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Methyl jasmonate alleviates chilling injury and regulates fruit quality in 'Midknight' Valencia orange

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ABSTRACT

Susceptibility of sweet oranges to chilling injury (CI) restricts the utilisation of cold storage to its full potential to extend storage life and maintain fruit quality. The present investigation examined the role of postharvest methyl jasmonate (MJ) dips and different cold storage temperatures on the incidence of CI and fruit quality of 'Midknight' Valencia over two years. The fruit were dipped for 1 min in aqueous emulsions containing different concentrations 0.10, 0.25 or 0.50 mM of MJ and 'Tween 20' (0.01%) as a surfactant. The untreated fruit were used as the control. The fruit were stored at 4 °C or 7 °C for 90 d followed by 10 d simulated shelf conditions. MJ treatments, irrespective of the concentration applied, reduced CI in the fruit. The fruit treated with 0.25 mM MJ followed by 90 d cold storage and 10 d simulated shelf conditions were free from CI, irrespective of the cold storage temperatures. Dip treatments of 0.25 or 0.50 mM MJ reduced soluble solids concentration (SSC) and titratable acidity (TA); however, the SCC/TA ratio was higher when fruit was dipped in 0.25 mM MJ as compared with all other treatments. 0.25 or 0.50 mM MJ reduced concentrations of vitamin C and total antioxidants compared with all other treatments. Overall, 0.25 mM MJ is recommended as a treatment to reduce CI, while maintaining fruit quality attributes.

1. Introduction

Cold storage at temperatures close to 0 °C is widely used to extend the postharvest life of various fruit and vegetables. Cold storage, however, is limited as a method to extend storage of tropical and subtropical fruit including sweet oranges because these fruit are prone to chilling injury (CI) when stored below 10–15 °C (Ladaniya, 2008). CI lowers the overall quality and marketability of many tropical and subtropical fruits and vegetables (Cao et al., 2009). Citrus fruit are usually stored at moderately low temperatures (6–10 °C), depending on cultivar, species, and storage duration (Schirra et al., 1998). CI in citrus fruit is expressed as rind staining, pitting, red blotches, scalding and watery breakdown on the flavedo (Sala and Lafuente, 1999; Reuther, 1989). Various factors affect CI susceptibility of the fruit such as cultivar, harvest date, fruit size, position of the fruit in the canopy, rind colour, microclimate and management practices (Paull, 1990). Intermittent warming reduced the level fatty acid unsaturation of the lipid fraction from the flavedo tissue of 'Olinda' oranges stored at 3 °C (Schirra and Cohen, 1999). CI reduction by heat-conditioning treatments was associated with repression of genes involved in the lipid degradation and regulated stress related proteins in 'Fortune' mandarin (Lafuente et al., 2017).

Previously, Cao et al. (2009) reported that application of 10 μmole L^{-1} MJ reduced CI symptoms in loquat fruit stored at 1 °C for 35 d was associated with reduced lipoxygenase (LOX) activity and maintenance of a high level of unsaturated/saturated fatty acid ratios. Furthermore, Martinez-Tellez and Lafuente (1992) associated the increased CI with the enhanced level of phenylalanine ammonia-lyase (PAL) in untreated 'Fortune' mandarin when stored at 2.5, 5 and 10 °C for 25 d.

MJ regulates many aspects of plant growth and development including fruit ripening, flowering and senescence (Creelman and Mullet, 1995). MJ is known as a signalling molecule which plays a role in biotic and abiotic stress responses such as pathogen/insect attack, drought, mechanical and CI (Creelman and Mullet, 1995; Hayat et al., 2007). MJ has also been widely known to induce defence mechanisms against a wide range of pathogens in many plant species (Penninckx et al., 1998). Dysfunction of the cell membrane occurs when fruit are stored at low temperature, eventually leads to the development of CI (Zhang and Tian, 2009). Jasmonic acid is a final product of the enzymatic oxidation

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of unsaturated fatty acids, and LOX is a pivotal enzyme in this pathway (Vick and Zimmerman, 1984). MJ plays an integral role in the intracellular signal-transduction cascade that operates in the plant to induce stress responses (Sembdner and Parthier, 1993).

Application of MJ prior to low-temperature storage has reduced the development of CI symptoms in various non-climacteric fruit such as lemon (2 °C) (Siboza et al., 2014), pomegranate (1.5 °C) (Mirdehghan and Ghotbi, 2014), pineapple (10 °C) (Nilprapruck et al., 2008), loquat (1 °C) (Cai et al., 2011), grapefruit (2 °C) (Meir et al., 1996) and guava (5 °C) (González-Aguilar et al., 2004). Recently, Siboza and Bertling (2013) reported that postharvest treatment with 10 µM MJ alone or in combination with 2 mM salicylic acid reduced CI and membrane lipid peroxidation; inhibited reactive oxygen species (ROS) production and enhanced antioxidant activity in the flavedo of 'Eureka' lemon. No research work has been reported on the effect of the postharvest application of MJ on extending postharvest life, reducing CI and maintaining quality in cold stored sweet oranges. To extend availability of fresh fruit, the cultivation of a late maturing 'Midknight' Valencia sweet orange (harvest season from September to December) has gained a great impetus in Western Australia (DAFWA, 2017). The extension of storage life of this late maturing cultivar will further extend the availability of sweet oranges to the consumers. It was surmised that lower temperature (4 °C) will be more effective in extending storage life of 'Midknight' Valencia orange but coupled with CI as compared to the optimum storage temperature (7 °C). Hence, we also evaluated the efficacy of MJ treatments to alleviate the CI in both cold storage temperatures. The present study aimed at investigating the effects of MJ application on reducing the incidence of CI and maintaining fruit quality in 'Midknight' Valencia sweet oranges stored at 4 °C or 7 °C for 90 d followed by 10 d simulated shelf conditions.

2. Materials and methods

2.1. Fruit material

Sweet orange cv. 'Midknight' Valencia (*Citrus sinensis* L. Osbeck) fruit were harvested at the physiological maturity (SSC 9.0% and juice content 38.0%) from seven-year old uniform sweet orange trees previously grafted to Carrizo citrange (*Citrus sinensis* (L.) Osbeck *x Poncirus trifoliata* Raf.) rootstock grown at a commercial orchard, Moora Citrus, at Dandaragan (30° 35′ S/115° 55′ E) in Western Australia. The trees were spaced 2.7 m tree to tree and 7.5 m between rows on north-south orientation. All the experimental trees received normal regimes of fertilisers, irrigation and plant protection (Moulds and Tugwell, 1999). The experiments were conducted on late maturing fruit in 2014 and 2015. Fruit of uniform size and free from disorders, diseases and blemishes were randomly harvested around the tree canopy on 15 October in 2014 and 28 October in 2015. Fruit were transported directly in a closed container to the Curtin Horticulture Research Laboratory, Curtin University, Perth, WA, within four hours of harvest and treated.

2.2. Experiment 1: effects of different concentrations of MJ dip treatments and cold storage temperature on CI incidence (2014)

Fruit were dipped for 1 min in an emulsion containing different concentrations (0.1, 0.25 or 0.50 mM) of MJ obtained from Sigma-Aldrich, (Saint Louis, USA). Tween® 20 (0.25%) was used as a surfactant. The untreated fruit were used as the control. Following the treatments, the fruit were kept for 6 h at 20 ± 1 °C and relative humidity (RH) of 60 ± 5%. After drying, fruit were packed in plastic crates (20 per crate) and stored at 4 °C or 7 °C for 90 d at 85–90% RH. The experiment was a completely randomised design with two factors (MJ concentration and storage temperature) with three replications, each with twenty fruit. CI incidence (%) was recorded following 90 d cold storage and 10 d simulated shelf conditions (21 ± 1 °C).

2.3. Experiment 2: effects of different concentrations of MJ dip treatments and cold storage temperature on CI incidence and fruit quality (2015)

In 2015, the first experiment was repeated with the same MJ concentrations; storage temperatures, time period and experimental design, but had four replications with twenty-five fruit per replication. In addition to the CI incidence (%), various fruit quality variables such as fruit firmness, SSC, TA, SSC/TA, vitamin C and total antioxidants were also determined from the fruit juice of the fruit stored at 4 °C and 7 °C for 90 d and followed by 10 d in simulated shelf conditions (21 ± 1 °C). Fruit weight loss (%) was recorded only after 90 d cold storage.

2.4. Assessments

2.4.1. CI incidence

Fruit were visually examined for the symptoms of CI.

2.4.2. Weight loss

Initial fruit weight of the 25 fruit per replicate was recorded at the start of cold storage time and final fruit weight was recorded after 90 d of cold storage by using a digital weigh balance.

2.4.3. Fruit firmness

Ten randomly selected fruit per replicate were used to determine fruit firmness by using a texture profile analyser (TPA Plus, AMETEK Lloyd Instruments Ltd, Fareham, UK) interfaced with Nexygen® 4.6 software. Individual fruit were placed between two horizontal plates with the stem axis perpendicular to the plate. The cross head speed was 200 mm min^{-1} and test was completed at a strain of 50% of fruit height. Fruit firmness was expressed in newtons (N).

2.4.4. Soluble solids concentration (SSC), titratable acidity (TA) and SSC/TA

Fresh juice was squeezed from randomly selected 10-fruit in each replication to determine the SSC and expressed as a percentage by using a digital refractometer (Atago-Palette PR 101, Atago CO. Ltd, Itabashi-Ku, and Tokyo, Japan). The TA was determined by titrating the juice with 0.1 N NaOH using 2–3 drops of phenolphthalein as an indicator to a pink colour end point. TA was calculated as percentage citric acid. SSC/TA was calculated by dividing SSC by the TA value.

2.4.5. Vitamin C and total antioxidant activity

Juice of 10 randomly selected fruit per replication were used for the determination of vitamin C and total antioxidant activity by following the methods of Rehman et al. (2018) and Brand-Williams et al. (1995) respectively, using a UV/VIS spectrometer (Jenway spectrophotometer Model 6405, Dunmow, Essex, UK). A standard curve of 98% L-ascorbic acid (range 0–55 µ g L^{-1}) was used to calculate ascorbic acid concentration, which was expressed as mg L^{-1} of fresh juice. The concentration of total antioxidants was calculated by using a standard curve (range 0–1000 µM) of 97% of 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) and expressed as µM Trolox equivalent antioxidant activity fresh juice basis.

2.5. Statistical analysis

Data were subjected to two-way analysis of variance (ANOVA) using GenStat 14th edition (release 14.1; Lawes Agricultural Trust, Rothamsted Experimental Station, Rothamsted, UK). Mean treatment differences were tested using least significant differences (LSD) following *F*-test at ($P = 0.05$).

Table 1
CI (%) of fruit treated with MJ and storage at 4 and 7 °C for 90 d followed by 10 d of simulated shelf conditions (21 ± 1 °C) in the 2014 and 2015 growing seasons.

CI (%)						
	2014			2015		
MJ (mM)	4 °C	7 °C	Average (Trt)	4 °C	7 °C	Average (Trt)
0	5.3	2.6	4.0	7.5	2.5	5.0
0.1	1.3	0.0	0.6	0.0	0.0	0.0
0.25	0.0	0.0	0.0	0.0	0.0	0.0
0.50	2.6	0.0	1.3	1.2	1.2	1.2
Mean (ST)	5.3	0.6		2.1	0.9	
LSD (P = 0.05)	Trt = 2.2, ST = 1.5, Trt x ST = ns			Trt = 1.9, ST = ns, Trt x ST = 2.7		

Trt = treatment, ST = storage temperature, n = 60 (20 fruit per replicate, 2014) and n = 100 (25 fruit per replicate, 2015).

3. Results

3.1. CI

Dip treatments of 0.1, 0.25 and 0.50 mM of MJ reduced CI as compared with that of control fruit in both years (Table 1). However, CI was lower at 7 °C (0.6%) compared with those at 4 °C (5.3%) in 2014, but there was no storage temperature effect in 2015. There was an interaction between MJ treatments and storage temperatures in 2015. 0.1, 0.2 or 0.50 MJ had lower % CI than the control (7.5%) at 4 °C, but not at 7 °C.

3.2. Weight loss

Weight loss of fruit was reduced by MJ irrespective of the concentration in 2015 (Table 2). Moreover, weight loss was higher (9.1%) when fruit was stored at 7 °C than at 4 °C (6.0%). All MJ concentrations had reduced weight loss compared with the control (8.8%) when stored at 4 °C, but not at 7 °C.

3.3. Fruit firmness

MJ dip treatments and cold storage temperatures did not significantly affect fruit firmness (N) after 90 d cold storage followed by 10 d simulated shelf conditions (Table 3). The interaction effect between MJ dip treatments and cold storage temperatures was non-significant.

3.4. SSC, TA and SSC/TA ratio

MJ at 0.25 or 0.50 mM changed SSC from 12.6 to 12.0% and TA from 0.65 to 0.67% respectively, compared with that of the control fruit (Table 4). The SCC/TA ratio was higher (19.4) when fruit was dipped in 0.25 mM MJ as compared to the control (17.8) and all other treatments. However, SSC and SSC/TA was higher in the fruit juice stored at 7 °C (12.9% and 18.9) than those kept at 4 °C (12.2% and 17.7), respectively. TA was not affected by cold storage temperatures. There was a significant interaction between MJ treatments and storage temperatures for SSC, TA and SSC/TA ratio. MJ (0.25 or 0.50 mM) had lower SSC compared with the control at 4 °C and 7 °C. Furthermore, all the MJ concentrations resulted in lower TA as compared to control (0.72%) in the fruit juice stored at 4 °C; whilst all the MJ treatments except (0.1 mM) exhibited lower TA as compared to control (0.72%) in the fruit juice stored at 7 °C. The SSC/TA ratio was higher (20.5) at 0.25 mM MJ treatment than other MJ concentrations and the control when stored at 7 °C.

Table 2
Percentage weight loss of fruit treated with MJ and storage at 4 and 7 °C for 90 d followed by 10 d of simulated shelf conditions (21 ± 1 °C) in the 2015 growing season. n = 4 replicates.

Weight loss (%)			
MJ (mM)	4 °C	7 °C	Average (Trt)
0	8.8	8.8	8.8
0.1	4.6	10.0	7.9
0.25	5.8	8.4	7.1
0.50	4.8	9.1	7.0
Mean (ST)	6.0	9.1	
LSD (P = 0.05)	Trt = 1.3, ST = 0.92, Trt x ST = 1.84		

Table 3
Fruit firmness of fruit treated with MJ and storage at 4 and 7 °C for 90 d followed by 10 d of simulated shelf conditions (21 ± 1 °C) in the 2015 growing season. n = 40 (10 fruit per replicate).

Firmness (N)			
MJ (mM)	4 °C	7 °C	Average (Trt)
Control	340.8	335.0	337.9
0.1	341.6	342.0	341.8
0.25	338.1	357.6	347.9
0.50	356.6	335.7	346.1
Mean (ST)	344.3	342.6	
LSD (P = 0.05)	Trt = ns, ST = ns, Trt x ST = ns		

Table 4
SCC, TA and SSC/TA ratio in the juice of fruit treated with MJ and storage at 4 and 7 °C for 90 d followed by 10 d of simulated shelf conditions (21 ± 1 °C) in the 2015 growing season. n = 4 replicates.

(MJ mM)	4 °C	7 °C	Average (Trt)
	SSC (%)		
Control	12.6	13.1	12.8
0.1	12.6	13.1	12.8
0.25	12.3	12.8	12.6
0.50	11.4	12.7	12.0
Mean (ST)	12.2	12.9	
LSD (P = 0.05)	Trt = 0.20, ST = 0.14, Trt x ST = 0.28		
	TA (%)		
Control	0.72	0.72	0.72
0.1	0.69	0.74	0.72
0.25	0.67	0.62	0.65
0.50	0.67	0.66	0.67
	0.69	0.69	
LSD (P = 0.05)	Trt = 0.02, ST = ns, Trt x ST = 0.02		
	SSC/TA		
Control	17.8	18.2	17.8
0.1	18.2	17.7	18.0
0.25	18.4	20.5	19.4
0.50	16.9	19.1	18.0
	17.7	18.9	
LSD (P = 0.05)	Trt = 0.62, ST = 0.44, Trt x ST = 0.87		

3.5. Vitamin C and total antioxidant activity

MJ at 0.25 and 0.50 mM reduced mean vitamin C (285.4 and 266.9 mg L^{-1}) and total antioxidant activities (406.7 and 396.7 μM L^{-1} Trolox) respectively, as compared to all other treatments (Table 5). However, the mean levels of vitamin C (301.4 mg L^{-1}) and total antioxidants (430.0 μM L^{-1} Trolox) were higher in the fruit which were stored at 4 °C as compared to those stored at 7 °C (283.3 mg L^{-1} and 409.7 μM L^{-1} Trolox). No interaction between factors was detected for vitamin C and total antioxidant activity.

Table 5
Vitamin C and total antioxidants in the juice of fruit treated with MJ and storage at 4 and 7 °C for 90 d followed by 10 d of simulated shelf conditions (21 ± 1 °C) in the 2015 growing season. n = 4 replicates.

Vitamin C (mg L^{-1})			
MJ (mM)	4 °C	7 °C	Average (Trt)
0	313	289	301
0.1	342	291	316
0.25	276	295	285
0.50	275	259	267
Mean (ST)	301	283	
LSD (P = 0.05)	Trt = 25.5, ST = 18.0, Trt x ST = ns		
Total Antioxidant activity (μM L^{-1} Trolox)			
0	443	415	429
0.1	457	437	447
0.25	418	395	407
0.50	402	392	397
	430	410	
LSD (P = 0.05)	Trt = 29.5, ST = 20.6, Trt x ST = ns		

4. Discussion

A 0.1–0.25 mM MJ dip treatment for 1 min was most effective in reducing CI in 'Midknight' Valencia fruit stored for 90 d at 4 °C or 7 °C followed by 10 d simulated shelf conditions in two seasons. The mechanism by which MJ induces chilling tolerance in sweet oranges is still unclear. The cell membrane is the main site for CI followed by membrane disruption and loss of membrane integrity (Li et al., 2012; Saltveit and Morris, 1990). Possibly, MJ application may have protected the fruit from membrane damage and enhanced chilling tolerance in fruit through the improved activity of PAL, total antioxidants and phenolics in the flavedo tissue. Previously, Siboza and Bertling (2013) and Siboza et al. (2014) also reported that activation of PAL and increased production of phenolics and total antioxidants were involved in reducing CI in the flavedo tissue of lemon during cold storage. Similarly, differences in susceptibility to CI in 'Fortune' mandarins (*Citrus reticulata* Blanco) and 'Navelina' (*Citrus sinensis* L. Osbeck) have been reported to be related to the activity of PAL than to peroxidase (PPO) and polyphenol oxidase (POD) (Martinez-Tellez and Lafuente, 1992). Additionally, no correlation was found between POD, PPO and the development of CI in 'Fortune' mandarins and 'Navelina' (Martinez-Tellez and Lafuente, 1992). The higher PAL activity is related to lower CI of fruit in cold storage (Lafuente et al., 2003).

MJ application may have protected the fruit from membrane damage, electrolyte leakage and membrane lipid peroxidation products, such as malondialdehyde (MDA) leading to the development of CI. Similarly, Jin et al. (2013) also reported that MJ application inhibits ion leakage and MDA content consequently protecting fruit from membrane damage and reducing CI in peach fruit. Zhang and Tian (2009) reported that 0.1 mM MJ reduced CI in peach fruit. In addition, MJ treatment reduced CI index and ion leakage percentage in guava fruit stored at 5 °C (González-Aguilar et al., 2003). Increased chilling tolerance in MJ treated loquat fruit was associated with reduced LOX activity and higher unsaturated/saturated fatty acid ratios (Cao et al., 2009). The accumulation of ROS and oxidative stress from over production are also one of the major reasons behind the incidence of CI. Chilling tolerance in horticultural crops has also been improved by activation of antioxidants (Cao et al., 2009). The antioxidant system, comprising enzymatic and non- enzymatic constituents, plays an important role in scavenging ROS and increases chilling tolerance in many fruit (Jimenez et al., 2002). The exact mechanism of MJ application in reducing CI in 'Midknight' Valencia sweet orange fruit warrants investigation.

MJ treatments of 0.1, 0.25 and 0.50 mM for 1 min reduced weight loss compared with that of the control after 90 d of storage in 2015. Nilprapruck et al. (2008) also reported that MJ reduced the weight loss in pineapple fruit with enhanced chilling tolerance when stored at 10 °C. In addition, González-Aguilar et al. (2001) revealed that MJ treated mango fruit also showed less weight loss when stored for 14 d at 10 °C. Roongruangsri et al. (2013) reported two tangerine cultivars 'Sai Num Phueng' and 'See Thong' storage at 5 °C showed reduced weight loss and moisture content of the peel as compared to storage at 25 °C. Reduced weight loss in fruit stored at 4 °C than at 7 °C may be ascribed to the reduced rate of respiration and transpiration, also reported earlier in fruit (Wills and Golding, 2016).

Fruit dipped in 0.1 mM MJ showed an increased level of total antioxidants (446.8 μM L^{-1} Trolox) in the juice as compared with other concentrations, except for the control (429.2 μM L^{-1} Trolox) in 2015. The trend was reversed when fruit was treated with higher concentrations. Similarly, Chanjirakul et al. (2006, 2007) have shown that MJ, through enhanced antioxidant activity, might improve functional properties of harvested fruit. Furthermore, Cao et al. (2009) found that postharvest application of MJ in loquat fruit exhibited higher levels of total phenolic and maintained higher antioxidant activity in the pulp as compared to the control fruit. The mode of action of dip application of MJ in regulating the levels of total antioxidants in cold stored fruit is yet to be investigated.

5. Conclusion

Postharvest dip application of 0.25 mM MJ for one minute reduced CI in 'Midknight' Valencia oranges irrespective of cold storage temperature in both years. All MJ treatments showed reduced weight loss after 90 d of cold stored fruit. MJ dip treatments also exhibited higher SCC/TA ratio and reduced vitamin C and total antioxidants.

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