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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 ($P = 0.05$).