX may play role in detoxifying ROS and reducing CI (Hariyadi and Parkin, 1991; Sala, 1998; Zheng *et al.*, 2008). Membranes are thought to be the primary sites for development of CI and this area deserves further study.

It was suggested that Spd might prevent CI by a mechanism involving protection of membrane lipids. Spd may inhibit CI by retarding lipid peroxidation (Buchereau *et al.* 1999). Inhibition of free Put accumulation, decrease in chilling tolerance and survival and an increase in electrolyte leakage may be reversed by the addition of Put (Lee *et al.*, 1997). Reduction in CI symptoms by PAs might be due to their capacity for preserving membrane integrity, both by lowering the membrane phase-transition temperature fluidity and retarding lipid peroxidation, resulting in increased cell viability (Drolet *et al.*, 1986; Kramer and Wang, 1989).

Similar to our results in the present study (Table 1), MJ-treated fruits showed less weight loss and pitting incidence as compared to non-treated control in mango (González-Aguilar *et al.* 2000) and guava (González-Aguilar *et al.,* 2004). In our study, untreated fruit were firmer than treated ones in all treatments (Table 1). This is because untreated fruits had higher WL (retained less water) resulting in smaller and firmer fruit (Table 1). Similar result was reported in other tree fruits (Cao *et al.,* 2009), strawberries (Pérez *et al.*, 1997), and peaches (Jin *et al.*, 2009).

Juice volume, juice density, vitamin C, SSC and SSC/TA were increased in all treated fruit in comparison with non-treated fruit, although differences were not always significant (Table 2). These observations are consistent with the reports in other fruit (Cao *et al.*, 2009; González-Aguilar *et al.,* 2000; Jin *et al.*, 2009).

Occasionally significant differences existed for some fruit quality attributes within treatments. Most noticeably, fruits treated with Put at 1 mgL^{-1} had lower ion and K leakage, perhaps because this treatment increased cell membrane integrity more than others. However, there were no significant firmness differences among different treatments at different rates (Table 1).

In conclusion, application of MJ and PAs can improve storage

Table 1. The effects of MJ, Spd and Put treatments on fruit chilling injury, decay, pitting, PHD, WL, firmness, ion leak and K^+ leak of 'Valancia' orange after 1.5 months of storage at 2 °C.

Treatment	Chilling injury (%)	Decay (%)	Pitting (%)	PHD (%)	WL (g)	Firmness (g/cm^2)	Ion leak. (%)	K^+ leak. (%)
Control	44.00a[Z]	13.33a	38.33a	57.33a	5.28a	928.27a	383.01a	276.71a
MJ (0.3 mM)	0.00b	0.00b	5.33b	17.33b	3.01b	708.67b	281.56b	192.64b
MJ (0.4 mM)	1.33b	0.00b	10.66b	14.67b	3.13b	663.00b	223.68c	219.04b
Spd (1 $mg.L^{-1}$)	0.00b	0.00b	1.33b	14.67b	2.23b	704.67b	272.52b	214.41b
Spd (1.5 $mg.L^{-1}$)	1.33b	1.33b	5.33b	20.00b	3.45b	674.83b	271.63b	219.49b
Put (1 mg. L^{-1})	0.00b	0.00b	6.66b	21.33b	2.64b	721.17b	128.90d	144.89c
Put (1.5 mg. L^{-1})	0.00b	0.00b	5.33b	12.00b	2.54b	753.50b	227.94c	194.28b

[Z] Mean separation within columns by Least Significant Difference (LSD) at P=0.05

Table 2. The effects of MJ, Spd and Put treatments on fruit juice volume, juice density, pH, SSC, vitamin C, TA, SSC/TA of 'Valancia' orange after 1.5 months of storage at 2 °C

Treatment	Juice Volume (%)	Juice Density (g/cm^3)	pH	SSC (%)	Vitamin C (mg ascorbic acid/100 mL juice)	TA (g citric acid/100 mL juice)	SSC/TA
Control	58.08c[z]	45.0a	3.94a	9.34b	95.6b	10.7a	869.0c
MJ (0.3 mM)	64.19b	44.0a	3.95a	10.58a	101.8ab	7.7c	1370.5a
MJ (0.4 mM)	69.78a	44.0a	3.96a	10.58a	102.8ab	8.1c	1254.5ab
Spd (1 $mg.L^{-1}$)	67.57ab	46.6a	3.95a	10.91a	106.3a	8.4bc	1202.2ab
Spd (1.5 $mg.L^{-1}$)	63.44b	45.0a	4.01a	10.58a	107.9a	9.8ab	1099.0b
Put (1 mg. L^{-1})	63.71b	48.0a	3.84b	11.16a	106.3a	10.0ab	1129.0b
Put (1.5 mg. L^{-1})	67.36ab	45.3a	3.99a	10.98a	105.6a	9.2abc	1191.7ab

[z] Mean separation within columns by Least Significant Difference (LSD) at P=0.05

life of oranges in cold temperatures by reducing low temperature injuries to the fruit. Most differences were observed between treated vs. non-treated fruit. Application of Put at 1 mg. L^{-1} significantly reduced ion leakage and pH and this observation warrants further detailed study with the chemical. In light of major impacts of these compounds on fruit quality attributes and cold injury, we strongly suggest that this study must continue with other fruit crops.

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